

Review

# Chemical Compounds, Bioactivities, and Applications of *Chlorella vulgaris* in Food, Feed and Medicine

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**Abstract:** This review presents the chemical composition, bioactive properties, and diverse applications of *Chlorella vulgaris*, a green microalga widely recognized for its exceptional nutritional value and therapeutic potential. The study emphasizes the presence of key nutrients, including high-quality proteins, essential vitamins, minerals, and an array of bioactive compounds such as carotenoids, chlorophyll, and polysaccharides. These compounds have been shown to exhibit a wide spectrum of biological activities, including potent antioxidant, anti-inflammatory, immunomodulatory, antiviral, anticancer, antidiabetic, lipid-lowering, and detoxifying effects. The review explores the multifaceted applications of *C. vulgaris* in various sectors, including its growing role as a functional food ingredient, a nutraceutical supplement in animal feed, and a promising therapeutic agent for combatting chronic diseases. This paper also highlights its potential for enhancing immune responses, mitigating oxidative stress, promoting detoxification of heavy metals, and improving overall health outcomes. However, current limitations in clinical evidence surrounding its medicinal efficacy present challenges that need to be addressed. Furthermore, significant obstacles remain in scaling up *C. vulgaris* production, including optimizing cultivation techniques and improving bioavailability. Additionally, this review identifies crucial research gaps, particularly in optimizing cultivation techniques, improving bioavailability, and validating the clinical efficacy of *C. vulgaris*. By addressing these challenges, *C. vulgaris* holds significant promise in contributing to global health, sustainable nutrition, and environmental conservation efforts by serving as a source of protein and bioactive components for a growing population while simultaneously having a lower environmental impact and requiring fewer resources in production compared to traditional ingredients like soybean meal.

**Keywords:** *C. vulgaris*; bioactive compounds; antioxidant activity; functional food; animal feed; therapeutic applications



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## 1. Introduction

Although *Chlorella vulgaris* (*C. vulgaris*) was described in 1890 by Martinus Beijerinck [1], it has only recently gained substantial attention. This renewed interest is largely due to its diverse chemical composition, extensive bioactive properties, and its potential as a significant protein source for both humans and animals, especially due to the growing global population [2]. *C. vulgaris* is known for being a rich source of key nutrients, including proteins, vitamins, minerals, and various bioactive compounds, such as carotenoids, chlorophyll, and polysaccharides, all of which contribute to its numerous therapeutic effects [3].

These compounds have been linked to antioxidant, anti-inflammatory, immunomodulatory, and detoxifying properties [4]. *C. vulgaris* has also shown promise in detoxifying heavy metals, enhancing the immune system, and supporting metabolic health, making it a strong candidate for nutraceutical and pharmaceutical applications [5–8].

In addition, *C. vulgaris*'s high protein content has positioned it as a viable alternative protein source in food products, comparable to other plant-based proteins like soybeans. *C. vulgaris* contains 43%-to-61% protein by dry weight, depending on growth conditions [9,10]. Approximately 20% of these proteins are associated with the cell wall, while about 50% are located within the cells. The remaining 30% of proteins are dynamic and may be involved in processes, such as cellular signaling or transport, contributing to various cellular functions as they move in and out of the cell [11]. Additionally, key pigments like chlorophyll have attracted significant interest due to their applications in functional foods, pharmaceuticals, and cosmetics [12]. Chlorophyll has been demonstrated to scavenge free radicals and inhibit lipid peroxidation [13], highlighting its valuable antioxidant properties in health-related products. Beyond its nutritional value, *C. vulgaris* is recognized for its bioactive compounds, which contribute to its role as a natural antioxidant, immune enhancer, and metabolic regulator [14–17].

Research on *C. vulgaris* has specifically investigated its antioxidant and anti-inflammatory activities. Studies suggest that its bioactive compounds, such as phenolic acids, chlorophylls, and carotenoids, may combat oxidative stress and reduce the risk of chronic diseases like cancer, cardiovascular diseases, and diabetes [18,19]. Regular consumption of fruits, vegetables, seaweed, microalgae, and similar foods can elevate dietary levels of chlorophyll and carotenoids. Among microalgae, *C. vulgaris* is particularly noteworthy due to its high protein content and abundance of biologically active substances [20].

Additionally, *C. vulgaris* has demonstrated potential in improving lipid metabolism, enhancing immune function, and detoxifying heavy metals from the body [21,22]. However, these health benefits can be influenced by factors, such as cultivation methods, environmental conditions, and processing techniques, which impact the stability and potency of its bioactive compounds. This remains an ongoing subject of investigation [23–25]. For instance, exposure to heat during processing may degrade certain bioactive molecules, reducing their efficacy in nutraceutical applications [26–29].

With the growing demand for sustainable, functional food sources, *C. vulgaris* emerges as a promising candidate for nutritional supplementation, medical applications, and animal feed [2]. This manuscript aims to provide a comprehensive assessment of the chemical composition, bioactivities, and diverse applications of *C. vulgaris* across the food, nutrition, medicine, and animal feed sectors. In doing so, it synthesizes current research from databases such as Google Scholar, PubMed, Scopus, and Web of Science to highlight the health-promoting effects of *C. vulgaris*'s bioactive compounds, including their antioxidant, anti-inflammatory, detoxifying, lipid-lowering, and immune-modulating properties. The study further discusses potential functional and therapeutic applications, identifies existing knowledge gaps, and explores future directions for research and industrial use [4].

## 2. Chemical Composition of *Chlorella vulgaris*

*C. vulgaris* is a unicellular green microalga recognized for its rich and complex chemical composition, making it highly valuable across various industries, including food, pharmaceuticals, nutraceuticals, and animal feed. It contains high levels of proteins, lipids, carbohydrates, vitamins, minerals, and pigments, all of which contribute to its wide range of biological activities [4]. This has led to significant interest in biotechnology, where *C. vulgaris* is seen as a sustainable source of these valuable biomolecules [19,20].

### 2.1. Proteins

Proteins are among the most abundant components of *C. vulgaris*, with studies reporting levels between 43% and 61%, depending on cultivation conditions [9,10,30]. This makes *C. vulgaris* a notable protein source among microalgae. The proteins in *C. vulgaris* are

considered high quality as they contain all essential amino acids, making them a complete protein source comparable to that found in animal products. The essential amino acids present include leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [31], which are often limiting in plant-based protein sources [3]. *C. vulgaris* also contains significant quantities of non-essential amino acids such as alanine, arginine, and glycine, further contributing to its nutritional profile [4]. This composition positions *C. vulgaris* as a competitive alternative to traditional protein sources like soybean meal, eggs, and meat.

Microalgae, including *C. vulgaris*, have been reported to have a protein digestibility-corrected amino acid score (PDCAAS) ranging from 0.63 to 0.77 [32]. This score can vary depending on cultivation conditions and processing methods [33]. For comparison, soybean meal typically scores around 0.91, eggs score a perfect 1.0, and meat ranges between 0.75 and 1.0, depending on the type [3]. Despite falling slightly below animal-based proteins, *C. vulgaris* is still regarded as a valuable source of plant-based protein due to its favorable PDCAAS.

Protein digestibility is also an important factor for *C. vulgaris*. Studies indicate that its digestibility is comparable to other plant proteins, with rates reported between 80% and 90% [30]. This digestibility is crucial for ensuring that amino acids are available for absorption in the human body. In contrast, animal proteins, such as those found in eggs and milk, often have higher digestibility rates, typically exceeding 90% [3]. When comparing *C. vulgaris* to other protein sources, it is essential to consider not only the protein content but also the overall nutritional profile. *C. vulgaris* is rich in vitamins, minerals, and omega-3 fatty acids, which enhances its value as a dietary supplement [3]. In contrast, while animal proteins provide high-quality protein, they may also come with higher levels of saturated fats and cholesterol, which are linked to an increased risk of cardiovascular diseases and other health concerns. [3].

While *C. vulgaris* provides valuable protein, its rigid cellulose cell wall can limit protein digestibility and bioavailability. To overcome this, pre-treatment processes are often necessary. Mechanical methods such as bead milling, sonication, and high-pressure homogenization have proven effective in disrupting the cell wall, thereby improving the accessibility of nutrients and enhancing the nutritional value of *C. vulgaris* proteins in various applications [27,34,35].

