*antioxidants*

MDPI

*Review*

# Beyond the Nutritional Use of Klamath Microalgae (*Aphanizomenon flos-aquae*): Safety, Regulatory Challenges and Emerging Health Benefits

**Lara Testai [1,2,3,*], Paola Nieri [1,2,3], Veronica Cocetta [4], Marco Biagi [5] and Monica Montopoli [4]**

[1] Department of Pharmacy, University of Pisa, 56126 Pisa, Italy; paola.nieri@unipi.it
[2] Marine Pharmacology Interdepartmental Center, University of Pisa, 56126 Pisa, Italy
[3] Interdepartmental Center Nutrafood "Nutraceuticals and Food for Health", Interdepartmental Center, University of Pisa, 56124 Pisa, Italy
[4] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 Padova, Italy; veronica.cocetta@unipd.it (V.C.); monica.montopoli@unipd.it (M.M.)
[5] Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy; marco.biagi@unipr.it
* Correspondence: lara.testai@unipi.it; Tel.: +39-050-2219596

**Abstract**

*Aphanizomenon flos-aquae* (AFA), commonly known as Klamath algae, is a freshwater cyanobacterium harvested mainly from Upper Klamath Lake (Oregon, USA) and widely marketed as a nutraceutical ingredient. Its biomass is characterized by a rich nutritional profile, including proteins, vitamins, minerals, phycocyanins, phenylethylamine (PEA), mycosporine-like amino acids (MAAs), and other bioactive compounds with antioxidant, anti-inflammatory, and neuromodulatory properties. Preclinical evidence indicates that AFA extracts may exert neuroprotective effects through the modulation of oxidative stress, neuroinflammation, amyloid aggregation, and monoaminergic neurotransmission. Beneficial activities have also been reported in experimental models of inflammatory bowel disease and obesity-related metabolic dysfunction, where AFA reduces inflammation, improves metabolic parameters, and modulates epigenetic pathways. Clinical evidence remains limited but suggests potential benefits as a source of bioavailable vitamin B12 and in improving mood and cognition-related symptoms. Despite its long history of use, safety remains closely linked to product quality, because commercial AFA is predominantly harvested from natural blooms, where contamination by cyanotoxins, heavy metals, and other microorganisms may occur. Overall, AFA represents a promising and sustainable nutraceutical resource, although larger clinical studies and rigorous quality-control strategies are required to fully validate its efficacy and safety.

**Keywords:** oxidative stress; inflammation; phycocyanin; phenylethylamine; mycosporines; mycosporine-like amino acids; toxicity

Received: 28 October 2026
Revised: 16 November 2026
Accepted: 18 November 2026
Published: 19 November 2026

**Copyright:** © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

## 1. Ecology, Production and Commercialization

*Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet and Flahault (AFA) is a filamentous cyanobacterium belonging to the order Nostocales and the family *Aphanizomenonaceae*. The species is characterized by its ability to fix atmospheric nitrogen and is naturally associated with freshwater ecosystems. Among the environments in which AFA can become

particularly abundant, Upper Klamath Lake in Oregon (USA) is of special importance (Figure 1). In this lake, favorable environmental conditions during the warmer months promote the development of large seasonal populations and surface accumulations of biomass.

The classification of *Aphanizomenon* has evolved considerably since the genus was first established near the end of the nineteenth century. Currently, eight species are classified in the genus: *A. flos-aquae*, *A. klebahnii*, *A. yezoense*, *A. paraflexuosum*, *A. flexuosum*, *A. slovenicum*, *A. platense*, and *A. hungaricum*.

Interest in AFA extends beyond its ecological role because its biomass has long been used as a commercially marketed food supplement. Its nutritional characteristics, together with the presence of compounds potentially relevant to nutraceutical applications, have contributed to its economic value. Commercial AFA is predominantly obtained from natural populations rather than from cultivation systems. It has been estimated that roughly 500–1000 tonnes of dry biomass are collected each year from blooms occurring in Upper Klamath Lake and subsequently processed for commercial distribution [1].

Since fluctuations in lake conditions may affect both the quantity and the reproducibility of the biomass obtained, the development of cultivation approaches has become an important research objective, particularly in view of the possible expansion of its biotechnological applications [2].

AFA shows considerable adaptability to its freshwater environment and, although mainly reported from temperate lakes, can tolerate a relatively broad range of physicochemical conditions. Temperature is one of the main factors influencing its growth, with particularly favorable values between approximately 23 and 29 °C [3]. Moreover, the cyanobacterium can grow under highly alkaline conditions, including environments with pH values close to 10 while it has been reported [4] to be unable to grow below pH 7.1.

Light availability is another important component for AFA production. Rather than remaining at a fixed depth, the filaments can modify their position in the water column as environmental conditions change, regulating the amount of solar radiation reaching the cells. Such behavior is particularly advantageous and may contribute to maintaining photosynthetic activity across a range of light conditions [5].

The occurrence of AFA is not restricted to Upper Klamath Lake, but populations have been documented in a further 40 freshwater lakes belonging to different geographical regions [2]. Its presence has also been described in the Baltic Sea, although only one genotype (reported as ITS1) was able to persist in this latter case [6]. In most of the documented sites, the biomass accumulated during bloom events is substantially lower than that observed in Upper Klamath Lake. The latter therefore remains currently relevant as the principal source of biomass used for commercial purposes.

The ability of AFA to attain high biomass concentrations in Upper Klamath Lake can be related to the interaction of several environmental factors rather than to a single limiting variable. The chemical characteristics of the lake, including its mineral composition and nutrient availability, occur in combination with temperature and pH conditions that can favor cyanobacterial proliferation. Nutrient dynamics are especially relevant because AFA is diazotrophic, utilizing not only available inorganic nitrogen, but also fixing atmospheric $N_2$.

During periods of high biomass accumulation, the surface material is collected using specialized harvesting boats. The recovered biomass then undergoes water removal and drying before being converted into a form suitable for further processing and commercialization. Since natural blooms may contain other microorganisms, quality control is therefore an essential component of the production process. Commercial biomass must be monitored for contaminants such as cyanotoxins, heavy metals, and microorganisms. The

potential presence of toxin-producing cyanobacteria, including *Microcystis* spp., is of particular relevance because it may influence the safety and quality of the final product [7].

Following dehydration, AFA biomass is generally processed into a finely milled powder. Depending on the intended application, the powder may be commercialized directly or incorporated into other formulations, including capsules and tablets (Figure 2). As already stated, there is growing interest in the controlled cultivation of AFA [2] which would facilitate a more consistent supply of material and provide greater control over environmental parameters, biomass composition, and the reproducible production of bioactive compounds to be investigated systematically. The transition from predominantly wild-harvested biomass toward reproducible cultivation could therefore contribute to the development of new applications for AFA in the food, nutraceutical, and biotechnology sectors.

The commercially available formulations are reported in Table 1. Klamin® is a proprietary extract of *Aphanizomenon flos-aquae* standardized for β-phenylethylamine (PEA), whereas AphaMax® is standardized for phycocyanins, and is a unique patented formulation by Nutrigea (San Marino, Italy). KlamExtra® combines both extracts to provide PEA and phycocyanin-rich fractions. Finally, AFA-B12 contains highly bioavailable vitamin B12 [8–11].

**Table 1.** Composition and standardization of commercially available formulations of Klamath microalgae.

| Product | Standardized Bioactive Component | Reported Standardization | References |
|---|---|---|---|
| Klamin® | β-Phenylethylamine (PEA) | 0.3–0.5% (*w*/*w*) | [8] |
| AphaMax® | Acquous extract of AFA, standardized in phycocyanins | ≥15% phycocyanins (*w*/*w*) | [10] |
| KlamExtra® | β-Phenylethylamine (PEA) + Phycocyanins | Combination of Klamin® and AphaMax® | [9] |
| AFA-B12® | Vitamin B12 | n/d | [11] |



**Figure 1.** Klamath lake image from the site: https://www.usgs.gov/media/images/view-upper-klamath-lake-or (accessed on15 October 2026).

