

A Review on Antioxidant Properties of *Spirulina*

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Abstract

Spirulina is free-floating filamentous microalgae growing in alkaline water bodies. As early as over 400 years ago, *Spirulina* was eaten as food by the Mayas, Toltec's and Kanembu in Mexico during the Aztec civilization. *Spirulina* is a well-known source of valuable food supplements, such as proteins, vitamins, amino acids, minerals, fatty acids, etc. It is widely used in human and animal nutrition, as well as in the cosmetic industry. Both in vivo and in vitro trials have shown effective and promising results in the treatment of certain cancers and allergies, anemia, hepatotoxicity, viral infection, vascular diseases, radiation protection, and obesity. The antioxidant activities of *Spirulina* were demonstrated in a large number of preclinical studies. Antioxidants in preventing many human diseases. Findings of this study showed *Spirulina* can be used as a source of antioxidants.

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Introduction

Cancer is now a major cause of mortality throughout the World [1]. In the developed world, it is generally exceeded only by cardiovascular disease but developing countries are responsible for the globally increasing trend. Over 10 million new cases and over 7 million deaths from cancer occurred worldwide in 2000. The contribution of developing countries was 53% for incidence and 56% for deaths. From 1990 to 2000, the incidence and deaths increased by 2.4% per annum by 2020, it is predicted that these diseases will be causing seven out of every 10 deaths in developing countries (Fig. 1) [2-5].

Cancer Worldwide	Males	Females	Persons	Year ²
Number of new per year	7,427,148	6,663,001	14,090,149	
Incidence rate per 100,000 population ¹	205.4	165.3	182.3	2012
Number of deaths per year	4,653,132	3,547,898	8,201,030	
Mortality rate per 100,000 population ¹	126.3	82.9	102.4	2012

1. World age-standardized 2. Latest estimates available

Figure 1. Static of cancer incidence and deaths in 2012.

Based on high costs cancer treatment strategies (Fig. 2) [6], in recent years antioxidants therapy has been developed to prevent cancer. Studies showed that free radicals

can lead to degenerative disease like cancer, aging, age related macular degeneration etc [7].

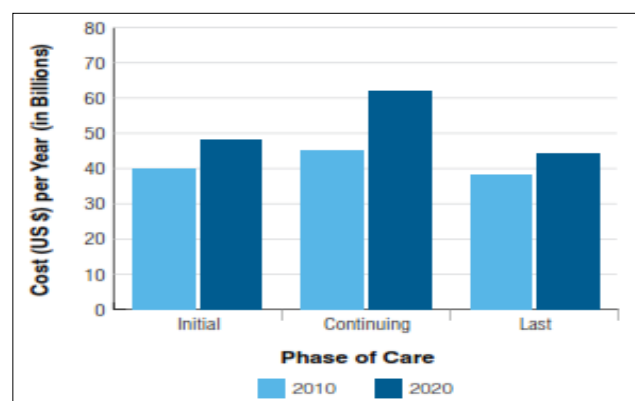


Figure 2. Cost of cancer care by phase of care, in 2010.

Antioxidants help to protect the body against free radicals; these are substances that neutralize free radicals or their actions. Antioxidants include carotenoids, flavonoids and related polyphenols, α -lipoic acid, glutathione etc. The main source of antioxidants for the body is vegetables and fruits. Unavailable of fruit and vegetables in many area of world, scientists rethought to provide antioxidants from other sources [8, 9]. Starting at middle 1980's, great efforts and extensive investigations have been turned to the development of nutraceuticals or functional food for preventing or managing various diseases [10-13]. The first



reports on antioxidants employed for food lipids were about using natural sources; in 1852, Wright reported that elm bark was effective in preserving butterfat and lard [8, 9, 13].

Spirulina was initially classified in the plant kingdom because of its richness in plant pigments as well as its ability of photosynthesis. It was later placed in the bacteria kingdom based on new understanding on its genetics, physiology and biochemical properties [14]. *Spirulina* naturally grows in high-salt alkaline water reservoirs in subtropical and tropical areas including America, Mexico, Asian and Central Africa [14, 15]. The nutritional value of *Spirulina* is well recognized with its unusual high protein content (60–70% by dry weight) and its richness in vitamins, minerals, essential fatty acids and other nutrients [15-17]. Recent studies suggest that *Spirulina*, a unicellular blue-green alga (Fig. 3), may have a variety of health benefits and therapeutic properties and is also capable of acting as an antioxidant and anti-inflammatory agent [18]. *Spirulina* is also used for health food, feed and for the biochemical products since 1980s. In fact, *Spirulina* is the most concentrated and nutritious whole food known to science. Moreover *Spirulina* has got no side effects and is non-toxic in nature [9, 14, 16].