## 2.2. Lipids

The lipid composition of *C. vulgaris* is of particular interest, as it can be used in various applications, such as the production of biofuels, nutritional supplements, and cosmetic ingredients [36]. Lipids in *C. vulgaris* represent around 5 to 58% [9] and are primarily composed of polyunsaturated fatty acids (PUFAs). The most prominent fatty acids include linoleic acid (18:2n-6), oleic acid (18:1c9), palmitic acid (16:0), and alpha-linolenic acid (18:3n-3), with linoleic and alpha-linolenic acids being the primary omega-6 and omega-3 fatty acids, respectively [37]. According to Zhang et al. [38], the fatty acid composition of *C. vulgaris* exhibits significant variability depending on the cultivation conditions, specifically between phototrophic and heterotrophic methods. Under phototrophic conditions, *C. vulgaris* displays a diverse fatty acid profile, with palmitic acid (C16:0) being the most abundant at  $44.99 \pm 0.21$  mg/g, followed by linoleic acid (C18:2) at  $25.4 \pm 0.29$  mg/g and alpha-linolenic acid (C18:3) at  $12.49 \pm 0.37$  mg/g. Additionally, oleic acid (C18:1) constitutes  $1.67 \pm 0.01$  mg/g, while stearic acid (C18:0) accounts for  $1.09 \pm 0.01$  mg/g. Conversely, under heterotrophic conditions, the fatty acid profile shifts; palmitic acid remains significant at  $28.01 \pm 0.77$  mg/g, while alpha-linolenic acid was not determined. Oleic acid experiences a notable increase, reaching  $32.89 \pm 0.10$  mg/g. This variability highlights the influence of cultivation strategies on the lipid composition of *C. vulgaris*, which is essential for its applications in food, feed, health-promoting products, and even biofuels. The lipid profile of *C. vulgaris* has garnered attention due to its associated health benefits, particularly its anti-inflammatory effects, which may help reduce chronic inflammation, and cardio-

vascular protective properties that can lower the risk of heart disease and improve overall heart health [31].

### 2.3. Carbohydrates and Fibres

Carbohydrates constitute approximately 12–20% of *C. vulgaris*'s dry weight [4]. These carbohydrates primarily exist as polysaccharides, which have been recognized for their immune-stimulating and antioxidant properties [39]. The polysaccharides in *C. vulgaris* are primarily composed of glucose, rhamnose, mannose, and galactose, and they include complex structures such as beta-glucans that contribute to the organism's biological activities and are particularly well-known for their significant immunomodulatory effects [40]. Additionally, *C. vulgaris* contains small amounts of simple sugars, such as glucose, as well as glycogen, a polysaccharide that serves as an energy reserve [4].

Furthermore, *C. vulgaris* contains sulphated polysaccharides, which are known for their antiviral and immunomodulatory effects. These sulphated polysaccharides may enhance the immune response and inhibit viral replication by blocking viral adsorption and entry into host cells, further contributing to the health benefits associated with *C. vulgaris* consumption [41–43].

In addition to its carbohydrate content, *C. vulgaris* is also a notable source of dietary fibers, which constitute a significant portion of its biomass [44]. These fibers, primarily composed of insoluble cellulose and hemicellulose, contribute to the structural integrity of the cell walls while also playing a beneficial role in human digestion and gut health [3]. The insoluble fibers in *C. vulgaris* promote bowel regularity by increasing stool bulk and facilitating movement through the digestive tract. They also provide a substrate for beneficial gut microbiota [7]. Furthermore, *C. vulgaris* contains soluble fibers such as pectins and beta-glucans, known for their ability to form viscous solutions that help regulate blood glucose levels and reduce cholesterol absorption [45].

When incorporated into animal feed, these fibers can improve digestive efficiency, promote gut health, and enhance nutrient absorption in livestock. The fermentable fibers in *C. vulgaris* provide a beneficial prebiotic effect, supporting the growth of beneficial gut bacteria, which may lead to improved immune function and overall health in animals. This combination of soluble and insoluble fibers not only enhances the nutritional profile of *C. vulgaris* for human consumption but also makes it a valuable component in animal feed formulations, contributing to improved digestion and health outcomes in livestock.

### 2.4. Pigments

*C. vulgaris* is rich in pigments, with chlorophyll being the most dominant, accounting for its characteristic green color. Chlorophyll serves not only as a key component in photosynthesis but also possesses antioxidant and detoxifying properties, making it a valuable functional compound in nutraceutical and medical applications [44,46]. In addition to chlorophyll, *C. vulgaris* contains carotenoids such as beta-carotene, lutein, and zeaxanthin, which are known for their roles in protecting against oxidative stress and promoting eye health. Specifically, lutein and zeaxanthin have been shown to accumulate in the retina, where they help protect the retinal pigment epithelium and photoreceptors from oxidative stress and blue light damage, significant contributors to age-related macular degeneration (AMD) [47–50]. Research indicates that these carotenoids' protective effect against oxidative stress can reduce the risk of progression of eye diseases, particularly AMD, by enhancing macular pigment density and improving visual function [47,48,51,52].

High serum levels of carotenoids, including lutein, zeaxanthin,  $\beta$ -carotene,  $\alpha$ -carotene, and cryptoxanthin, have been associated with a reduced risk of neovascular AMD, with odds ratios ranging from 0.3 to 0.5 for individuals with sufficient intake of these compounds [53]. Although not all studies have consistently demonstrated a correlation between carotenoid intake and AMD risk, the role of lutein and zeaxanthin in retinal health is well-established. As *C. vulgaris* provides a rich source of these beneficial pigments, it holds promise as a functional food for eye health support.

### 2.5. Vitamins and Minerals

*C. vulgaris* is a rich source of essential vitamins and minerals. It is particularly high in B-complex vitamins, including thiamine (B1), riboflavin (B2), niacin (B3), and folic acid (B9), all of which are important for energy metabolism and cellular function [36]. Additionally, it contains high levels of vitamins A (beta-carotene) and E (tocopherols), which function as antioxidants and support skin and eye health [20,36,54]. *C. vulgaris* not only provides these vital vitamins but also serves as a significant source of essential minerals, including iron, calcium, magnesium, and zinc, all of which contribute to its health-promoting properties. The high iron content, in particular, has made *C. vulgaris* a potential supplement for individuals with iron deficiencies [31].

### 2.6. Nucleotides

The bioactivity of nucleotides derived from *C. vulgaris* contributes to various health benefits, including immunomodulation, antioxidant effects, and potential anticancer properties. Nucleotides play a crucial role in cellular metabolism and are essential for DNA and RNA synthesis. They are involved in energy transfer through adenosine triphosphate (ATP) and serve as signaling molecules. The presence of nucleotides in *C. vulgaris* has been linked to enhanced immune responses and improved gut health. For instance, studies have shown that nucleotide supplementation can enhance the proliferation of immune cells, thereby improving overall immune function [55]. This immunomodulatory effect is particularly beneficial in both human health and animal nutrition, where it can lead to improved growth performance and disease resistance in livestock [56]. In addition to their immune-boosting properties, nucleotides from *C. vulgaris* exhibit antioxidant activity. The microalga is rich in various antioxidants, including carotenoids and chlorophyll, which work synergistically with nucleotides to scavenge free radicals and reduce oxidative stress [57]. This antioxidant capacity is crucial in preventing cellular damage and may play a role in cancer prevention. Research has indicated that extracts from *C. vulgaris* can exert antiproliferative effects on cancer cells, suggesting that its nucleotides may contribute to these protective effects [58]. The combination of nucleotides and other bioactive compounds in *C. vulgaris* enhances its potential as a functional food and nutraceutical. Moreover, the incorporation of *C. vulgaris* in animal feed has been shown to improve growth performance and feed efficiency. The nucleotides present in *C. vulgaris* can support gut health by promoting the growth of beneficial gut microbiota, which is essential for nutrient absorption and overall health in animals [56]. This is particularly relevant in aquaculture, where *C. vulgaris* is used as a feed supplement to enhance the pigmentation and health of fish, as well as to improve their immune responses.

### 2.7. Secondary Metabolites

In addition to its major components, *C. vulgaris* contains secondary metabolites such as polyphenols, sterols, and terpenes, which contribute to its overall bioactivity. Polyphenolic compounds, including catechins and chlorogenic acids, have been identified in *C. vulgaris* and are known for their antioxidant, anti-inflammatory, and antidiabetic properties [22,31]. These secondary metabolites, though present in smaller quantities, significantly enhance the therapeutic potential of *C. vulgaris*.

## 3. Bioactivity of *Chlorella vulgaris* Compounds

*C. vulgaris* has garnered significant attention for its bioactive compounds, which exhibit numerous health-promoting properties. These bioactivities are primarily attributed to their proteins, polysaccharides, lipids, pigments, and secondary metabolites. The therapeutic potential of these compounds includes antioxidant, anti-inflammatory, immunomodulatory, antiviral, anticancer, antidiabetic, and lipid-lowering effects. This section details the specific bioactivities of key compounds found in *C. vulgaris*.