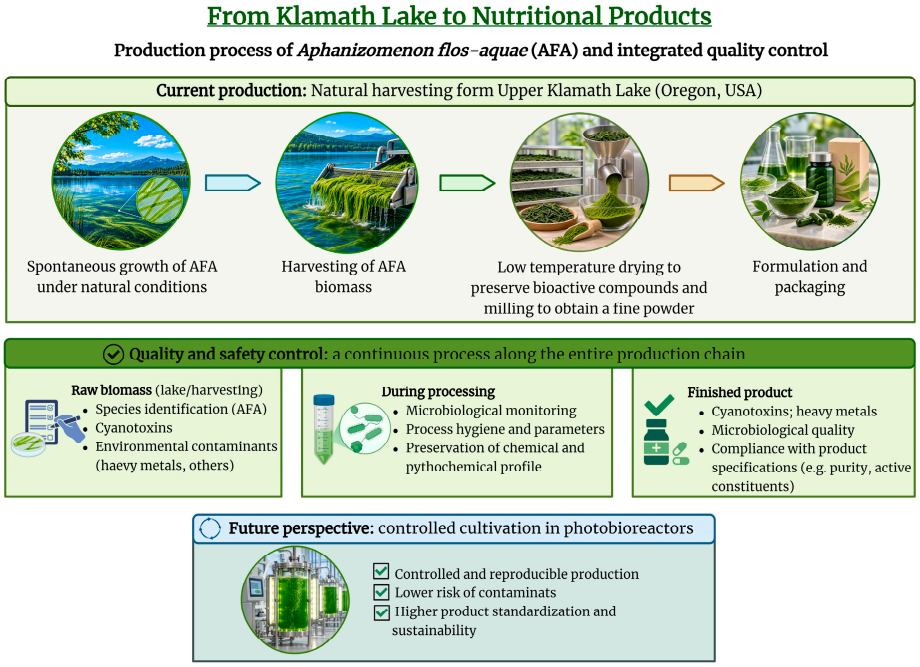



**Figure 2.** Schematic overview of the production workflow, quality and safety control, and future perspectives for *Aphanizomenon flos-aquae* (AFA)-based nutritional products. Created in BioRender. Cocetta, V. (2026) https://BioRender.com/fabb4f5. Selected graphical elements were generated using ChatGPT (GPT-6, OpenAI, 2026).

## 2. Beneficial Properties of Klamath Algae

### *2.1. Bioactive Compounds and Mechanisms Implicated in Biological Action*

AFA is an edible freshwater cyanobacterium that displays a rather distinctive nutritional profile, being rich in vitamin B12, minerals, such as molybdenum and tungsten (cofactors for several antioxidant enzymes), and a variety of pigments, including carotene, beta-carotene, chlorophylls, and phycocyanins [8,12,13]. Several preclinical studies have shown that AFA-derived extracts, particularly the patented formulations Klamin®, AphaMax® and their combination KlamExtra®, can exert beneficial effects across different pathological conditions, such as neurodegeneration, intestinal inflammation, as well as obesity-related metabolic dysfunction. The mechanisms involved appear to be mainly antioxidant, anti-inflammatory, neuromodulatory, and, more recently, epigenetic [8].

Specifically,, oxidative stress is widely recognized as one of the earliest and most important pathogenic events in chronic age-related diseases. Excessive production of reactive oxygen species (ROS) in fact induces lipid peroxidation, DNA oxidation, protein aggregation, mitochondrial dysfunction, and ultimately neuronal apoptosis; therefore, scavenger activity is viewed as an effective preventive strategy. Beyond direct antioxidant activity, the stimulation of endogenous defense systems, including the Nrf2/ARE pathway, which regulates the transcription of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1), emerges as a crucial aspect in preventing the progression of chronic diseases [12]. Interestingly, several bioactive compounds have been identified in AFA extracts and associated with biological activity. Among them are mycosporine-like amino acids (MAAs), polyphenols, essential fatty acids, and several bioactive peptides, which act synergistically on multiple cellular targets [13–17].

Mycosporines and MAAs are ultraviolet (UV)-absorbing compounds, naturally present in several organisms, including cyanobacteria, microalgae, fungi, macroalgae, corals,

lichens, and both freshwater and marine animals; they have evolved as photoprotective agents against chronic UV radiation exposure. Numerous studies have demonstrated that in general, MAAs exhibit significant antioxidant activity and effectively protect cells from oxidative stress through UV-filtering, as well as direct and indirect reactive oxygen species (ROS)-quenching mechanisms. Their antioxidant potential has been investigated using a variety of assays, including oxygen radical absorbance capacity (ORAC), inhibition of lipid peroxidation, DPPH and ABTS radical scavenging, singlet oxygen quenching, superoxide anion scavenging, and hydrogen peroxide neutralization. Despite the growing interest in the cosmeceutical and pharmaceutical fields, especially for skin protection and to promote wound healing, the precise molecular mechanisms underlying the antioxidant activity of MAAs remain incompletely understood and warrant further investigation (Table 2) [18–20]. Regarding the MAAs identified in Klamath extracts, they are mainly porphyra-334 and shinorine [21]. Gacesa and colleagues, examining the antioxidant properties of porphyra-334 and shinorine, demonstrated in 2018 that the two MAAs are activators of the Nrf2-Keap1 pathway, either in tube or in vitro assays (Table 2) [22].

Phycocyanins, especially phycobiliproteins, are main constituents of blue–green algae; they are characterized by an intense blue color due to their non-protein unit known as phycocyanobilin. This natural pigment reduces the production of ROS and reactive nitrogen species (RNS) and prevents oxidative damage, exerting beneficial effects in physiological and pathological conditions—in particular as concerns wound healing, accelerating wound closure by direct action on fibroblast migration and indirect effect on keratinocyte migration (Table 2) [23]. Currently, the clinical use of phycobiliproteins is limited by their low bioavailability, although there are some studies in which new formulations have been developed, including liposomes [24], nanoformulations [25], and more recently, phycocyanin-encapsulating hyalurosomes [26].

Interestingly, AFA contains a fair amount of phenylethylamine (PEA, approximatively 1–3 mg/g in the raw alga), a dopaminergic neuromodulator able to promote catecholaminergic neurotransmission by stimulating dopamine release and acting as a fast inhibitor of catecholamine reuptake. It also appears to modulate serotonergic transmission, although this effect is somewhat less characterized [14]. PEA is, however, rapidly degraded by peripheral monoamine oxidase B (MAO-B), which substantially limits its efficacy unless the compound is protected by MAO-B inhibiting agents. This is particularly relevant, considering that MAO-B is also the main enzyme responsible for dopamine catabolism within the central nervous system; nevertheless, Scoglio et al. demonstrated that the AFA extract Klamin®, containing PEA, phycocyanins and MAAs, acts as a selective MAO-B inhibitor. Specifically, phycocyanins displayed a mixed-type inhibition mechanism, with a selectivity more than 3.75-fold higher for MAO-B than for MAO-A, while MAAs (low molecular weight, blood–brain barrier (BBB)-permeable compounds) showed, for the first time, a competitive-type inhibition of MAO-B (Table 2) [14]. These findings are of relevant interest, suggesting a synergy among different bioactive molecules that can improve the translational value of AFA extracts.

### *2.2. Neuroprotective Effects*

Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and other age-related neurological disorders, represent one of the major global health challenges due to the progressive loss of neuronal function and the absence of effective disease-modifying therapies. Although these disorders differ in their clinical manifestations, they share several pathogenic mechanisms, including oxidative stress, mitochondrial dysfunction, chronic neuroinflammation, protein misfolding, impaired autophagy, and dysregulated neuronal energy metabolism. Therefore, increasing attention has been given to naturally derived compounds able to simultaneously target multiple

molecular pathways involved in neurodegeneration. ROS accumulation and an impairment of antioxidant defenses are the earliest events in neurodegenerative processes; besides them, accumulating evidence indicates that chronic neuroinflammation plays a central role in the progression of neurodegenerative disorders: for instance, persistent activation of microglia and astrocytes promotes the release of pro-inflammatory cytokines, including tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin (IL)-1$\beta$, IL-6, nitric oxide, and ROS, establishing a self-perpetuating cycle of neuronal injury [27,28].