Figure 3. Microscopic view of *Spirulina*.

Spirulina have well-documented protective effects against viral and bacterial infections, cancer, allergies, diabetes, inflammation, and hyper lipidemia in addition, due to its concentrated nutrition, *Spirulina* was recommended by both National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) as one of the primary foods during long-term space missions. *Spirulina* has been orally administered to patients as an anti-cancer and anti-viral agent although the molecular mechanism by which *Spirulina* acts on the immune system remains largely undefined. Recent study showed that when the *Spirulina* was enriched with Selenium and combined with the anticancer drugs, a significant decrease of the proliferation rate and an increase of apoptosis rate were observed [9, 13, 17, 19, 20]. *Spirulina* is a complete food resource of Chlorophyll, Phycocyanin, & Carotenoids. It is

also has an application as a natural dye in food industry, cosmetic and pharmaceutical industry [21].

Phytonutrients	
Beta-Carotene	6.8 mg
Zeaxanthin	9 mg
Chlorophyll	30 mg
Total Carotenoids	15 mg
C-Phycocyanin	240 mg
Total Phycocyanins	519 mg
Superoxide Dismutase	1080 units

Figure 4. Amount of pigment in one gram *Spirulina* powder.

Chlorophyll

Chlorophyll is an essential compound in many everyday products. It is used not only as an additive in pharmaceutical and cosmetic products but also as a natural food coloring agent. Additionally, it has antioxidant and antimutagenic properties (Fig. 5) [22, 23].

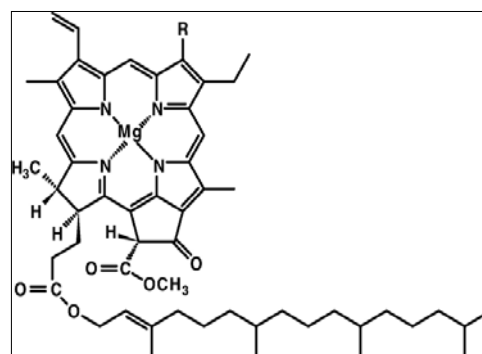


Figure 5. Structure of chlorophyll.

Chlorophyll has been found to accelerate wound healing by more than 25% in some studies. Since chlorophyll stimulates tissue growth; it prevents the advancement of bacteria and speeds up the wound healing process. Chlorophyll is similar in chemical structure to hemoglobin and, as such, is predicted to stimulate tissue growth in a similar fashion through the facilitation of a rapid carbon dioxide and oxygen interchange. Because of this property, chlorophyll is used not only in the treatment of ulcers and oral sepsis but also in proctology [23-25].

Chlorophyll derivatives such as pheophorbide *b* and pheophytin *b* have always been known as strong antioxidants. However, these derivatives exist in very low concentrations in fruits and vegetables. The most significant activity of chlorophyll derivatives in the prevention of cancer is the trapping of mutagens in the gastrointestinal tract. Furthermore, the ability of both natural and commercial chlorophyll derivatives to act as photosensitizers have enabled their utilization as effective agents in photodynamic-therapy of cancer. *in vitro* and *in vivo* consistent with the

prevention of cancer including antioxidant activity, anti-mutagenic activity, modulation of xenobiotic metabolizing enzymes, and induction of apoptotic events in cancer cell lines [23, 25].

Carotenoids

Spirulina extracts containing carotenes and various carotenoids (Fig. 6) are frequently used as natural coloring materials [19]. Carotenoids are vitally important antioxidants. Numerous studies have indicated that people whose diets contain a lot of foods rich in carotenoids lower their risk of developing various types of cancer [10].

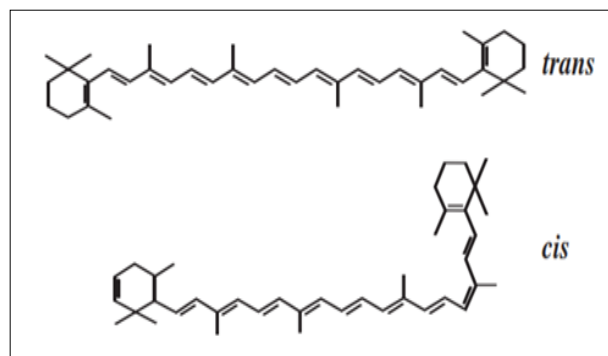


Figure 6. Molecular structure of nature's two forms of beta-carotene.