### 3.1. Antioxidant Activity

The antioxidant potential of *C. vulgaris* is largely attributed to its diverse array of bioactive compounds, including chlorophyll, carotenoids (such as lutein and  $\beta$ -carotene), polysaccharides, and polyphenols. These compounds collectively contribute to neutralizing harmful reactive oxygen species (ROS) and reducing oxidative stress, thereby lowering the risk of chronic diseases like cancer, cardiovascular disorders, and neurodegenerative conditions [18,44,54,59]. Antioxidants generally function through two primary mechanisms: non-enzyme-promoted and enzyme-promoted antioxidants. Non-enzyme-promoted antioxidants work directly by neutralizing free radicals themselves, while enzyme-promoted antioxidants support the body's natural antioxidant defenses by enhancing the activity of key enzymes involved in the breakdown of ROS. Understanding this distinction is essential for appreciating the full range of protective effects provided by *C. vulgaris*. To better understand these effects, the antioxidant mechanisms can be divided into two categories: non-enzyme-promoted antioxidants and enzyme-promoted antioxidants.

#### 3.1.1. Non-Enzyme-Promoted Antioxidants

Chlorophyll, a key component of *C. vulgaris*, has been shown to scavenge free radicals, offering protection against oxidative stress and its associated health risks, such as neuroinflammation and cell damage [18,59]. Carotenoids like lutein and  $\beta$ -carotene are also crucial non-enzyme-promoted antioxidants that protect cells, particularly in the skin and eyes, by neutralizing ROS [44,54]. Lutein, in particular, has been linked to improved cognitive function and neuroprotection, suggesting its role in mitigating the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's [59,60].

The polyphenols found in *C. vulgaris* enhance antioxidant defense mechanisms and protect neurons through both antioxidant and anti-inflammatory actions. Although *C. vulgaris* contains relatively lower levels of omega-3 fatty acids like  $\alpha$ -linolenic acid compared to marine algae, these essential fats still contribute to brain health and help prevent cognitive decline [59,60]. These combined non-enzyme-promoted antioxidant properties of *C. vulgaris* not only support brain health but also protect skin cells from UV-induced damage, making this microalga a promising candidate in both neuroprotection and dermatological applications [61].

#### 3.1.2. Enzyme-Promoted Antioxidants

In addition to its non-enzyme-promoted antioxidants, *C. vulgaris* contains polysaccharides that support the body's natural antioxidant defenses by stimulating key antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). These enzymes play a vital role in ROS neutralization by converting superoxide radicals into less harmful molecules. The mechanisms by which SOD and CAT alleviate oxidative stress are well established: at the cellular level, SOD catalyzes the dismutation of superoxide anions ( $O_2^-$ ) into oxygen and hydrogen peroxide, reducing oxidative stress, while CAT further breaks down hydrogen peroxide into water and oxygen, mitigating its potential to harm cellular structures like proteins, lipids, and DNA. For instance, Tsiplakou et al. [62] reported a notable increase in SOD (10.31%) and CAT (18.66%) activities in the blood plasma of goats supplemented with *C. vulgaris*, highlighting its potential to enhance the antioxidant defense mechanisms in livestock. Similarly, Sikiru et al. [63] demonstrated that *C. vulgaris* supplementation in pregnant New Zealand White rabbits led to improved antioxidant enzyme activities, thereby reducing oxidative stress markers such as malondialdehyde. These findings are corroborated by Panahi et al. [64], whose studies have shown that the antioxidant properties of *C. vulgaris* extend to human health, with clinical trials indicating its potential to reduce oxidative stress markers in smokers. Moreover, the anti-inflammatory and immunomodulatory properties of these polysaccharides further protect against oxidative stress and inflammation, particularly in the brain [54,59]. This dual action of enzyme-promoted antioxidants and non-enzyme-promoted antioxidants highlights the comprehensive antioxidant capability of *C. vulgaris*, supporting its therapeutic potential in various health applications.

### 3.2. Anti-Inflammatory Effects

*C. vulgaris* exhibits significant anti-inflammatory properties due to its bioactive compounds, particularly carotenoids and polysaccharides. These compounds help suppress inflammatory pathways by reducing the production of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) and Interleukin-6 (IL-6) while also inhibiting the activity of inflammatory enzymes like Cyclooxygenase-2 (COX-2). Studies have shown that extracts from *C. vulgaris* can downregulate the expression of these cytokines in various cell models, suggesting its potential for therapeutic application in inflammatory diseases [65,66]. Additionally, the bioactive compounds in *C. vulgaris* can inhibit COX-2 expression and activity, thereby reducing the synthesis of inflammatory mediators like prostaglandins [20,65,67,68], making *C. vulgaris* beneficial in managing chronic inflammatory conditions such as arthritis and cardiovascular diseases. The COX pathway represents a pivotal enzymatic cascade in the regulation of inflammatory processes. It is mediated by the COX enzyme, which catalyzes the conversion of arachidonic acid into bioactive lipid mediators, specifically prostaglandins and thromboxane [68,69]. Prostaglandins serve as key modulators in the pathophysiology of inflammation, mediating vasodilation, pyrexia, and nociception, while thromboxane plays a crucial role in platelet aggregation and hemostasis. This pathway is a major regulator of the inflammatory response, with both homeostatic and pathogenic outcomes depending on the extent and regulation of the inflammatory stimulus.

*Chlorella*-11 peptide, a bioactive compound from *C. vulgaris* (Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe) [67,70], inhibits pro-inflammatory cytokines such as TNF- $\alpha$  and reduces the production of prostaglandins via the COX pathway. Additionally, it suppresses another key mediator of inflammation, nitric oxide (NO), which is derived from L-arginine through the action of inducible NOS (iNOS) [71].

Inflammatory activity of *C. vulgaris* extracts has been reported in some in vitro and in vivo studies [65,70,72]. Sibi et al. [72] examined the in vitro anti-inflammatory effects of *C. vulgaris* and found that treatment with these microalga extracts decreased the production of inflammatory mediators (PGE<sub>2</sub>, and IL6) and NO in LPS-activated RAW 264.7 cells, and suppressed inflammation. *Chlorella*-11 peptide, a component isolated from *C. vulgaris* (Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe), has also been studied in vitro and in vivo. *C. vulgaris* also modulates the (NF- $\kappa$ B) pathway, helping to reduce inflammation at a molecular level [65,67,73]. NF- $\kappa$ B is a key regulator of inflammatory processes and plays a crucial role in the pathogenesis of various chronic inflammatory diseases [68,74]. Additionally, it promotes the production of anti-inflammatory cytokines like IL-10, further aiding in tissue repair and healing [66]. This makes *C. vulgaris* a promising agent for addressing both acute and chronic inflammation in various conditions.

### 3.3. Immunomodulatory Activity

*C. vulgaris* is known for its ability to enhance both innate and adaptive immune responses, largely through the activation of cytokine production. Its polysaccharides stimulate the release of key cytokines like interferon-gamma (IFN- $\gamma$ ) and interleukin-2 (IL-2), which are essential for activating immune cells such as natural killer cells and T cells [20]. IFN- $\gamma$  enhances macrophage and natural killer (NK) cell activity, while IL-2 promotes T cell activation and proliferation, boosting the body's defense against infections and cancer cells [65,67]. This immunomodulatory effect of *Chlorella* has been observed in both animal studies and human clinical trials, making it a promising natural supplement for supporting overall immune function [73]. For instance, Ramírez-Coronel et al. [75] found that *C. vulgaris* supplementation improved immune responses in common carp, indicating its potential benefits in aquaculture. This aligns with findings from Velankanni et al. [7], who noted that *Chlorella* can modulate gut microbiota and enhance immune responses, suggesting its utility in managing autoimmune conditions. Such immunomodulatory effects, combined with its antioxidant capabilities, make *C. vulgaris* a promising candidate for dietary therapies aimed at reducing oxidative stress and enhancing overall health. *C. vulgaris* also improves the function of macrophages, NK cells, and cytotoxic T cells, enhanc-

ing immune surveillance against pathogens and cancer cells [73]. Additionally, it helps regulate the balance between pro-inflammatory and anti-inflammatory cytokines, increasing anti-inflammatory cytokines like IL-10, which helps control inflammation and prevent tissue damage [65,67]. By modulating the NF- $\kappa$ B pathway and boosting IL-10 production, *C. vulgaris* effectively serves as both an anti-inflammatory agent and an immunomodulator.