In this context, the use of Klamin®, containing both PEA, capable of positively modulating monoaminergic neurotransmission, and phycocyanin and MAAs, inhibitors of MAOs, is supposed to enhance dopaminergic synaptic activity by a synergistic mechanism linked to a direct effect on dopaminergic synapses and limiting the formation of neurotoxic byproducts generated by MAO-B-mediated oxidation of dopamine and PEA [14].

In an in vitro study on LAN5 neuronal cells, Nuzzo et al. showed that Klamin® was able to counteract neuronal toxicity induced by the oxidant agent tert-butylhydroperoxide (TBH) through a direct ROS-scavenging activity that also prevented TBH-induced mitochondrial dysfunction [29]. Beyond this, Klamin® exerted a protective effect against A$\beta$-peptide-induced neurotoxicity, interfering with amyloid aggregation through several complementary mechanisms: inhibition of fibril extension, promotion of shorter and less toxic aggregates, and destabilization of preformed fibrils. Notably, this anti-amyloidogenic activity was paralleled by a clear anti-inflammatory effect, with reduced production of pro-inflammatory cytokines such as IL-6 and IL-1$\beta$ following A$\beta$ stimulation (Table 2) [29].

The antioxidant and adaptogenic properties of Klamin® were also confirmed in vivo, in a study on premature senescent (PS) mice [15]. Using the Morris water maze test, the authors found that PS mice displayed impaired spatial learning compared to non-prematurely senescent (NPS) controls. This effect was prevented by high-dose Klamin® supplementation (100 mg/kg/day), which, interestingly, also enhanced performance in NPS animals. At the biochemical level, high-dose supplementation restored cerebral thiol levels and normalized malondialdehyde (MDA), suggesting a dose-dependent antioxidant effect; the lower dose (20 mg/kg/day), by contrast, only reduced MDA levels. Furthermore, only the high dose induced an upregulation of the cerebral erythropoietin/erythropoietin receptor (EPO/EPOR) system in both groups, without any corresponding change in plasma EPO, pointing to a centrally mediated mechanism, rather than a systemic one. This finding is particularly noteworthy, as EPO is increasingly recognized as a multifunctional neuroprotective cytokine that promotes neuronal survival, inhibits apoptosis, enhances neurogenesis, preserves mitochondrial function, and attenuates inflammatory responses (Table 2). Taken together, these findings suggest that the cognitive and antioxidant benefits of Klamin® may not be limited to its already known PEA-, phycocyanin- and MAA-related effects, but could also involve activation of this additional neuroprotective pathway.

Galizzi et al. investigated the neuroprotective potential of KlamExtra® in a high-fat diet (HFD) mouse model of obesity-induced neurodegeneration [11]. Supplementation significantly reduced both body weight and plasma triglycerides, and partially improved glucose metabolism and insulin resistance. At the central level, KlamExtra® preserved brain insulin signaling and attenuated HFD-induced neuroinflammation, evidenced by reduced GFAP and TREM2 expression and prevention of astrogliosis.

These findings are consistent with the broader idea that AFA may act upstream of neuronal degeneration, primarily by modulating the inflammatory microenvironment rather than acting exclusively as a direct antioxidant shield for neurons. In line with this, histopathological and TUNEL analyses further revealed reduced neuronal degeneration

and preserved expression of synaptic proteins (PSD95, synaptophysin). Interestingly, the expression of BACE1 and PSN1, both involved in amyloid precursor protein (APP) processing and found upregulated in HFD-fed mice, was also reduced following AFA supplementation, although this effect did not reach statistical significance (Table 2) [9].

Taken together, the evidence reviewed here suggests that AFA extracts exert multitarget neuroprotective effects, acting through direct antioxidants [8,13] and several complementary mechanisms: selective MAO-B inhibition and dopaminergic modulation [16]; anti-amyloidogenic activity [8,13]; stimulation of the cerebral EPO/EPOR system, with a consequent improvement in spatial learning and memory in a model of premature senescence [15]; and modulation of central insulin signaling and neuroinflammation in the context of diet-induced obesity [9]. While these results are certainly promising, further studies will be needed to fully elucidate the underlying molecular mechanisms and confirm the translational relevance of these findings.

### *2.3. Inflammatory Bowel Diseases*

AFA and its derived extracts have shown promising activity on intestinal function and inflammation, acting both on gut motility and on the molecular mechanisms underlying inflammatory bowel disease (IBD) [30]. IBD comprises a group of chronic inflammatory conditions of the gastrointestinal tract, characterized by dysregulated immune responses, epithelial barrier disruption, and altered gut motility, including Crohn's disease and ulcerative colitis. The gastrointestinal tract is not only a target of chronic inflammation but also a key regulator of systemic immune homeostasis, with important bidirectional connections to the nervous system (gut–brain axis) and metabolic regulation (gut–liver axis) [31].

Notably, the neuromodulatory effects of AFA are not limited to the central nervous system but also appear to involve the enteric nervous system (ENS). Amato et al. investigated the effects of Klamin® (particularly concentrated in β-phenylethylamine (β-PEA, 8–20 mg/g)) on human distal colon contractility, using the organ bath technique [32]. Klamin® reduced the amplitude of spontaneous colonic contractions in a concentration-dependent manner, an effect mediated by β-PEA through activation of the trace-amine-associated receptor 1 (TAAR1), as confirmed by significant antagonism with the selective TAAR1 antagonist EPPTB. Consistently, AphaMax®, which contains considerably less β-PEA, failed to modify colonic contractility, further supporting this mechanistic interpretation. The relaxant effect was completely abolished by tetrodotoxin (TTX), pointing to a neurally mediated mechanism, whereas it remained unaffected by L-NAME, thus ruling out any involvement of nitric oxide. Methysergide, a serotonin receptor antagonist, significantly blocked the response instead, implicating serotonergic signaling in this pathway. Interestingly, RT-PCR and immunohistochemistry revealed, for the first time, TAAR1 expression in the human colon, co-localized with 5-HT-positive myenteric neurons. Taken together, these results indicate that Klamin® exerts a spasmolytic effect on human colonic contractility through a β-PEA/TAAR1-mediated release of serotonin from myenteric neurons. This is a particularly relevant finding, given the well-established role of ENS dysfunction in IBD pathogenesis (Table 2) [32].

Beyond its effects on motility, the anti-inflammatory and antioxidant properties of AFA extracts have also been directly investigated in experimental colitis. Zizzo et al. evaluated AphaMax® in a rat model of DNBS-induced colitis [10]. Preventive oral treatment with 20, 50, or 100 mg/kg/day for 14 days attenuated colitis severity, improving body weight loss, diarrhea, and the colonic weight/length ratio, while also preserving histological architecture. At the molecular level, AphaMax® reduced myeloperoxidase (MPO) activity and inhibited NF-κB activation, with a parallel decrease in COX-2, iNOS, IL-6 and IL-1β expression, as well as reduced ROS and nitrite levels. These effects can be plausibly

attributed to the remarkably strong antioxidant activity of AFA-phycocyanins, reported to be 75- to 200-fold greater than that of other common phycocyanins. Interestingly, AphaMax® had no effect on sham animals, suggesting a selective action restricted to inflammatory conditions, an effect that may also involve the stabilization of tight junction proteins such as occludin, claudins, and ZO proteins (Table 2). This intestinal anti-inflammatory profile appears to mirror the anti-neuroinflammatory effects reported by Galizzi et al., raising the possibility that AFA may exert a broader, systemic anti-inflammatory action across multiple organ systems [9].

More recently, AFA's anti-inflammatory activity has also been linked to a novel epigenetic mechanism. Naselli et al. provided the first evidence that AFA can modulate DNA methylation in inflammation-related pathways, using colon tissue from DNBS-treated rats as well as Caco-2 cells [33]. In vivo, DNBS-induced colitis caused global DNA demethylation, whereas AphaMax® produced a hypermethylation pattern, most pronounced at the 100 mg/kg dose. In Caco-2 cells, when AFA extract, obtained by solubilizing the powder of microalgae in phosphate-buffered solution (1 mg/mL), was administered after IL-1β, it consistently restored global hypermethylation, particularly at lower concentrations (10 μg/mL), suggesting a possible role in stabilizing DNA and counteracting inflammation-induced instability (Table 2) [33].