They possess antioxidant activity, especially in the presence of light. Carotenoids also have important metabolic functions in animals and man, including conversion to Vit A, enhancement of the immune response and protection against diseases such as cancer by way of scavenging free radicals, β -carotene, as a major carotenoid of *Spirulina* is a potential antioxidant having anti-carcinogenic and radio-protective effects. *Spirulina* contains up to 2,000 IU/g dry weight of β -carotene [26-28]. Eating foods rich in antioxidants such as carotenoids, phycocyanin, superoxide dismutase and vitamins C and E is another great way to help prevent cancer. [7, 29]. Numerous studies have indicated that people whose diets contain a lot of foods rich in carotenoids lower their risk of developing various types of cancer [30], most people get only 25-30% of the daily dietary carotenoid intake recommended in a cancer-preventive diet; and many people are unwilling to make radical dietary changes [19]. *Spirulina* is a whole food with its beta-carotene in a naturally chelated food matrix. And *Spirulina* is not only rich in beta-carotene, it contains other very important carotenoids like zeaxanthin and beta-cryptoxanthin as well as lesser known carotenoids such as myxoxanthophyll and echinenone. *Spirulina* extracts are capable in inhibiting carcinogenesis [31]. In one study found that *Spirulina* stabilized the liver disease and prevented the disease from progressing to cirrhosis. Another mouse study showed that *Spirulina* reduced both skin and stomach tumors significantly. *Spirulina* was shown to reduce both the size of the tumors and to reduce the incidence of tumors. They concluded that diets containing carotenoid-rich fruits and vegetables may be protective against prostate cancer [19]. Many earlier

studies have found similar relationships between carotenoid intake and other forms of cancer. Beta-carotene may also help to protect the skin against the damaging effects of sunlight and help to prevent skin cancers [26]. The results of the trial were surprising in that contrary to the hypothesis, the beta-carotene supplemented group had a small (statistically insignificant) increase in the incidence of lung cancer. Interestingly, the group in the study with the highest blood levels of beta carotene from dietary sources had the lowest incidence of lung cancer [26]. *Spirulina* is the richest beta carotene food known, having over ten times more beta carotene than any other food, including carrots. Beta carotene is one of the most effective substances for deactivating free radicals, which damage cells, leading to cancer. Free radicals are molecular fragments from environmental pollution, toxic chemicals, drugs, and physical and emotional stress [32]. Beta carotene prevents free radicals from reacting, and decreases incidence of lung cancer, prevents chemically induced tumors in animals, prevents precancerous pre-chromosome damage and enhances immunological resistance. Evidence linking natural beta carotene and cancer prevention is impressive [7, 26]. For those who do not eat 4-9 servings of fruits and vegetables daily, *Spirulina* will add natural carotene insurance [19]. According to the findings of the National Cancer Institute, United States of America, an intake of 6.0 mg β -carotene daily may be effective in minimizing the risk of cancer. If anybody takes 4.0 g *Spirulina* daily, that is sufficient to get 6 mg β -carotene [28].

Beta carotene was found to significantly inhibit the formation of squamous cell carcinoma of hamster buccal pouch when a solution was applied topically three times weekly for 22 weeks in an experiment with 40 hamsters. In a second experiment with 80 hamsters, beta carotene was found to inhibit oral carcinogenesis in an initiation-promotion hamster buccal pouch system. Beta carotene inhibited both initiation and promotion [33]. Studies among preschool children in India have demonstrated *Spirulina* fusiformis to be an effective source of dietary vitamin A. Supplementation with *Spirulina* did not increase serum concentrations of retinal or beta carotene, nor was it associated with toxicity [32, 34].

Another study showed that beta-carotene reduces the size of tumors that were already present in hamsters and slowed new tumor growth, extending the hamsters' survival time [10, 33]. Numerous studies have shown that people whose diets are high in beta-carotene have a lower incidence of various cancers. Smokers, who are especially vulnerable, should maintain their beta-carotene levels. Low beta-carotene levels in the blood of smokers have been connected with the later appearance of lung cancer. Researchers at Albert Einstein College of Medicine have shown that beta-carotene exerts a protective effect against the development and progression of cervical cancer. Beta-carotene may also help to protect the skin against the damaging effects of sunlight and help to prevent skin cancers. In the early 1980's a landmark study by the US National Science Foundation entitled Diet, Nutrition and cancer, concluded on the basis of epidemiological

evidence that diets rich in beta carotene were correlated with a reduction in the incidence of cancer. In fact, over 200 studies of dietary consumption of beta-carotene indicated a reduction of a range of cancers [19, 26].