In vitro trials and animal studies have shown that *Chlorella* and its extracts play a significant role in modulating immune responses to tumors, as well as bacterial and viral infections [76–83]. Research indicates that these organisms can enhance the activity of immune cells, boost cytokine production, and improve overall immune function, suggesting their potential as natural immunotherapeutic agents. As for human trials, Kwak et al. [65] demonstrated that an 8-week intervention with *C. vulgaris* resulted in significant increases in serum concentrations of interferon- $\gamma$  ( $p < 0.05$ ) and interleukin-1 $\beta$  ( $p < 0.001$ ), along with a tendency for interleukin-12 levels to rise ( $p < 0.1$ ) compared to the placebo group. Additionally, NK cell activity was notably enhanced in the *Chlorella* group, correlating positively with the serum levels of interleukin-1 $\beta$  ( $r = 0.280$ ,  $p = 0.047$ ) and interferon- $\gamma$  ( $r = 0.271$ ,  $p < 0.005$ ). These findings suggest that short-term *Chlorella* supplementation may provide beneficial immunostimulatory effects by enhancing NK cell activity and promoting the production of Th-1 cell-induced cytokines, including interferon- $\gamma$ , interleukin-12, and interleukin-1 $\beta$ , in healthy individuals.

### 3.4. Antiviral and Antimicrobial Activity

Research indicates that *C. vulgaris* has antiviral properties, particularly against enveloped viruses such as herpes simplex virus, hepatitis C virus, and HIV. Sulphated polysaccharides from *C. vulgaris* have been shown to prevent viral entry into host cells by interfering with viral attachment mechanisms [21,67]. This antiviral activity suggests *C. vulgaris* could be used as a supplementary treatment for viral infections, though further studies are needed to confirm its clinical efficacy [4].

In addition to its antiviral effects, *C. vulgaris* has shown notable antimicrobial activity against a wide range of bacterial and fungal pathogens. Certain bioactive compounds in *Chlorella*, including peptides, fatty acids, and polysaccharides, have been demonstrated to inhibit the growth of Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* [84]. This antimicrobial potential makes *C. vulgaris* an appealing natural alternative to synthetic antibiotics, especially in an era of increasing antibiotic resistance.

Moreover, *C. vulgaris* has demonstrated antifungal properties, particularly against pathogenic fungi such as *Candida albicans* and *Aspergillus niger*, which are responsible for various infections in humans and animals [85]. The antifungal effect is attributed to bioactive polysaccharides and fatty acids in *Chlorella*, which interfere with fungal cell wall integrity, leading to cell death.

The antimicrobial properties of *C. vulgaris* have significant implications for its use in the food industry as a natural preservative. Its ability to prevent the growth of spoilage microorganisms can extend the shelf life of food products, while its safety profile makes it suitable for use in functional foods and nutraceuticals [21].

### 3.5. Anticancer Activity

*C. vulgaris* has demonstrated promising anticancer effects, particularly through its capacity to induce apoptosis in cancer cells and inhibit tumor proliferation. Bioactive compounds such as polysaccharides and carotenoids—especially lutein and beta-carotene—have been shown to suppress carcinogenesis by protecting against DNA damage and blocking pathways that encourage tumor growth [18]. Additionally, chlorophyll contributes to cancer prevention by detoxifying carcinogens and hindering tumor development [4]. The polysaccharides derived from *C. vulgaris* exhibit immunomodulatory and antioxidant properties, which further aid in reducing the risk of cancer. These compounds help mitigate

oxidative stress and prevent DNA damage, both of which are critical factors in cancer progression [86,87].

Carotenoids, particularly lutein and beta-carotene, are prominent in *C. vulgaris* and have been linked to anticancer effects. These compounds act as potent antioxidants, neutralizing free radicals and thereby reducing oxidative damage to cellular components, including DNA [88,89]. Studies indicate that carotenoids extracted from *C. vulgaris* can inhibit the proliferation of various cancer cell lines, including colon cancer cells, by inducing cell cycle arrest and apoptosis [88,90].

The presence of lutein, the major carotenoid in *C. vulgaris*, has been specifically noted for its ability to suppress cancer cell growth, making it a valuable component in cancer-prevention strategies [89]. Additionally, chlorophyll, another significant component of *C. vulgaris*, plays a role in detoxifying carcinogens and preventing tumor development. Chlorophyll has been shown to bind to potential carcinogens, facilitating their excretion from the body and thereby reducing the risk of cancer [91,92].

The detoxifying properties of chlorophyll, combined with its ability to enhance the antioxidant capacity of the body, contribute to the overall anticancer potential of *C. vulgaris* [55,92]. In summary, the anticancer activity of *C. vulgaris* is primarily mediated through its secondary metabolites, including polysaccharides, carotenoids, and chlorophyll. These compounds work synergistically to induce apoptosis in cancer cells, inhibit tumor growth, and protect against DNA damage, highlighting the potential of *C. vulgaris* as a functional food in cancer prevention and management.

### 3.6. Antidiabetic Effects

*C. vulgaris* has been shown to enhance glycemic control, especially in individuals with type 2 diabetes. Its bioactive compounds, including polysaccharides and carotenoids, play a key role in regulating blood glucose levels and improving insulin sensitivity [93]. Studies indicate that supplementation with *C. vulgaris* can lead to reductions in fasting blood glucose and HbA1c levels, highlighting its potential as an effective adjunct in diabetes management [94–98]. These compounds are believed to contribute to the hypoglycemic effects of *C. vulgaris* through various mechanisms.

One of the primary mechanisms involves the modulation of insulin-signaling pathways. Research indicates that *C. vulgaris* can enhance insulin sensitivity and prevent insulin resistance, particularly in the context of high-fat diets [94]. This modulation is likely facilitated by the activation of key signaling molecules in the insulin pathway, which promotes glucose uptake and utilization in peripheral tissues [99]. Moreover, the antioxidant properties of *C. vulgaris* play a crucial role in its ability to regulate blood glucose levels. The presence of polyphenolic compounds in *C. vulgaris* has been shown to reduce oxidative stress, a condition often associated with insulin resistance and type 2 diabetes [100]. By mitigating oxidative stress, *C. vulgaris* may help preserve pancreatic  $\beta$ -cell function, thereby improving insulin secretion and action [101]. Additionally, the antioxidant enzymes, such as SOD and CAT, are significantly upregulated in response to *C. vulgaris* supplementation, further enhancing the body's defense against oxidative damage [102]. Furthermore, the polysaccharides found in *C. vulgaris* may also contribute to glycemic control by influencing the gut microbiota and enhancing the absorption of glucose. These polysaccharides can act as prebiotics, promoting the growth of beneficial gut bacteria that are associated with improved metabolic health [103]. This interaction between *C. vulgaris* and gut microbiota may lead to enhanced glucose metabolism and reduced postprandial blood glucose levels. In summary, the bioactive compounds in *C. vulgaris*, including polysaccharides and carotenoids, regulate blood glucose levels through multiple mechanisms: enhancing insulin sensitivity, reducing oxidative stress, and potentially modulating gut microbiota. These findings underscore the potential of *C. vulgaris* as a functional food in the management of type 2 diabetes.

### 3.7. Lipid-Lowering and Cardiovascular Effects

Several studies suggest that *C. vulgaris* can lower lipid levels, contributing to cardiovascular health. Its consumption has been associated with reductions in total cholesterol, LDL cholesterol, and triglycerides, while it may either increase HDL cholesterol or have no significant effect on it [68,103,104]. According to Sherafati et al. [105], a systematic review and dose-response meta-analysis assessed the impact of *C. vulgaris* supplementation on blood lipids. The analysis revealed a significant reduction in total cholesterol (TC) and LDL levels, with a mean difference of  $-7.47$  mg/dL for TC and  $-7.71$  mg/dL for LDL. However, there was no significant effect on triglycerides and HDL levels. The dose-response analysis further indicated that the LDL lowering effect was most pronounced at dosages up to 1500 mg/day, with no significant benefits at higher doses. These lipid-lowering effects, along with the antioxidant and anti-inflammatory properties of *C. vulgaris*, make it an effective agent in preventing atherosclerosis and promoting heart health [44].

The mechanisms by which *C. vulgaris* exerts these lipid-lowering effects are multifaceted and involve various biochemical pathways. One of the primary mechanisms is the presence of omega-3 fatty acids in *C. vulgaris*, which are known to have beneficial effects on lipid metabolism. Omega-3 fatty acids can reduce triglyceride levels in the bloodstream by promoting their utilization and oxidation in the liver [106]. In a study involving Wistar strain rats, it was observed that the administration of *C. vulgaris* led to a significant reduction in triglyceride levels, suggesting that the omega-3 content plays a crucial role in this process [106]. Additionally, the PUFAs found in *C. vulgaris* may help improve the lipid profile by enhancing the activity of lipoprotein lipase, an enzyme that facilitates the breakdown of triglycerides [106].