Gene-specific analysis further revealed site-specific effects on the IL-6 and IL-8 promoters, while AFA reduced DNMT3A and DNMT1 expression but increased DNMT3B, indicating a preferential effect on de novo methylation rather than maintenance methylation. Taken together, these findings represent, to our knowledge, the first demonstration that a microalga can modulate DNA methylation in a human/animal system, raising the intriguing possibility of sustained anti-inflammatory effects extending well beyond the immediate pharmacodynamic window—with potentially broader implications for inflammatory pathways relevant not only to IBD, but also to neurodegeneration and metabolic dysfunction.

Overall, the studies reviewed in this section indicate that AFA extracts exert multilevel beneficial effects on the gastrointestinal tract, such as modulation of intestinal motility via a β-PEA/TAAR1/serotonin pathway [32], attenuation of colonic inflammation and oxidative stress through the NF-κB/COX-2/iNOS axis [10], and, more recently, regulation of inflammation-related genes at the epigenetic level [33]. Although further studies are necessary, these findings collectively support the potential of AFA-derived extracts such as Klamin® and AphaMax® as complementary nutraceutical agents in IBD management.

### *2.4. Obesity and Metabolic Dysregulation*

Obesity and its associated metabolic dysfunctions represent a major global health burden, characterized by adipose tissue dysregulation, chronic low-grade inflammation, insulin resistance, dyslipidemia, and systemic metabolic imbalance. Obesity-associated chronic inflammation is now increasingly recognized as a key upstream driver of both neurodegeneration and intestinal dysfunction, effectively placing metabolic health at the pathophysiological intersection of the biological systems discussed throughout this review [34,35]. Beyond its neuroprotective and intestinal anti-inflammatory properties, AFA has also been investigated for its potential to counteract obesity-related metabolic dysfunctions.

Terzo et al. evaluated the effects of KlamExtra® in a mouse model of diet-induced obesity (DIO) [36]. In this study, mice were fed a high-fat diet (HFD) for 10 weeks, followed by 8 weeks of AFA supplementation, either continuing the HFD or switching to a standard normocaloric diet to assess both the preventive potential of AFA and its ability to accelerate metabolic recovery. Under continued HFD conditions, AFA supplementation reduced body weight gain without affecting caloric intake (pointing to a peripheral,

rather than central mechanism) and also reduced visceral adipose tissue hypertrophy, plasma triglycerides, and hepatic fat accumulation.

At the molecular level, AFA prevented the HFD-induced upregulation of key lipogenic factors (such as PPAR-γ, SREBP-1c, FAS, and FAT-P) in adipose tissue, and downregulated SREBP-1c in the liver as well, evidencing a potential epigenetic modulation of lipid metabolism genes by the microalga-derived extract.

AFA supplementation also improved glucose homeostasis, reducing fasting glycemia and insulinaemia, while enhancing glucose tolerance and insulin sensitivity. These metabolic improvements were paralleled by a reduction in hepatic and adipose tissue inflammation with decreased IL-6, TNF-α, and NF-κB, and increased IL-10, potentially involving additional pathways such as AMPK activation, PPARα modulation, and NLRP3 inflammasome inhibition. A concurrent reduction in oxidative stress (lower ROS levels and restored SOD activity) was also observed, likely attributable to the antioxidant carotenoids, phycoerythrins, and phycocyanins contained in AFA. Interestingly, upon dietary normalization, AFA no longer provided additional benefit on body weight or steatosis but significantly accelerated the recovery of glucose metabolism and the resolution of inflammation and oxidative stress (Table 2).

Taken together, these metabolic improvements, along with the concurrent reduction in systemic inflammatory markers, reinforce the close interplay between metabolic dysregulation and chronic inflammation in obesity [36]. They also appear consistent with the neuroprotective effects reported by Galizzi et al. in an HFD model of neurodegeneration [9], suggesting that the metabolic and neuroprotective actions of AFA may converge on shared inflammatory and oxidative stress pathways.

Similarly, the improvements in gut barrier integrity and intestinal inflammation described may represent an additional factor contributing to these metabolic benefits, particularly considering the close link of gut permeability and microbiota dysbiosis to obesity and insulin resistance.

Overall, the evidence discussed here supports the potential of AFA extracts as multitarget nutraceutical agents in the management of obesity-related dysmetabolism, with mechanisms of action that appear to converge with their neuroprotective and intestinal protective effects [36]. Further studies will be needed to confirm the translational relevance of these findings in overweight and obese individuals.

### *2.5. In Silico Approach for Identification of New Bioactive Compounds*

Recent advances in bioinformatics have paved the way for the discovery of additional bioactive compounds from AFA. Pedroni et al. developed an in silico pipeline aimed at identifying biologically active peptides from algae and cyanobacteria, successfully discovering several sequences with inhibitory activity against pancreatic α-amylase [16]. Although this study focused primarily on a metabolic target, the methodology itself represents a valuable platform that could be extended to the identification of peptides targeting neurodegenerative pathways like β-secretase (BACE1), acetylcholinesterase (AChE), or inhibitors of α-synuclein aggregation. Given the remarkable proteome diversity of AFA, systematic peptide mining may reveal novel compounds capable of modulating oxidative stress, protein aggregation, neuroinflammation, synaptic plasticity, or mitochondrial function.

## 3. Clinical Evidence and Therapeutic Potential

Current clinical evidence on the beneficial effects of AFA extracts remains limited, as only a small number of studies have been conducted. Most trials enroll few participants, resulting in weak statistical power. In addition, the available studies are highly heterogeneous because they employ different, non-standardized extracts. These premises

highlight the need for more comprehensive and rigorously designed investigations. The most relevant clinical evidence available to date is summarized below.

### *3.1. Source of Vitamin B12*

The American Dietary Guidelines recommend vitamin B12 supplementation to all people over the age of 50, since one-third of the older population loses the ability to assimilate it from food. Indeed, vitamin B12 deficiency is associated with hyperhomocysteinemia, a significant risk factor for cardiovascular diseases [37–39]. Interestingly, elevated homocysteine levels and vitamin B12 deficiency are more frequently observed in adult women and may critically contribute to cardiovascular risk. Although there are other conditions that can raise homocysteine concentration, such as a deficiency of vitamin B6 and/or B9, these are very rare in a well-balanced diet, even in a vegan one, and usually occur only in a situation of general malnutrition [40]. In contrast, vitamin B12 is the only vitamin that humans cannot obtain in adequate amounts from plant foods, which may pose a challenge for vegans. To prevent vitamin B12 deficiency, vegans generally rely on vitamin B12 supplements produced by bacterial synthesis, which are ethically compatible with veganism, given their non-animal origin. On the other hand, many plant-derived foods traditionally considered a source of vitamin B12, such as certain fermented products, are unreliable, as they mainly contain biologically inactive, non-cobalaminic analogs that cannot be absorbed [41]. In this regard, AFA has been proposed as a source of "true" vitamin B12; indeed, Miyamoto and collaborators confirmed this, reporting that Klamath algae contains approximately 6 μg/g of cobalamin, an amount markedly higher compared with other marine sources, including *Spirulina* and *Chlorella* (1–2 μg/g) [42]. Baroni and colleagues first published a clinical trial in which they observed in subjects supplemented with a Klamath algae product (called AFA-B12) a positive response, with an increase in blood levels of vitamin B12 and reduction in homocysteine [11]. Interestingly, the integrative use of vitamin B12, particularly in combination with folate, was reported by several studies to protect different tissues against oxidative conditions, particularly with protection against DNA damage [43].

### *3.2. Post-Menopausal Symptoms*

A clinical trial investigating supplementation with a Klamath algae extract (Klamin®) reported significant improvements in several biochemical markers of oxidative stress, as well as in subjective well-being among post-menopausal women. Postmenopausal symptoms are well recognized for their detrimental impact on quality of life. Therefore, alongside hormone replacement therapy, considerable efforts have been directed toward the identification of effective non-conventional strategies to alleviate the most common vasomotor symptoms, as well as the psychological and physical manifestations associated with menopause [44]. Although hormonal markers such as cortisol, LH, and FSH did not change significantly, a 2-month supplementation period reduced plasma MDA levels and improved psychological symptoms—specifically mood, anxiety, and depression—commonly associated with menopause. Such beneficial effects could be due to the peculiar phytochemical composition of the commercial product, containing phycocyanins and neuromodulators, including phenylethylamine and MAO-B inhibitors (Table 2) [45,46].