Phycocyanin

Phycocyanin is one of the key ingredients that make *Spirulina* such a wonderful Super food, and a vital difference between *Spirulina* and other green foods like chlorella, wheat grass and barley [35]. The Japanese have found that phycocyanin protects the liver and the kidneys during detoxification, as well as activating the immune system. Researchers at the Osaka Medical Center for cancer and Cardiovascular Diseases said "*Spirulina* is surmised to potentiate the immune system leading to suppression of cancer development and viral infection [36]. *Spirulina* selectively inhibited development of some tumoral cell lines, showing potential anticancer activity against human stomach cancer cells (AGS), human liver cancer cells (Hep3B), human lung cancer cells (A549), and breast cancer cells (MCF-7) [16]. Phycocyanin is a powerful water soluble antioxidant [27, 37]. A great deal of research has been done in Japan on phycocyanin. The Japanese have found that phycocyanin protects the liver and the kidneys during detoxification, as well as activating the immune system. Researchers at the Osaka Medical Center for Cancer and Cardiovascular Diseases said "*Spirulina* is surmised to potentiate the immune system leading to suppression of cancer development and viral infection [19]. C-phycocyanin (C-PC) is one of the major Bili proteins of *Spirulina* with antioxidant and radical scavenging properties (Fig. 7) [38].

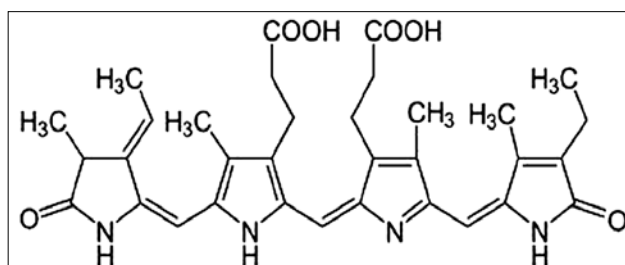


Figure 7. Chemical structure of C-phycocyanin.

C-PC, a selective cyclooxygenase-2 inhibitor, induces apoptosis in lipopolysaccharide-stimulated RAW 264.7 macrophages. It is also known to exhibit anti-inflammatory and anticancer properties. Phycocyanin has the ability to scavenge free radicals, including alkoxyl, hydroxyl and peroxy radicals. It also decreases nitrite production, suppresses inducible nitric oxide synthase (iNOS) expression, and inhibits liver microsomal lipid peroxidation. Phycocyanin of *Spirulina* inhibits the growth of human leukemia K562 cells [35].

Serum glutamate oxaloacetate and serum glutamate pyruvate transaminase activity along with increase in liver GSH level. The activities of antioxidants enzymes superoxide dismutase, catalase and Glutathione-S-transferase were also concomitantly restored to near

normal level by *Spirulina* supplementation to mercuric chloride intoxicated mice. The results clearly demonstrate that *Spirulina* treatment augments the antioxidants defense mechanism in mercuric chloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases [39, 40]. phycocyanin is a potent peroxy radical scavenger with an IC(50) of 5.0 μ M and the rate constant ratios obtained for phycocyanin and uric acid (a known peroxy radical scavenger) were 1.54 and 3.5, respectively. These studies clearly suggest that the covalently linked chromophore, phycocyanobilin, is involved in the antioxidant and radical scavenging activity of phycocyanin [7, 41].

Other studies of *Spirulina* application in cancers

Liver fibrosis is a chronic liver disease that will further develop to cirrhosis if severe damage continues to form. A potential treatment for liver fibrosis is to inhibit activated hepatic stellate cell (HSC) proliferation and, subsequently, to induce HSC apoptosis. It has been reported that antioxidants are able to inhibit the proliferation of HSCs. In this study, the aqueous extract of *Spirulina* was chosen as the source of antioxidant to investigate the inhibitory effect on the proliferation of HSC [42].