Another important mechanism is the fiber content of *C. vulgaris*, which can decrease intestinal fat absorption. The dietary fiber present in *C. vulgaris* binds to dietary fats in the gastrointestinal tract, thereby reducing their absorption into the bloodstream. This effect can lead to lower overall lipid levels and improved metabolic health [17]. Furthermore, the presence of bioactive compounds, such as peptides and polysaccharides, may also contribute to the modulation of lipid metabolism by influencing gut microbiota composition and activity, which in turn can affect lipid absorption and metabolism [17]. *C. vulgaris* has also been shown to exert anti-inflammatory effects, which can further aid in lipid regulation. Chronic inflammation is often associated with dyslipidemia and metabolic syndrome, and the anti-inflammatory properties of *C. vulgaris* may help mitigate these conditions. By reducing inflammation, *C. vulgaris* can improve insulin sensitivity and promote better lipid metabolism, leading to lower lipid levels in the body [17]. Moreover, the antioxidant properties of *C. vulgaris* may play a role in its lipid-lowering effects. Antioxidants help reduce oxidative stress, which is linked to lipid peroxidation and the development of atherosclerosis. By neutralizing free radicals, *C. vulgaris* can protect lipids from oxidative damage, thereby maintaining a healthier lipid profile.

### 3.8. Neuroprotective Effects

The neuroprotective effects of *C. vulgaris* are largely due to its rich content of bioactive compounds, including carotenoids, polyphenols, PUFA, and polysaccharides. Carotenoids like lutein, along with polyphenols, enhance the body's antioxidant defense mechanisms, protecting neurons from oxidative stress and reducing the risk of neurodegenerative diseases [107]. These compounds also have strong anti-inflammatory effects, which further shield neurons from damage. Polyunsaturated fatty acids, especially omega-3s, contribute to neuron protection by maintaining cell membrane integrity and reducing inflammation.

Polysaccharides, such as beta-glucans, play a role in modulating the immune response and lowering neuroinflammation, while *Chlorella* growth factor supports neuronal repair and regeneration [9]. Tryptophan, an amino acid found in *C. vulgaris*, boosts serotonin production, improving mood and cognitive function. According to Panahi et al. [108], *C. vulgaris* extract, an antioxidant-rich algal product, was found to have beneficial effects when used as an adjunctive therapy for patients with depression. In a 6-week exploratory

trial, *C. vulgaris* extract supplementation significantly improved anxiety, as well as physical and cognitive symptoms of depression. Clinical reports also show increased serum concentrations of antioxidants following short-term supplementation with extracts from this microalga [64,99,108]. The reduction of oxidative stress could be regarded as a plausible mechanism contributing to its observed antidepressant properties. Depression is often accompanied by a depletion in total antioxidant status and deregulated activity of antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase. *C. vulgaris* extract contains a complex mixture of antioxidants, including chlorophyll,  $\beta$ -carotene,  $\alpha$ -carotene, ascorbic acid,  $\alpha$ -tocopherol, lutein, lycopene, and zeaxanthin. Additionally, it provides trace elements such as zinc, copper, and magnesium, which are crucial for the activity of antioxidant metalloenzymes.

The synergistic effect of these bioactive compounds, combined with essential vitamins and minerals like B vitamins, magnesium, and zinc, make *C. vulgaris* a potent neuroprotective agent, offering antioxidant, anti-inflammatory, and regenerative benefits for brain health [9,19,107,109,110].

### 3.9. Detoxification and Heavy Metal Chelation

*C. vulgaris* has garnered considerable attention for its ability to bind and eliminate heavy metals and toxins from the body, demonstrating strong detoxification and chelation capabilities. This detoxification potential is attributed primarily to its cell wall structure, which is rich in polysaccharides, proteins, and glycoproteins that can bind to heavy metals and other toxins. By doing so, *C. vulgaris* can effectively reduce the bioavailability of harmful substances, promoting their excretion and mitigating their toxic effects.

Studies have shown that *Chlorella* can facilitate the removal of heavy metals such as mercury, lead, cadmium, and arsenic [111–114]. For instance, research has demonstrated that *C. vulgaris* supplementation significantly reduced mercury levels in animal models exposed to mercury contamination, thereby lowering the accumulation of this toxic metal in tissues such as the liver, kidneys, and brain [113,115]. These findings suggest that *C. vulgaris* can serve as a protective agent in individuals exposed to environmental mercury, such as those who consume large amounts of fish or live near industrial areas.

Similarly, *C. vulgaris* has been studied for its effectiveness in reducing lead accumulation. A study by Queiroz et al. [116] found that *C. vulgaris* supplementation decreased lead levels in the blood and tissues of lead-exposed rats while mitigating oxidative stress caused by lead toxicity. These results indicate the potential of this microalgae in preventing lead poisoning, which remains a significant public health concern in many parts of the world.

Cadmium is another heavy metal that poses severe health risks, including kidney damage and bone demineralization. *C. vulgaris* has demonstrated the ability to chelate cadmium, reducing its absorption and promoting its excretion. A study by Farag et al. [117] found that cadmium-exposed rats treated with *C. vulgaris* showed lower cadmium concentrations in their tissues and reduced oxidative stress markers. This chelating ability highlights *C. vulgaris* as a potential intervention for populations exposed to cadmium through industrial pollutants or contaminated food and water.

In addition to heavy metals, *C. vulgaris* has been shown to aid in the detoxification of organic pollutants, including polychlorinated biphenyls (PCBs), dioxins, and pesticides. The complex structure of *Chlorella's* cell walls allows for the adsorption and elimination of these harmful compounds from the body. Studies have shown that *C. vulgaris* supplementation decreased levels of dioxins and PCBs in the blood of individuals exposed to these environmental toxins, highlighting its potential role in reducing the burden of persistent organic pollutants [118,119].

Table 1 summarizes key chemical compounds and their bioactivities of *C. vulgaris*, showcasing this microalga's various benefits.

**Table 1.** *Chlorella vulgaris*'s major chemical compounds, associated bioactivities, and utilization.

Compound(s)	Use	Bioactivity	Description	Reference(s)
Carotenoids (lutein, beta-carotene)	Food, Feed, Medicine	Antioxidant, Anti-inflammatory, Anticancer, Neuroprotective, Color enhancer	Neutralizes reactive oxygen species and protects cells from oxidative damage, particularly in the skin and eyes. Enhances the coloration of skin and egg yolks in poultry and other animals	[44,54,59–61,120]
Chlorophyll	Food, Medicine	Antioxidant, Anticancer, Detoxification, Immune function	Scavenges free radicals, reduces oxidative stress, protects against chronic diseases, and aids in detoxifying carcinogens.	[4,13,25,39,120]
Fatty Acids	Food, Medicine	Antimicrobial, Anti-inflammatory, Lipid-lowering effect	Inhibits bacterial and fungal growth, reduces inflammation, and lowers cholesterol and triglyceride levels.	[31,68,85,103,104]
Proteins	Food, Feed, Medicine	Cell growth and repair, Immunomodulatory, Anticancer	Stimulates immune responses, including cytokine production (IFN- $\gamma$ , IL-2), and enhances the activity of macrophages, NK cells, and T cells.	[20,65,67,73]
Glycoproteins	Medicine	Detoxification	Binds and eliminates heavy metals (e.g., mercury, lead, cadmium) from the body and mitigates toxic effects of pollutants.	[111,112,116,117,121]
Peptides	Food, Feed, Medicine	Antimicrobial, Cell growth and repair,	Inhibits the growth of various bacterial pathogens, including <i>Staphylococcus aureus</i> and <i>E. coli</i> .	[84]
Nucleotides	Medicine	Immunomodulatory, Antioxidant, Anti-cancer Cell repair,	Cellular metabolism improves immune function and cell repair, and it contributes to DNA and RNA synthesis and participates in energy transfer. Improves growth performance and disease resistance. Anti-cancer cell's proliferative effect.	[55–58]
Polysaccharides	Food, Feed, Medicine	Antioxidant, Anti-inflammatory, Immunomodulatory, Antidiabetic, Antiviral, Antimicrobial, Detoxification	Enhances antioxidant defenses, suppresses pro-inflammatory cytokines, promotes immune cell activation, and regulates blood glucose levels.	[20,65–67]
Dietary Fibres	Food, Feed, Medicine	Digestive health, Prebiotic effect, Cholesterol-lowering effect, Weight management	Promotes regular bowel movements, stimulates beneficial gut bacteria, binds with cholesterol, and provides a sense of satiety.	[3,7,17,45]
Secondary Metabolites (e.g., polyphenols, phytosterols)	Medicine	Antioxidant, Anti-inflammatory, Anticancer, Cholesterol-lowering effects	Protects against oxidative damage, suppresses inflammation, reduces cholesterol absorption in the intestines, and promotes apoptosis in cancer cells.	[18,22,31]
Sulphated Polysaccharides	Medicine	Antiviral	Prevents viral entry into host cells, effective against enveloped viruses such as HSV, hepatitis C, and HIV.	[41–43]

#### 4. Applications of *Chlorella vulgaris* in Food, Feed, and Medicine

With its high protein content, essential fatty acids, vitamins, minerals, and bioactive compounds [122,123], *C. vulgaris* is increasingly utilized as a functional ingredient in various industries. This section will explore its prominent roles and potential uses across these fields, highlighting its contributions to both human and animal health.