### *3.3. Attention Deficit/Hyperactivity Disorder (ADHD)*

ADHD is a neurodevelopmental disorder characterized by pervasive and persistent patterns of inattention, hyperactivity, and/or impulsivity beginning in childhood. It is associated with substantial impairment in social, academic, and occupational functioning. Comorbidity is present in 70–80% of cases, commonly including oppositional defiant disorder, anxiety and depressive disorders, obsessive–compulsive disorder, and tic

disorders, which complicate both diagnosis and treatment. Psychostimulants, including phenylethylamine, appear particularly effective in enhancing certain neuropsychological functions, such as the inhibition of responses, working memory and processes of discrimination of stimuli, resulting in an improvement in attention, vigilance, verbal and visual learning, and short-term memory.

Klamath algae extract has been evaluated in ADHD due to its high PEA content and the presence of MAO-B inhibitors, which facilitate its central availability. In a pilot clinical trial, 13 children diagnosed with ADHD received Klamin® supplementation for six months, with doses adjusted according to body weight (125–600 mg/day during the first month and 250–1200 mg/day thereafter). ADHD symptoms were assessed using validated international scales, including the Child Global Assessment Scale (C GAS), SNAP IV, Conners®Parent Rating Scale R, the oppositional defiant disorder subscale (SNAP IV), short-term verbal memory (WISC III digit span), and arithmetic reasoning and mental calculation (WISC III). Despite the limited sample size, the study reported positive preliminary results that support further investigation in larger cohorts (Table 2) [47].

**Table 2.** Antioxidant effects and their mechanism/s reported in the literature for AFA algae-derived compounds or extracts.

| Compounds/Formulation | Experimental Model | Biological Effects | Reference |
|---|---|---|---|
| MAAs | In vitro on keratinocytes | UV-absorbing effect | [18–20] |
| MAAs | In tube | Aspecific antioxidant effect | [18–20] |
| Klamath-derived MAAs | In tube and cell-based models | Antioxidant effect through Nrf2-keap1 pathway | [22] |
| Phycocyanins | In vitro on fibroblasts | Reduction in ROS and RNS | [23] |
| Phycocyanin and MAAs isolated from Klamin® | In vitro on MAO-A and MAO-B assay kits | Relativelly selective MAO-B inhibition | [14] |
| Klamin® | In vitro on LAN5 neuronal cells | ROS scavenger and mito-preventive effects | [29] |
| Klamin® (100 mg/Kg) | In vivo on premature senescent mice | Improvement of spatial learning and reduction in MDA | [15] |
| KlamExtra® | In vivo on High Fat Diet-mice | Reduction in neuronflammation (TREM2 and GFAP) | [9] |
| Klamin® | Ex vivo on human distal colon | Spasmolytic effect (by TAAR1 activation) | [32] |
| AphaMax (20–50–100 mg/Kg for 14 days) | In vivo model on rat with DNBS-induced colitis | Reduction in MPO activity and NF-kB inhibition, improvement of occludin and claudin expression | [10] |
| AFA extract and AphaMax® | In vitro on Caco-2 cells ILβ-stimulated and ex vivo on colon from DNBS-treated mice | DNA methylation modulation | [33] |
| KlamExtra® | In vivo model on mice with diet-induced obesity | Prevention of upregulation of lipogenic factors (i.e., PPAR-γ, SREBP-1c, FAS, FAT-P). Improvement of glucose homeostasis, reducing of fasting glycemia, insulinaemia, enhancing glucose tolerance and insulin sensitivity | [36] |
| Klamin® | Clinical trial on post-menopausal women | Reduction in MDA | [45,46] |
| Klamin® | ADHD patients | Improvement of ADHD symptoms | [47] |

## 4. Safety and Regulatory Aspects

AFA has long been marketed as an ingredient in food supplements in the United States, particularly following the development of commercial operations established around Klamath Lake, in Oregon [2]. Under the US regulatory framework, like many other supplements, those containing AFA do not require product-specific pre-market approval by the Food and Drug Administration (FDA). However, manufacturers and distributors are responsible for ensuring their safety, identity and purity. Particular regulatory attention has been paid to cyanotoxin contamination because wild-harvested AFA may be collected together with toxin-producing cyanobacteria, especially species belonging to the genus *Microcystis* [48–50]. Due to this concern, the Oregon Health Division established a provisional limit for total microcystin, expressed as MC-LR equivalents, of 1 μg/g dry weight for supplements containing blue–green algae such as AFA [48,49].

A specific limit for toxins has also been established in Canada and producers and suppliers should demonstrate that total microcystins in the raw material do not exceed 1 μg/g [51].

AFA is also widely used as an ingredient of food supplements in the European Union (EU). Products containing AFA must comply with the general requirements of EU food law and Directive 2002/46/EC on food supplements. EU legislation does not establish a harmonized maximum level for microcystins or other cyanotoxins in AFA-based food supplements. This regulatory gap is particularly relevant because commercially available AFA products have repeatedly been found to contain microcystins, occasionally at concentrations exceeding the provisional 1 μg/g benchmark [50,52]. Some Member States have therefore introduced national specifications. In France, AFA preparations in food supplements are marketable only when analytical reports demonstrate a microcystin concentration below 1 μg/g and the absence of other relevant toxins [53]. Italy has adopted a comparable, and apparently broader, provision; AFA is included among the botanicals and related substances permitted in food supplements under Annex 1 to the Italian Ministerial Decree of 10 August 2018, as subsequently updated. The applicable specification requires "microcystins and other cyanotoxins" to be below 1 μg/g [54].

Of note, the Italian Ministry of Health also associates AFA with the physiological health claim "normal mood". This represents formal recognition of the health-related positioning of AFA in the Italian food supplement framework.

Despite a long history of real-world exposure, no well-documented cases of human poisoning have been specifically attributed to the consumption of uncontaminated AFA at the recommended doses, nor has its widespread use generated a clear clinical safety signal [55]. Consistently, consumer-oriented safety assessments consider contaminant-free blue–green algae products as possibly safe for short-term use; reported adverse effects are generally mild and nonspecific, including nausea, gastrointestinal discomfort, headache, fatigue and dizziness [56]. However, the available evidence is derived mainly from market experience and relatively small or short-term clinical studies rather than from systematic post-marketing surveillance or adequately designed long-term safety trials. Therefore, the absence of reported serious adverse events is reassuring and supports the apparent tolerability of AFA under customary conditions of use, but it should not be interpreted as definitive evidence of safety, particularly for prolonged exposure or use in vulnerable populations.

Despite this apparently reassuring real-world background, several safety concerns remain and deserve careful consideration regarding AFA. These concerns arise from the biological characteristics of cyanobacteria, their uncontrolled growth in a natural environment, the possible occurrence of toxin-producing strains and species, and the consequent variability and potential contamination of the harvested biomass.

The first and best-documented safety concern is the occurrence of microcystins in AFA-based food supplements. Since AFA is harvested from a natural ecosystem, the collected biomass may include toxin-producing cyanobacteria, particularly *Microcystis* spp., which share the same habitat.

Concern about microcystins is toxicologically justified because these cyanobacterial metabolites are potent hepatotoxins that inhibit protein phosphatases PP1 and PP2A and promote oxidative stress, cytoskeletal disruption, and hepatocyte injury [57,58]. Their documented occurrence in some AFA supplements therefore represents a real potential hazard [49].

An extensive review commissioned by EFSA [49] identified microcystins in commercial AFA products marketed in several European and non-European countries, although concentrations varied considerably among products and batches.

Among the studies considered analytically reliable, mean concentrations of 2.15–10.89 μg/g were reported in products from the United States [48], while maximum concentrations of 5.20, 4.00 and 2.53 μg/g were found in products analyzed in Italy, Switzerland and Ireland, respectively [50,59,60]. Notably, an Italian survey detected microcystins in 40% of tested AFA products at concentrations exceeding 1 μg/g [50].