The growth inhibitory effects of aqueous *Spirulina* and chlorella extract on human liver cancer cells, HepG2, were also studied and compared in pairs. Results indicated that the total phenol content of *Spirulina* was almost five times greater than that of chlorella (6.86 \pm 0.58 vs 1.44 \pm 0.04 mg tannic acid equivalent/g of algae powder, respectively). The aqueous extracts of these two algae both showed anti-proliferative effects on HSC and HepG2, but *Spirulina* was a stronger inhibitor than chlorella [42].

Water-soluble polysaccharides of *Spirulina* appear to display antioxidant, anticancer, and antiviral effects [36]. In addition, *Spirulina* does not have secondary undesirable effects, not being described episodes of occasional human or animal death as occurred with other microalgae, mainly due to the presence in those microalgae of hepato and neurotoxins. In parallel with those initial antiviral and anticancer studies, *Spirulina* extracts were tested for their immunomodulatory capacities. Since then, enhancement of the immune system by *Spirulina* has turned into a key research line for future uses of this organism. Recently, the Food and Drug Administration of the United States of America has included *Spirulina* in the group of Generally Recognized as Safe (GRAS) products [16, 20].

It is well established that exercise promotes the production of reactive oxygen and nitrogen species, which contribute to skeletal muscle fatigue and damage. Two clinical trials were conducted to investigate the effects of *Spirulina* on preventing exercise induced skeletal muscle fatigue and damage through its antioxidant property. In one study with 16 student volunteers, intake of a diet containing 5% *Spirulina* for 3 weeks resulted in a significant reduction of plasma oxidative marker malondialdehyde (MDA) with a concurrent increase in the blood superoxide dismutase (SOD) activity [11, 29, 43].

In a study from the Department of Microbiology and Immunology at Hokkaido University in Japan, *Spirulina* extract was found to excite antitumor natural killer cell

response. For their study, the researchers transplanted tumors into a specific species of mice. After orally administering hot water extracts of *Spirulina* to the mice, they watched for molecular signals in the major histocompatibility complex of the mice cells. At first, a sub cell line showed slight expressions of NT response, but as the study furthered, they noticed natural killer cell activation signals going off through expressions of Rae-1, a ligand for NK activation. With natural killer cells now arriving onto the scene of the tumor-ridden cells, the implanted melanoma slowly regressed, until the natural killer cell defense effectively eliminated the entire tumor [36, 37].

In China, the influence of water soluble polysaccharide from *Spirulina platensis* on excision repair of DNA was investigated by means of endonuclease assay and radio autography. The results showed that the presence of the polysaccharide enhanced significantly both the repair activity of radiation damaged DNA excision and the unscheduled DNA synthesis (UDS). During the examination of the time course of the excision process, it was found that the presence of polysaccharide of *Spirulina* not only increased the initial rates of the damaged DNA excision and the UDS, but also postponed the saturations of both important reactions of excision and repair DNA synthesis [44, 45].

Researchers in both Japan and China have examined the potential of Spirulina's polysaccharides in cancer therapy. In a study titled "Inhibition of tumor invasion and metastasis by Calcium Spirulan," scientists at Japan's Toyama Medical and Pharmaceutical University found that lung metastasis was significantly reduced by Calcium Spirulan by inhibiting tumor invasion of the cell membranes [46].

The results of the present study clearly point to the therapeutic potential of *Spirulina* in Cadmium-induced teratogenicity and probably through its antioxidant activity. Organic foods often contain high levels of cadmium it is a little-known fact that the USDA organic program does not regulate, or measure, or in any way limit the concentration of heavy metals in "certified organic" foods. Natural News has found that many "organic" foods contain alarming levels of heavy metals such as cadmium, lead, mercury, arsenic and even lighter metals like aluminum [47, 48].

The iron chelating properties of *Spirulina* was discovered when human neuroblastoma cells in vitro were exposed to toxic amounts of iron and then to *Spirulina*, which revealed that the iron induced oxidative stress was reduced. Geriatric patients administered *Spirulina* for 16 weeks showed a remarkable improvement in the antioxidant potential, as measured by the increased levels of antioxidant status in plasma of these individuals. A double-blinded, placebo controlled study performed on individuals after exercise, showed decreased amount of creatine kinase, (an indicator of muscular breakdown) when they were supplemented with *Spirulina*. Moreover, their exhaustion time in the treadmill exercise increased by 52 seconds. This could be explained by the antioxidant potential of *Spirulina* [32].