##### 4.1. Applications in Food

*C. vulgaris* has gained significant popularity in the food industry, primarily as a dietary supplement and functional food ingredient due to its impressive nutrient density and

bioactive properties. With protein content ranging from 50–60% of its dry weight, *C. vulgaris* provides an excellent plant-based protein source, which is especially valuable in vegetarian and vegan diets as a sustainable alternative to animal-based proteins. It offers a complete protein profile, including all essential amino acids, making it comparable in quality to conventional protein sources like soy and meat [4].

Due to its versatile form, *C. vulgaris* is commonly available as powder and tablets, which are easily incorporated into a variety of food products such as smoothies, protein bars, health drinks, and snacks. These products cater to health-conscious consumers seeking nutrient-rich ingredients with additional health benefits [34,120]. Furthermore, because of its high chlorophyll content, *C. vulgaris* is used as a natural food colorant, providing food products with a vibrant green color that is appealing in the health food market. Chlorophyll also contributes antioxidant benefits, adding another layer of value to *Chlorella*-infused foods [25].

Beyond its use as a protein supplement, *C. vulgaris* has a broad spectrum of nutritional and health benefits. Its rich composition of essential amino acids, vitamins (particularly B-complex vitamins and beta-carotene), and minerals (such as iron, magnesium, and calcium) supports nutritional fortification. For individuals suffering from nutrient deficiencies, particularly in regions where malnutrition is prevalent, *Chlorella* supplementation can enhance dietary intake and improve overall health [102]. Its high content of bioactive compounds, such as polysaccharides and polyunsaturated fatty acids, contributes to its potential health benefits, including the enhancement of dietary intake and overall health improvement [3]. Studies show that food products enriched with *Chlorella* can help lower cholesterol levels and improve blood sugar management, making it a valuable component in diets aimed at preventing cardiovascular diseases and managing conditions like diabetes [18]. For instance, a study found that daily consumption of *Chlorella* led to reductions in total cholesterol and low-density lipoprotein cholesterol (LDL-C) in mildly hypercholesterolemic adults [104]. Similarly, Fallah et al. [124] reported that *Chlorella* intake resulted in decreased cholesterol levels in patients with hypercholesterolemia, highlighting its antilipidemic properties [125]. The mechanisms behind these effects may involve the inhibition of intestinal cholesterol absorption and the enhancement of fecal steroid excretion, as suggested by several studies [65,73,126]. Other studies indicate that the consumption of *Chlorella* can positively influence glucose metabolism and insulin sensitivity. For example, research by Sun et al. [96] evaluated the effects of *Chlorella pyrenoidosa* on diabetes management, suggesting its potential as a functional food for glycemic control. Furthermore, the presence of dietary fiber and bioactive compounds in *C. vulgaris* may contribute to its anti-diabetic properties, as these components are known to modulate glucose absorption and metabolism [127].

In addition to its nutritional benefits, *C. vulgaris*'s antioxidant, antimicrobial, and detoxifying properties offer further advantages when incorporated into food products. The antioxidants present in this microalga, including chlorophyll, carotenoids, and polyphenols, help neutralize free radicals and reduce oxidative stress in the body, which can lead to chronic diseases such as cancer and heart disease. These antioxidants also help preserve the food itself, extending its shelf life by preventing the oxidation of fats and oils, making *Chlorella* an attractive natural preservative in processed foods [34].

Moreover, the antimicrobial properties of *C. vulgaris* are beneficial in food preservation. The bioactive compounds in *C. vulgaris*, such as peptides and fatty acids, have been shown to inhibit the growth of harmful microorganisms, including bacteria and fungi, which contribute to food spoilage. By incorporating *C. vulgaris* into food products, manufacturers can potentially reduce the reliance on synthetic preservatives, offering consumers more natural and cleaner-label products [21].

With increasing consumer demand for sustainable, plant-based, and functional food options, *C. vulgaris* is positioned as a versatile and highly beneficial ingredient in the food industry. Its comprehensive nutritional profile, combined with its health-promoting properties, makes it a key player in the development of innovative food products that not only

support health but also cater to environmental and ethical considerations. Table 2 summarizes *C. vulgaris*'s nutritional compounds for food and associated effects.

**Table 2.** Nutritional compounds in *Chlorella vulgaris* for food and their associated benefits.

Compound	Benefit	Reference(s)
Protein	Excellent plant-based protein source for dietary supplementation	[4]
Amino Acids	Complete protein profile, supports muscle and tissue health	[4]
Polyunsaturated Fatty Acids	Contribute to heart health and anti-inflammatory effects	[3]
Polysaccharides	Immunomodulatory properties, antioxidant benefits, support gut health	[3,25]
Vitamins (B-complex, beta-carotene)	Nutritional fortification supports energy metabolism, promotes eye health	[102]
Minerals (iron, magnesium, calcium)	Enhances dietary intake, supports bone health and metabolic processes	[102]
Chlorophyll	Antioxidant properties, acts as a natural food colorant, detoxifies carcinogens	[25]
Carotenoids (lutein, beta-carotene)	Antioxidant benefits support skin and eye health, enhance cognitive function	[44,59]
Dietary Fiber	Modulates glucose absorption, improves insulin sensitivity, enhances satiety	[3,7,127]
Antioxidants (chlorophyll, carotenoids, polyphenols)	Neutralizes free radicals, reduces oxidative stress, extends food shelf life	[34]
Antimicrobial Compounds (peptides, fatty acids)	Inhibits growth of harmful microorganisms, reduces reliance on synthetic preservatives	[21]
Bioactive Compounds	Cholesterol management, improves glucose metabolism, potential anti-diabetic properties	[96,124]

#### 4.2. Applications in Feed

*C. vulgaris* has garnered significant attention as a valuable ingredient in animal feed due to its high protein content, essential fatty acids, and other bioactive compounds [123]. It is incorporated into livestock, poultry, aquaculture, and pet feeds, offering benefits such as enhanced growth, improved immune function, and better resistance to diseases [34,128].

In livestock and poultry feed, *C. vulgaris* is typically incorporated as a protein-rich supplement, providing all essential amino acids needed for animal growth and development [129,130]. The most common inclusion level may range from 0.1% to 5%, depending on the species and production goals, with formulations often in powdered or pelletized forms to ensure easy mixing with traditional feed ingredients like corn and soybean meal. Studies have shown that supplementation with *C. vulgaris* in diets for chickens, pigs, and cattle improves weight gain, feed-conversion efficiency, and overall growth performance [31,128,131]. Moreover, it has been observed to enhance the nutritional quality of animal products by increasing beneficial fatty acid content in meat and eggs [132,133].

In aquaculture, *C. vulgaris* is used as a powdered or microencapsulated feed additive, particularly for species such as tilapia, carp, and salmon [131]. These formulations are usually added in combination with fish meal or plant-based feeds, and microalgae can be utilized directly as feed for the cultured organisms or indirectly as feed for other live-feed organisms, such as rotifers and *Artemia* [134]. The direct use of *C. vulgaris* as a feed for fish is primarily limited to herbivorous plankton feeders, such as cyprinids and cichlids. However, the indirect use of *C. vulgaris* as feed for live-feed organisms is more widespread, particularly in the culture of marine finfish larvae, which rely heavily on live feed during their early stages of development.

The inclusion of *C. vulgaris* in aquaculture feeds typically ranges from 5% to 20% of the total diet, depending on the target species and production objectives [20,36]. At these levels, *C. vulgaris* has been shown to significantly enhance growth rates, feed efficiency, and pigmentation in fish [135]. The carotenoids present in *C. vulgaris* improve skin and flesh pigmentation, which is a key factor in enhancing the aesthetic quality of fish—an impor-

tant consideration in commercial aquaculture [4]. Additionally, its immunomodulatory properties have been shown to boost immune function in fish and shrimp, which helps reduce the need for antibiotics and supports sustainable aquaculture practices [135]. The proper formulation, particularly regarding pellet size and stability in water, is crucial to ensure optimal absorption and minimize feed waste.

In pet food, *C. vulgaris* is commonly formulated as a powdered supplement added to both dry and wet feeds, typically at concentrations under 3%. It is used to promote overall health and well-being in companion animals by supporting immune function, digestion, and vitality [136].