Microcystins are not the only cyanotoxins of potential relevance, as anatoxin-a has also been detected in AFA-containing products. Moreover, safety concerns are not limited to cyanotoxins but include possible contamination by heavy metals and potentially harmful microorganisms [61].

The growing attention devoted to cyanotoxin contamination and the continuing development of increasingly sensitive targeted and suspect-screening analytical methods are providing a progressively more accurate characterization of AFA products, thereby supporting effective quality control and their safer use [62,63].

## 5. Conclusions

Klamath microalgae represent a promising nutraceutical resource owing to their rich content of proteins, mycosporines and mycosporine-like amino acids, vitamins, minerals, and pigments. Their reported antioxidant, anti-inflammatory, immunomodulatory, and neuroprotective properties highlight their potential role in promoting health and preventing chronic diseases. However, further clinical studies are needed to confirm their efficacy, establish optimal dosages, and evaluate long-term safety.

Moreover, significant heterogeneity exists among commercial AFA preparations due to differences in harvesting conditions, extraction procedures, and concentrations of biologically active constituents, including phycocyanins, carotenoids, PEA, and MAAs. This variability complicates the comparison of findings across studies and underscores the need for standardized products and quality-control procedures. Additional limitations concern the scarce information regarding the bioavailability, metabolism, and tissue distribution of AFA-derived compounds in humans. Although several mechanisms, the including modulation of oxidative stress, inflammatory signaling, and stem cell mobilization, have been proposed, direct mechanistic validation remains incomplete [64–66].

More broadly, microalgae are emerging as sustainable and versatile sources of bioactive compounds for functional foods and dietary supplements. Their nutritional value, combined with environmentally friendly production processes, makes them attractive candidates for next-generation nutraceutical applications. Nevertheless, future research should prioritize the standardization of cultivation, quality control, and regulatory harmonization, which remain essential to fully realize their potential and ensure safety.

**Author Contributions:** Conceptualization, L.T. and M.M.; methodology, V.C. and P.N.; original draft preparation, V.C., P.N. and M.B.; writing—review and editing, M.M. and L.T.; supervision, L.T. and M.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** No new data were created or analyzed in this study. Data sharing is not applicable to this article.

**Acknowledgments:** V.C. was supported by Fondazione Umberto Veronesi. During manuscript preparation, the authors used ChatGPT (GPT-6, OpenAI, 2026) to generate selected graphical elements of Figure 2, subsequently assembled using BioRender. The authors reviewed the final figure and take full responsibility for its content.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Scoglio, S.; Scoglio, G.D. Klamath Lake *Aphanizomenon Flos-Aquae*: Wild-Harvesting, Extracts and Benefits. In *Insights Into Algae-Fundamentals, Culture Techniques and Biotechnological Uses of Microalgae and Cyanobacteria*; IntechOpen: London, UK, 2024. https://doi.org/10.5772/intechopen.1004405.
2. Scoglio, G.D.; Jackson, H.O.; Purton, S. The commercial potential of *Aphanizomenon flos-aquae*, a nitrogen-fixing edible cyanobacterium. *J. Appl. Phycol.* **2024**, *36*, 1593–1617. https://doi.org/10.1007/s10811-024-03214-0.
3. Tsujimura, S.; Ishikawa, K.; Tsukada, H. Effect of temperature on growth of the cyanobacterium *Aphanizomenon flos-aquae* in Lake Biwa and Lake Yogo. *Phycol. Res.* **2001**, *49*, 275–280. https://doi.org/10.1046/j.1440-1835.2001.00255.x.
4. Yamamoto, Y.; Nakahara, H. Life Cycle of Cyanobacterium *Aphanizomenon flos-aquae*. *Taiwania* **2009**, *54*, 113. https://doi.org/10.6165/tai.2009.54(2).113.
5. Yamamoto, Y.; Nakahara, H. The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: The importance of pH, water temperature, and day length. *Limnology* **2025**, *6*, 1–6. https://doi.org/10.1007/s10201-004-0138-1.
6. Laamanen, M.J.; Forsström, L.; Sivonen, K. Diversity of *Aphanizomenonflos-aquae* (Cyanobacterium) Populations along a Baltic Sea Salinity Gradient. *Appl. Environ. Microbiol.* **2002**, *68*, 5296–5303. https://doi.org/10.1128/AEM.68.11.5296-5303.2002.
7. Schaeffer, D.J.; Malpas, P.B.; Barton, L.L. Risk Assessment of Microcystin in Dietary *Aphanizomenon flos-aquae*. *Ecotoxicol. Environ. Saf.* **1999**, *44*, 73–80. https://doi.org/10.1006/eesa.1999.1816.
8. Nuzzo, D.; Contardi, M.; Kossyvaki, D.; Picone, P.; Cristaldi, L.; Galizzi, G.; Bosco, G.; Scoglio, S.; Athanassiou, A.; Di Carlo, M. Heat-Resistant *Aphanizomenon flos-aquae* (AFA) Extract (Klamin®) as a Functional Ingredient in Food Strategy for Prevention of Oxidative Stress. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 9481390. https://doi.org/10.1155/2019/9481390.
9. Galizzi, G.; Deidda, I.; Amato, A.; Calvi, P.; Terzo, S.; Caruana, L.; Scoglio, S.; Mulè, F.; Di Carlo, M. *Aphanizomenon flos-aquae* (AFA) Extract Prevents Neurodegeneration in the HFD Mouse Model by Modulating Astrocytes and Microglia Activation. *Int. J. Mol. Sci.* **2023**, *24*, 4731. https://doi.org/10.3390/ijms24054731.
10. Zizzo, M.G.; Caldara, G.; Bellanca, A.; Nuzzo, D.; Di Carlo, M.; Scoglio, S.; Serio, R. AphaMax®, an Aphanizomenon Flos-Aquae Aqueous Extract, Exerts Intestinal Protective Effects in Experimental Colitis in Rats. *Nutrients* **2020**, *12*, 3635. https://doi.org/10.3390/nu12123635.
11. Baroni, L.; Scoglio, S.; Benedetti, S.; Bonetto, C.; Pagliarani, S.; Benedetti, Y.; Rocchi, M.; Canestrari, F. Effect of a Klamath algae product ("AFA-B12") on blood levels of vitamin B12 and homocysteine in vegan subjects: A pilot study. *Int. J. Vitam. Nutr. Res.* **2009**, *79*, 117–123. https://doi.org/10.1024/0300-9831.79.2.117.
12. Jomova, K.; Raptova, R.; Alomar, S.Y.; Alwasel, S.H.; Nepovimova, E.; Kuca, K.; Valko, M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Arch. Toxicol.* **2023**, *97*, 2499–2574. https://doi.org/10.1007/s00204-023-03562-9.
13. Amato, A.; Terzo, S.; Mulè, F. Natural Compounds as Beneficial Antioxidant Agents in Neurodegenerative Disorders: A Focus on Alzheimer® Disease. *Antioxidants* **2019**, *8*, 608. https://doi.org/10.3390/antiox8120608.