Studies the influence of *Spirulina* on IgA levels in human saliva and demonstrated that it enhances IgA production, suggesting a pivotal role of micro alga in mucosal immuni-

ty. A Japanese team identified the molecular mechanism of the human immune capacity of *Spirulina* by analyzing blood cells of volunteers with pre- and post-oral administration of hot water extract of *Spirulina platensis*. IFN- γ production and Natural Killer (NK) cell damage were increased after administration of the micro alga extracts to male volunteers. In a recent double-blind, placebo controlled [49].

Study from Turkey evaluating the effectiveness and tolerability of Spirulina for treating patients with allergic rhinitis, Spirulina consumption significantly improved the symptoms and physical findings compared with placebo ($P < .001$), including nasal discharge, sneezing, nasal congestion and itching [49].

Spirulina can also be exploited for the production of various enzymes especially antioxidant enzymes. It has a very high amount of the superoxide dismutase enzyme, which is an important free radical scavenging enzyme. This enzyme can be used therapeutically for the treatment of various diseases related to oxidative stress or as a component in anti-wrinkle skin lotions and face masks as aging is believed to a consequence of oxidative stress [9, 50].

Reactive oxygen species (ROS) can be generated in a biological system by processes such as irradiation. These affect various systems like digestive, hematopoietic, immune etc. Defense mechanisms include antioxidant enzymes such as super oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase, as well as the non-enzymatic antioxidants like beta-carotene, glutathione, vitamin E etc. Radiation protection offered by *Spirulina* may be due to the phytopigments (carotenoids, chlorophyll, phycocyanin) as well as polysaccharides [51].

Another mouse study showed that *Spirulina* reduced both skin and stomach tumors significantly. *Spirulina* was shown to reduce both the size of the tumors and to reduce the incidence of tumors [39]. The study determined the histopathologic and hematologic effects of *Spirulina platensis* and *Moringa oleifera* Lam., and their synergism on tumor-induced Sprague-Dawley rats. Induction of tumors was done through intraperitoneal administration of 1,2-dimethylhydrazine and 7,12-dimethylbenzanthracene for four weeks. Readily available capsules and tablets of *Spirulina* and malunggay mixed with water were used to achieve the desired 50% and 75% concentration. Intraperitoneal administration of the extracts and intravenous administration of 5-fluorouracil as the positive control treatment were done for two weeks post tumor induction. After treatment, representatives of each treatment groups were drawn blood and dissected for hematologic and histopathologic examination, respectively. Results show anti-tumor activity of all extracts in the liver in contrast to their ineffectiveness in the lungs. Furthermore, all but the 75% *Spirulina* extract were effective against gastric tumor development while both concentrations of *Spirulina* and its 75% concentration successfully countered development of tumors of the small intestine and large intestine, respectively. All of the extracts caused a general negative effect on the complete blood count. In conclusion, *S. platensis* proved to be the more effective against tumor development [52].

Thus Selenium-enriched *Spirulina* as a vector for anti-cancer drug delivery enables to associate any anticancer drug with Se. This process allows high drug delivery and produce significant anti-proliferative effect the functionalization of selenium nanoparticles (SeNPs) with *Spirulina* polysaccharides (SPS) that has been developed in a study. Results suggest that the strategy to use SPS as a surface decorator could be an effective way to enhance the cellular uptake and anticancer efficacy of nanomaterials. SPS-SeNPs may be a potential candidate for further evaluation as a chemo preventive and chemotherapeutic agent against human cancers [53].

Also study of antimicrobial activities of *Spirulina* extract against *Staphylococcus aureus* (Gram positive bacterium), *Escherichia coli* (Gram negative bacterium), *Candida albicans* (yeast) and *Aspergillus niger* (fungus) showed that *C. albicans* is the most sensitive microorganism to all *Spirulina* fractions, which were obtained by the supercritical fluid extraction. This antimicrobial activity could be related to a synergic effect of fatty acids.

Conclusion

Spirulina is a potent mixture of antioxidants and most of *Spirulina*'s health benefits are associated with its antioxidant pigments. These are carotenoids (mixture of carotenes and xanthophylls), chlorophyll and the unique blue pigment phycocyanin. A little of its usage in medicine has been established by numerous studies still more of its hidden properties are yet to be explored. Some of its properties such as Anti oxidant, Anti inflammatory, Anti cancer, Anti aging (prevents cell death), Drug delivery system., etc. Along with these actions in Humans has been described briefly in this Review. Hope this review will serve the purpose of aiding in future Research work to unleash the further components present in *Spirulina*.

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