Its nutrient-dense composition supports immune function and digestion in dogs, while its antioxidant properties help reduce inflammation and oxidative stress, particularly in ageing pets and those with chronic health conditions [123,136]. While lower doses do not typically impact the palatability of pet foods, higher concentrations—above 1.5%—may alter sensory traits, such as taste and texture, making them less appealing to animals [137]. Therefore, careful attention to formulation is required to balance health benefits with palatability.

#### 4.3. Applications in Medicine

Beyond its nutritional benefits, *C. vulgaris* exhibits significant potential for therapeutic applications, spanning from immune modulation to cancer prevention [9]. Its bioactive compounds, including chlorophyll, carotenoids, and polysaccharides, have been extensively studied for their anti-inflammatory, antioxidant, antiviral, and anticancer properties [4,123].

In particular, the anti-inflammatory properties of *C. vulgaris* are crucial, especially considering that inflammation is a key factor in developing chronic diseases like arthritis and cardiovascular diseases. Persistent inflammation can lead to tissue damage and exacerbate disease progression. *C. vulgaris* offers a potent anti-inflammatory solution by inhibiting pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6, and downregulating COX-2 activity. Additionally, it modulates the NF- $\kappa$ B pathway, increasing the production of anti-inflammatory cytokines like IL-10, which aids in tissue repair and reduces inflammation. This comprehensive approach may not only alleviate symptoms but also target the root causes of inflammation, positioning *C. vulgaris* as a promising candidate for integrative treatment strategies in managing chronic conditions. Another notable medicinal application of *C. vulgaris* is its ability to support immune health. Studies suggest that regular consumption can enhance innate and adaptive immunity by stimulating the activity of NK cells, macrophages, and T cells. This makes it valuable in preventing infections and supporting immune function, particularly in immunocompromised individuals or those facing chronic illnesses [21].

Additionally, *C. vulgaris* has demonstrated notable antiviral activity, particularly against enveloped viruses such as herpes simplex virus (HSV), hepatitis C virus, and HIV. Research indicates that sulphated polysaccharides can effectively prevent viral adsorption and replication, thereby blocking the initial stages of viral infection [41,42]. This mechanism is particularly relevant for enveloped viruses, which rely on specific interactions with host cell receptors for entry. The sulphating of polysaccharides enhances their ability to mimic heparan sulphate, a natural component on cell surfaces that many viruses exploit for attachment [41]. Antiviral activity is often correlated with the degree of sulphating, indicating that modifications to the polysaccharide structure can enhance their efficacy against these viruses [43].

Sulphated polysaccharides isolated from *Chlorella* have been shown to inhibit viral replication by preventing viruses from penetrating host cells. This antiviral activity highlights *Chlorella* as a promising candidate for the development of antiviral therapies, especially in an era where emerging viral threats pose global health challenges [4].

In terms of anticancer potential, *C. vulgaris* compounds like carotenoids and chlorophyll play a key role. These compounds have been shown to induce apoptosis (programmed cell death) in cancer cells and inhibit tumor proliferation. *Chlorella's* ability to reduce ox-

oxidative stress, inhibit angiogenesis (the formation of new blood vessels that feed tumors), and activate pro-apoptotic pathways positions it as a potential adjunct therapy in cancer treatment. Research has shown that these compounds can reduce DNA damage, inhibit tumor growth, and enhance the body's natural defenses against cancer [18].

Furthermore, *C. vulgaris* has demonstrated significant benefits in managing chronic diseases such as cardiovascular conditions and type 2 diabetes [17]. Its lipid-lowering effects help reduce total cholesterol, LDL cholesterol, and triglycerides while simultaneously increasing HDL cholesterol. These effects, combined with its anti-inflammatory properties, contribute to the prevention of atherosclerosis and promote heart health [3,86]. Additionally, *C. vulgaris* supplementation has been associated with improved glycemic control in individuals with type 2 diabetes. Studies show reductions in fasting blood glucose and HbA1c levels, highlighting its potential as an adjunct in diabetes management [3,120]. Table 3 presents the medicinal applications of *C. vulgaris* compounds.

**Table 3.** Medicinal applications of compounds in *Chlorella vulgaris* and associated health effects.

Compound	Benefit	Reference(s)
Chlorophyll	Antioxidant, reduces oxidative stress Neuroprotective effects	[4]
Carotenoids (lutein, beta-carotene)	Induces apoptosis in cancer cells Neuroprotective effects	[18]
Sulphated Polysaccharides	Antiviral activity against enveloped viruses	[41,42]
Polysaccharides	Enhances immune function, boosts NK cell activity Anti-inflammatory properties Inhibits viral adsorption and replication Potential adjunct in cancer treatment Improves glycemic control in diabetes Blocks tumor proliferation Neuroprotective effects	[3,4,18,43,120,123]
Lipids	Reduces total cholesterol, LDL, and triglycerides	[17]
Bioactive compounds	Prevents infections and supports immune function	[21]
Dietary Fiber	Lower lipid absorption Modulates cholesterol levels Enhances Gastrointestinal Motility	[3,7,40]

## 5. Potential Side Effects

While *C. vulgaris* is recognized for its nutritional benefits, it also presents potential side effects and toxicological concerns, particularly regarding heavy metal accumulation. Metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), copper (Cu), and zinc (Zn) are particularly concerning due to their toxicity and ability to bioaccumulate in organisms, including microalgae.

Research indicates that *C. vulgaris* absorbs heavy metals from its environment, which raises apprehensions about its safety as a dietary supplement. For instance, exposure to copper and zinc can negatively impact metabolic processes, leading to decreased photosynthetic pigment content and overall cellular health [138]. Studies have shown that *C. vulgaris* can accumulate significant levels of cadmium and lead, potentially exceeding safe consumption limits when sourced from contaminated waters [139]. Additionally, it

has been demonstrated that cadmium accumulation occurs in a dose-dependent manner; concentrations as low as 10  $\mu\text{M}$  adversely affect growth, while levels of 100  $\mu\text{M}$  can cause significant toxicity and cell death [140]. Lead accumulation has also been documented, with concentrations of 10  $\mu\text{M}$  resulting in observable physiological stress in algal cells [138].

To protect consumer health, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set guidelines for acceptable levels of these metals in food products. The maximum allowable limits for cadmium and lead are 0.1 mg/kg and 0.2 mg/kg, respectively, while mercury is regulated at a maximum level of 0.5 mg/kg in fish and seafood [141].

To mitigate these risks, producers of *C. vulgaris* supplements need to implement rigorous quality controls, including regular monitoring of water quality, heavy metal testing, and adherence to cultivation protocols such as good agricultural practices (GAP) and ISO certifications. These measures help ensure that *C. vulgaris* products are safe for consumer use and free from harmful contaminants.

Studies have shown that pollutants, including pesticides like cypermethrin, can inhibit the growth of *C. vulgaris* at relatively low concentrations, suggesting that environmental contaminants may compromise its safety as a food supplement [142]. Producers employ controlled environments, such as closed bioreactors, to reduce exposure to pollutants, while third-party certifications like organic or non-GMO labels offer additional consumer assurance. Furthermore, the presence of ROS and oxidative stress induced by various nanoparticles has been documented to negatively impact the growth and metabolic functions of *C. vulgaris*, highlighting the need for careful monitoring of environmental conditions during cultivation [143,144].

Another aspect to consider is the antinutritional factors present in *C. vulgaris*. While it is rich in essential nutrients, some studies suggest that certain compounds may interfere with nutrient absorption or metabolism. For instance, the presence of phytates and oxalates in microalgae can bind minerals, potentially reducing their bioavailability. Moreover, the lipid profile of *C. vulgaris*, while beneficial in many respects, can also lead to imbalances in fatty acid consumption if not properly managed, particularly in populations with specific dietary needs [106]. Toxicological assessments of *C. vulgaris* have revealed that its consumption can lead to adverse health effects under certain conditions. For example, high doses of *C. vulgaris* have been associated with hepatotoxicity and nephrotoxicity in animal models, particularly when combined with other substances like paracetamol [145]. For example, in a study where Wistar rats were administered high doses of *C. vulgaris*, significant alterations in biochemical parameters indicative of liver and kidney function were observed, including elevated levels of serum creatinine and urea [146]. The concept of a “high dose” can vary depending on the context of consumption and the specific health outcomes being assessed. In animal studies, doses of *C. vulgaris* have been administered in a range of 250 mg/kg to 2500 mg/kg body weight. For instance, a study indicated that a dose of 2500 mg/kg body weight led to increased levels of creatinine and urea, markers of kidney function, suggesting potential nephrotoxic effects [146]. Conversely, lower doses, such as 250 mg/kg, have been associated with protective effects against liver and kidney damage induced by toxic agents [145]. The safe range for consumption of *C. vulgaris* in humans is less clearly defined, but dietary supplements typically recommend dosages ranging from 3-to-10 g per day. This range is generally considered safe and is supported by various studies that have not reported significant adverse effects at these levels [106,147].