14. Scoglio, S.; Benedetti, Y.; Benvenuti, F.; Battistelli, S.; Canestrari, F.; Benedetti, S. Selective monoamine oxidase B inhibition by an *Aphanizomenon flos-aquae* extract and by its constitutive active principles phycocyanin and mycosporine-like amino acids. *Phytomedicine* **2014**, *21*, 992–997. https://doi.org/10.1016/j.phymed.2014.03.006.
15. Sedriep, S.; Xia, X.; Marotta, F.; Zhou, L.; Yadav, H.; Yang, H.; Soresi, V.; Catanzaro, R.; Zhong, K.; Polimeni, A.; et al. Beneficial nutraceutical modulation of cerebral erythropoietin expression and oxidative stress: An experimental study. *J. Biol. Regul. Homeost. Agents.* **2011**, *25*, 187–194. PMID:21880207.
16. Pedroni, L.; Perugino, F.; Galaverna, G.; Dall@Asta, C.; Dellafiora, L. An In Silico Framework to Mine Bioactive Peptides from Annotated Proteomes: A Case Study on Pancreatic Alpha Amylase Inhibitory Peptides from Algae and Cyanobacteria. *Nutrients* **2022**, *14*, 4680. https://doi.org/10.3390/nu14214680.
17. Shick, J.M.; Dunlap, W.C. Mycosporine-Like Amino Acids and Related Gadusols: Biosynthesis, Accumulation, and UV-Protective Functions in Aquatic Organisms. *Annu. Rev. Physiol.* **2002**, *64*, 223–262.
18. Lawrence, K.P.; Gacesa, R.; Long, P.F.; Young, A.R. Molecular photoprotection of human keratinocytes in vitro by the naturally occurring mycosporine-like amino acid palythine. *Br. J. Dermatol.* **2018**, *178*, 1353–1363.
19. Geraldes, V.; Pinto, E. Mycosporine-Like Amino Acids (MAAs): Biology, Chemistry and Identification Features. *Pharmaceuticals* **2021**, *14*, 63. https://doi.org/10.3390/ph14010063.
20. Rosic, N.N. Recent advances in the discovery of novel marine natural products and mycosporine-like amino acid UV-absorbing compounds. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 7053–7067. https://doi.org/10.1007/s00253-021-11467-9.
21. Righi, V.; Parenti, F.; Schenetti, L.; Mucci, A. Mycosporine-like Amino Acids and Other Phytochemicals Directly Detected by High-Resolution NMR on Klamath (*Aphanizomenon flos-aquae*) Blue-Green Algae. *J. Agric. Food. Chem.* **2016**, *64*, 6708–6715. https://doi.org/10.1021/acs.jafc.6b02615.
22. Gacesa, R.; Lawrence, K.P.; Georgakopoulos, N.D.; Yabe, K.; Dunlap, W.C.; Barlow, D.J.; Wells, G.; Young, A.R.; Long, P.F. The mycosporine-like amino acids porphyra-334 and shinorine are antioxidants and direct antagonists of Keap1-Nrf2 binding. *Biochimie* **2018**, *154*, 35–44. https://doi.org/10.1016/j.biochi.2018.07.020.
23. Madhyastha, H.; Madhyastha, R.; Nakajima, Y.; Omura, S.; Maruyama, M. Regulation of growth factors-associated cell migration by C-phycocyanin scaffold in dermal wound healing. *Clin. Exp. Pharmacol. Physiol.* **2012**, *39*, 13–19.
24. Manconi, M.; Pendás, J.; Ledón, N.; Moreira, T.; Sinico, C.; Saso, L.; Fadda, A.M. Phycocyanin liposomes for topical anti-inflammatory activity: In vitro in vivo studies. *J. Pharm. Pharmacol.* **2009**, *61*, 423–430.
25. Caddeo, C.; Chessa, M.; Vassallo, A.; Pons, R.; Diez-Sales, O.; Fadda, A.M.; Manconi, M. Extraction, purification and nanoformulation of natural phycocyanin (from *Klamath algae*) for dermal and deeper soft tissue delivery. *J. Biomed. Nanotechnol.* **2013**, *9*, 1929–1938. https://doi.org/10.1166/jbn.2013.1741.
26. Castangia, I.; Manca, M.L.; Catalán-Latorre, A.; Maccioni, A.M.; Fadda, A.M.; Manconi, M. Phycocyanin-encapsulating hyalurosomes as carrier for skin delivery and protection from oxidative stress damage. *J. Mater. Sci. Mater. Med.* **2016**, *27*, 75. https://doi.org/10.1007/s10856-016-5687-4.
27. Teleanu, D.M.; Niculescu, A.G.; Lungu, I.I.; Radu, C.I.; Vladâcenco, O.; Roza, E.; Costăchescu, B.; Grumezescu, A.M.; Teleanu, R.I. An Overview of Oxidative Stress,Neuroinflammation, and Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 5938. https://doi.org/10.3390/ijms23115938.
28. Jomova, K.; Alomar, S.Y.; Valko, R.; Nepovimova, E.; Kuca, K.; Valko, M. Oxidative stress and inflammation in neurodegenerative disorders. *Arch. Toxicol.* **2026**, *100*, 4367–4445. https://doi.org/10.1007/s00204-026-04518-5.
29. Nuzzo, D.; Presti, G.; Picone, P.; Galizzi, G.; Gulotta, E.; Giuliano, S.; Mannino, C.; Gambino, V.; Scoglio, S.; Di Carlo, M. Effects of the *Aphanizomenon flos-aquae* Extract (Klamin®) on a Neurodegeneration Cellular Model. *Ox. Med. Cell. Long.* **2018**, *2018*, 9089016. https://doi.org/10.1155/2018/9089016.
30. Petracco, G.; Faimann, I.; Reichmann, F. Inflammatory bowel disease and neuropsychiatric disorders: Mechanisms and emerging therapeutics targeting the microbiota-gut-brain axis. *Pharmacol. Ther.* **2025**, *269*, 108831. https://doi.org/10.1016/j.pharmthera.2025.108831.
31. Kaplan, G.G. The global burden of IBD: From 2015 to 2025. *Nat. Rev. Gastroenterol. Hepatol.* **2015**, *12*, 720–727. https://doi.org/10.1038/nrgastro.2015.150.
32. Amato, A.; Terzo, S.; Marchesa, P.; Maffongelli, A.; Martorana, M.; Scoglio, S.; Mulè, F. Spasmolytic Effects of Aphanizomenon Flos Aquae (AFA) Extract on the Human Colon Contractility. *Nutrients* **2021**, *13*, 3445. https://doi.org/10.3390/nu13103445.
33. Naselli, F.; Volpes, S.; Cardinale, P.S.; Micheli, S.; Cicio, A.; Scoglio, G.D.; Chiarelli, R.; Zizzo, M.G.; Picone, P.; Caradonna, F.; et al. First evidence of epigenetic modulation of human gene methylation by microalga *Aphanizomenon flos-aquae* (AFA) in inflammation-related pathways in intestinal cells. *Clin. Epigenet.* **2025**, *17*, 44. https://doi.org/10.1186/s13148-025-01849-5.