Beyond paracetamol, there are concerns that *C. vulgaris* might interact with other commonly used medications or supplements, such as immunosuppressants, anticoagulants, or cholesterol-lowering drugs. These interactions could potentially affect liver or kidney function, though more research is needed to fully understand these effects. Consumers should consult healthcare professionals before combining *C. vulgaris* with medications, especially in high doses or over long periods. This suggests that while *C. vulgaris* may offer protective effects against some toxins, it can also exacerbate toxicity under specific

circumstances, necessitating further research into safe consumption levels and potential interactions with other dietary components [145].

## 6. Future Perspectives and Research Directions

As research on *C. vulgaris* advances, its applications in food, medicine, and animal feed are expected to grow significantly [146]. Despite the existing knowledge of its bioactive compounds and nutritional value, many areas remain underexplored, and future research will be essential to fully realize the potential of this microalga in various industries.

One of the key areas for future development lies in optimizing the cultivation and production of *C. vulgaris* to meet the increasing global demand. Current methods face substantial challenges, as they are often resource-intensive, sensitive to specific environmental conditions, and costly, which restricts large-scale production. Advances in biotechnological processes, such as photobioreactor design, automation, and genetic engineering, could dramatically improve *C. vulgaris* yields while reducing costs and environmental impact [31,34]. However, specific challenges remain, such as increasing light utilization efficiency in photobioreactors and improving nutrient-delivery systems during cultivation. Future research should focus on addressing these bottlenecks by developing innovative reactor designs and nutrient formulations tailored to different strains of *C. vulgaris*. Optimizing growth conditions such as nutrient availability, light intensity, and temperature could also enhance the concentrations of valuable bioactive compounds like chlorophyll, carotenoids, and polysaccharides. Additionally, research should focus on overcoming the technical challenge of scaling up production while maintaining the consistency of bioactive compound yields, including developing cost-effective and sustainable nutrient sources for large-scale cultivation. Integrating *C. vulgaris* production with waste recycling and renewable energy sources offers a promising pathway for making production more sustainable and less resource-intensive [148]. While *C. vulgaris* is already established as a dietary supplement and functional food ingredient, there remains significant potential to expand its applications in these sectors. Future research should investigate the development of innovative food products that incorporate *C. vulgaris* not only for its nutritional benefits but also for its therapeutic properties. In particular, researchers could explore novel encapsulation techniques to improve the stability and bioavailability of its key bioactive compounds during food processing and digestion. For instance, functional foods targeting specific health outcomes, such as immune support, cardiovascular health, or diabetes management, could be enhanced by incorporating *C. vulgaris* [4]. Additionally, research should address how to optimize the extraction efficiency of bioactive compounds from *C. vulgaris*, as well as develop new methods for enhancing their bioavailability in humans. Techniques such as nanoencapsulation and emulsification are promising areas to explore in this regard. As an ingredient in animal feed, future research is focusing on optimizing the inclusion rates and identifying the best formulation approaches for each species.

The therapeutic potential of *C. vulgaris* has been demonstrated in numerous in vitro and in vivo studies, particularly regarding its antioxidant, anti-inflammatory, and immunomodulatory effects [127,149,150]. However, clinical evidence supporting these effects remains limited, as most studies to date lack large-scale human trials. Key research questions include determining optimal dosing regimens for clinical efficacy, understanding the long-term effects of supplementation, and elucidating the molecular mechanisms underlying its bioactivities, including the specific signaling pathways affected by *C. vulgaris* compounds. Future research should prioritize randomized controlled trials (RCTs) to better assess the clinical efficacy and safety of *C. vulgaris* in treating chronic diseases such as cancer, cardiovascular diseases, diabetes, and immune disorders. Additionally, research should explore strategies to enhance the reproducibility and reliability of clinical data since current findings are often inconsistent. While *C. vulgaris* offers a wide range of health benefits and applications, its consumption is not without risks. Concerns regarding the potential for heavy metal accumulation, the presence of antinutritional factors, and the risk of toxicological interactions with other products [138] highlight the need for comprehensive

safety evaluations. Research should also focus on developing purification techniques to minimize heavy metal contamination during production and assessing the long-term safety of *C. vulgaris* consumption at different dosages.

As research on *C. vulgaris*'s bioactive compounds progresses, there is growing interest in exploring its use in novel medical applications. For example, *C. vulgaris*-derived compounds could be investigated as potential drug candidates for antiviral therapies, particularly against emerging viral threats [4]. Specific studies could focus on identifying the exact antiviral mechanisms of *C. vulgaris* compounds, such as whether they block viral entry or replication. Its anti-inflammatory properties also hold promise for treating chronic inflammatory conditions, such as arthritis and inflammatory bowel disease. Future research should explore the specific pathways involved in its anti-inflammatory effects and how these can be harnessed for therapeutic development. Additionally, due to rising concerns about antibiotic resistance, investigating the antimicrobial properties of *C. vulgaris* could reveal promising natural alternatives to conventional antibiotics, which are beneficial for both human and veterinary medicine. In light of global challenges such as climate change, food insecurity, and malnutrition, *C. vulgaris* offers a promising solution as a sustainable, nutrient-dense food source. Future research should focus on its potential role in addressing these issues, particularly in developing regions where malnutrition is prevalent [34]. Due to its ability to thrive in diverse environments, *C. vulgaris* can be integrated into local agricultural systems to enhance food security. Additionally, its potential for use in bio-fuel production, water purification, and carbon capture offers exciting opportunities for contributing to sustainability and environmental preservation [86,150,151].

Recent advances in genetic and metabolic engineering present exciting opportunities to enhance the bioactive properties of *C. vulgaris*. By identifying and manipulating key genes involved in the biosynthesis of compounds such as carotenoids, polysaccharides, and fatty acids, researchers can develop strains of *C. vulgaris* with higher yields of specific nutrients or therapeutic compounds [4]. Future research should focus on overcoming technical challenges in the metabolic engineering of *C. vulgaris*, such as improving gene-editing tools to target specific metabolic pathways without affecting the overall cell viability. While promising, genetic engineering of *C. vulgaris* still faces significant barriers, particularly in ensuring stable modifications that do not compromise cell health or yield. Moreover, genetic engineering can enable *C. vulgaris* to be used as a biofactory for producing pharmaceutical compounds, enzymes, and other high-value products, further expanding its industrial and therapeutic applications [31].

## 7. Conclusions

This review highlights the exceptional chemical composition and diverse bioactivities of *C. vulgaris*, emphasizing its immense potential across the fields of food, medicine, and animal feed. The microalga's rich protein content, abundance of essential vitamins, minerals, and fatty acids, and its wide array of bioactive compounds, particularly chlorophyll, carotenoids, and polysaccharides, make it a promising candidate for both nutritional supplementation and therapeutic use.

The well-documented antioxidant, anti-inflammatory, immunomodulatory, antiviral, anticancer, antidiabetic, lipid-lowering, and detoxifying properties of *C. vulgaris* position it as a valuable agent for the prevention and treatment of various chronic diseases, including cancer, cardiovascular conditions, diabetes, and immune disorders. Additionally, its capacity to enhance immune function, combat oxidative stress, and promote detoxification further reinforces its status as a versatile functional food and therapeutic compound.

Beyond its applications in human health, *C. vulgaris* has demonstrated significant potential in animal nutrition, contributing to enhanced growth performance, improved immune responses, and increased disease resistance in livestock, poultry, and aquaculture species. This versatility underscores its importance in promoting sustainable animal nutrition and improving the overall health and resilience of animal populations.

However, challenges remain in optimizing *C. vulgaris* production at a large scale, as current cultivation methods are often resource-intensive and rely on specific environmental conditions, which can limit its accessibility and affordability. Addressing these production barriers is essential to fully realize the global potential of *C. vulgaris* in various sectors. Additionally, while numerous preclinical studies support its therapeutic properties, clinical evidence remains limited, with few large-scale human trials confirming the medicinal efficacy of *C. vulgaris* for chronic disease prevention or treatment. Further research is essential to optimize cultivation methods, improve bioavailability, and validate the therapeutic efficacy of *C. vulgaris* through rigorous clinical trials. Future studies should also investigate innovative applications in biotechnology and sustainability, solidifying *C. vulgaris* as a critical player in addressing global health, food security, and environmental sustainability challenges.

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