34. Masenga, S.K.; Kabwe, L.S.; Chakulya, M.; Kirabo, A. Mechanisms of Oxidative Stress in Metabolic Syndrome. *Int. J. Mol. Sci.* **2023**, *24*, 7898. https://doi.org/10.3390/ijms24097898.
35. Varra, F.N.; Varras, M.; Varra, V.K.; Theodosis-Nobelos, P. Molecular and pathophysiological relationship between obesity and chronic inflammation in the manifestation of metabolic dysfunctions and their inflammation-mediating treatment options. *Mol. Med. Rep.* **2024**, *29*, 95. https://doi.org/10.3892/mmr.2024.13219.
36. Terzo, S.; Calvi, P.; Giardina, M.; Gallizzi, G.; Di Carlo, M.; Nuzzo, D.; Picone, P.; Puleio, R.; Mulè, F.; Scoglio, S.; et al. Positive Impacts of Aphanizomenon Flos Aquae Extract on Obesity-Related Dysmetabolism in Mice with Diet-Induced Obesity. *Cells* **2023**, *12*, 2706. https://doi.org/10.3390/cells12232706.
37. Manson, J.E.; Bassuk, S.S. Vitamin and Mineral Supplements: What Clinicians Need to Know. *JAMA* **2018**, *319*, 859–860. https://doi.org/10.1001/jama.2017.21012.
38. Lonn, E.; Yusuf, S.; Arnold, M.J.; Sheridan, P.; Pogue, J.; Micks, M.; McQueen, M.J.; Probstfield, J.; Fodor, G.; Held, C.; et al. Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N. Engl. J. Med.* **2006**, *354*, 1567–1577. https://doi.org/10.1056/NEJMoa060900.
39. Eikelboom, J.W.; Lonn, E.; Genest, J., Jr.; Hankey, G.; Yusuf, S. Homocyst(e)ine and cardiovascular disease: A critical review of the epidemiologic evidence. *Ann. Intern. Med.* **1999**, *131*, 363–375. https://doi.org/10.7326/0003-4819-131-5-199909070-00008.
40. Herbert, V. Staging vitamin B-12 (cobalamin) status in vegetarians. *Am. J. Clin. Nutr.* **1994**, *59*, 1213S–1222S. https://doi.org/10.1093/ajcn/59.5.1213S.
41. Areekul, S.; Pattanamatum, S.; Cheeramakara, C.; Churdchue, K.; Nitayapabskoon, S.; Chongsanguan, M. The source and content of vitamin B12 in the tempehs. *J. Med. Assoc. Thai.* **1990**, *73*, 152–156.
42. Miyamoto, E.; Tanioka, Y.; Nakao, T.; Barla, F.; Inui, H.; Fujita, T.; Watanabe, F.; Nakano, Y. Purification and characterization of a corrinoid-compound in an edible cyanobacterium phanizomenon flos-aquae as a nutritional supplementary food. *J. Agric. Food Chem.* **2006**, *54*, 9604.
43. Halczuk, K.; Kaźmierczak-Barańska, J.; Karwowski, B.T.; Karmańska, A.; Cieślak, M. Vitamin B12—Multifaceted In Vivo Functions and In Vitro Applications. *Nutrients* **2023**, *15*, 2734. https://doi.org/10.3390/nu15122734.
44. Monteleone, P.; Mascagni, G.; Giannini, A.; Genazzani, A.R.; Simoncini, T. Symptoms of menopause—Global prevalence, physiology and implications. *Nat. Rev. Endocrinol.* **2018**, *14*, 199–215. https://doi.org/10.1038/nrendo.2017.180.
45. Genazzani, A.D.; Chierchia, E.; Lanzoni, C.; Santagni, S.; Veltri, F.; Ricchieri, F.; Rattighieri, E.; Nappi, R.E. Effects of Klamath Algae extract on psychological disorders and depression in menopausal women: A pilot study. *Minerva Ginecol.* **2010**, *62*, 381–388.
46. Scoglio, S.; Benedetti, S.; Canino, C.; Santagni, S.; Rattighieri, E.; Chierchia, E.; Canestrari, F.; Genazzani, A.D. Effect of a 2-month treatment with Klamin, a Klamath algae extract, on the general well-being, antioxidant profile and oxidative status of postmenopausal women. *Gynecol. Endocrinol.* **2009**, *25*, 235–240.
47. Cremonte, M.; Sisti, D.; Maraucci, I.; Giribone, S.; Colombo, E.; Rocchi, M.B.L.; Scoglio, S. The Effect of Experimental Supplementation with the Klamath Algae Extract Klamin on Attention-Deficit/Hyperactivity Disorder. *J. Med. Food.* **2017**, *20*, 1233–1239.
48. Gilroy, D.J.; Kauffman, K.W.; Hall, R.A.; Huang, X.; Chu, F.S. Assessing potential health risks from microcystin toxins in blue-green algae dietary supplements. *Environ. Health Perspect.* **2000**, *108*, 435–439. https://doi.org/10.1289/ehp.00108435.
49. Testai, E.; Buratti, F.M.; Funari, E.; Manganelli, M.; Vichi, S.; Arnich, N.; Biré, R.; Fessard, V.; Sialehaamoa, A. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. *EFSA Support. Publ.* **2016**, *13*, 998E.
50. Vichi, S.; Lavorini, P.; Funari, E.; Scardala, S.; Testai, E. Contamination by Microcystis and microcystins of blue-green algae food supplements (BGAS) on the Italian market and possible risk for the exposed population. *Food Chem. Toxicol.* **2012**, *50*, 4493–4499. https://doi.org/10.1016/j.fct.2012.09.029.
51. Available online: https://www.canada.ca/en/health-canada/services/drugs-health-products/natural-non-prescription/legislation-guidelines/guidance-documents/quality-guide.html (accessed on 15 October 2026).
52. Roy-Lachapelle, A.; Solliec, M.; Bouchard, M.F.; Sauvé, S. Detection of Cyanotoxins in Algae Dietary Supplements. *Toxins* **2017**, *9*, 76. https://doi.org/10.3390/toxins9030076.
53. Available online: https://www.legifrance.gouv.fr/loda/id/JORFTEXT000029254516/2026-10-02 (accessed on 15 October 2026).
54. Available online: https://www.salute.gov.it/new/it/tema/alimenti-fini-medici-speciali-ed-integratori/sostanze-e-preparati-vegetali/ (accessed on 15 October 2026).

55. Lyon-Colbert, A.; Su, S.; Cude, C. A Systematic Literature Review for Evidence of *Aphanizomenon flos-aquae* Toxigenicity in Recreational Waters and Toxicity of Dietary Supplements: 2000–2017. *Toxins* **2018**, *10*, 254. https://doi.org/10.3390/toxins10070254.
56. Available online: https://www.webmd.com/vitamins-supplements/blue-green-algae#uses (accessed on 15 October 2026).
57. Arman, T.; Clarke, J.D. Microcystin Toxicokinetics, Molecular Toxicology, and Pathophysiology in Preclinical Rodent Models and Humans. *Toxins* **2021**, *13*, 537. https://doi.org/10.3390/toxins13080537.
58. Li, T.; Fan, X.; Cai, M.; Jiang, Y.; Wang, Y.; He, P.; Ni, J.; Mo, A.; Peng, C.; Liu, J. Advances in investigating microcystin-induced liver toxicity and underlying mechanisms. *Sci. Total Environ.* **2023**, *905*, 167167. https://doi.org/10.1016/j.scitotenv.2023.167167.
59. Ortelli, D.; Edder, P.; Cognard, E.; Jan, P. Fast screening and quantitation of microcystins in microalgae dietary supplement products and water by liquid chromatography coupled to time of flight mass spectrometry. *Anal. Chim. Acta.* **2008**, *617*, 230–237. https://doi.org/10.1016/j.aca.2008.03.033.
60. Vinogradova, T.; Danaher, M.; Baxter, A.; Moloney, M.; Victory, D.; Haughey, S.A. Rapid surface plasmon resonance immunobiosensor assay for microcystin toxins in blue-green algae food supplements. *Talanta* **2011**, *84*, 638–643. https://doi.org/10.1016/j.talanta.2011.01.036.
61. Sánchez-Parra, E.; Boutarfa, S.; Aboal, M. Are Cyanotoxins the Only Toxic Compound Potentially Present in Microalgae Supplements? Results from a Study of Ecological and Non-Ecological Products. *Toxins* **2020**, *12*, 552. https://doi.org/10.3390/toxins12090552.
62. Carbonell-Rozas, L.; Aparicio-Muriana, M.M.; Romero-González, R.; Frenich, A.G.; García-Campaña, A.M.; Olmo-Iruela, M.D. Application of Targeted and Suspect Screening Workflows for Cyclic Peptide Cyanotoxin Profiling in Spirulina- and Klamath-Based Food Supplements. *Foods* **2025**, *14*, 2969. https://doi.org/10.3390/foods14172969.
63. Miller, T.R.; Xiong, A.; Deeds, J.R.; Stutt, W.L.; Samdal, I.A.; Lovberg, K.E.; Miles, C.O. Microcystin Toxins at Potentially Hazardous Levels in Algal Dietary Supplements Revealed by a Combination of Bioassay, Immunoassay, and Mass Spectrometric Methods. *J. Agric. Food Chem.* **2020**, *68*, 8016–8025. https://doi.org/10.1021/acs.jafc.0c02024.
64. Pugh, N.D.; Ross, S.A.; ElSohly, H.N.; ElSohly, M.A.; Pasco, D.S. Characterization of the immune-enhancing polysaccharides from *Aphanizomenon flos-aquae*. *Phytother. Res.* **2001**, *15*, 168–173. https://doi.org/10.1002/ptr.689.
65. Jensen, G.S.; Hart, A.N.; Zaske, L.A.; Drapeau, C.; Gupta, N.; Schaeffer, D.J.; Cruickshank, J.A. Mobilization of human CD34+ CD133+ and CD34+ CD133(-) stem cells in vivo by consumption of an extract from *Aphanizomenon flos-aquae*—related to modulation of CXCR4 expression by an L-selectin ligand? *Cardiovasc. Revasc. Med.* **2007**, *8*, 189–202. https://doi.org/10.1016/j.carrev.2007.03.004.
66. Di Pierro, F.; Rapacioli, G.; Ferrara, T.; Togni, S. Potential therapeutic effects of *Aphanizomenon flos-aquae* extracts in neuroprotection and immune modulation. *Minerva Med.* **2008**, *99*, 481–496. PMID: 18827803.


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.