Spirulina (Arthrospira Platensis): Anti-viral effects and potential symptom mitigation in case of viral infection

Shauder L[1], Avital T, & Dach B[1]

Spirulina (*Arthrospira platensis*) is being widely studied for its possible anticancer, antibacterial and antiparasitic properties, and for several medical conditions such as allergies, ulcers, anemia, heavy-metal poisoning, and radiation poisoning. S. Platensis has also been studied for its antiviral properties, which seem to be related to its unique sulfated polysaccharide named Calcium-Spirulan (Ca-SP). Furthermore, antioxidative characteristics of its Phycobiliproteins (e.g. Phycocyanin) been demonstrated to reduce oxidative stress which is suggested to aid in reducing viral proliferation and possibly life-threatening collateral symptoms such as severe inflammation. Reviewing the current scientific evidence for antiviral properties along with the beneficial effects on the immune system, Spirulina might serve as a highly potential natural resource for multi-functional nutritional treatment and support in face of the novel coronavirus (COVID-19) outbreak. In that context, fresh Spirulina biomass, which contains higher concentrations of heat-sensitive compounds such as Phycocyanin, is probably the most potent form for the suggested function.

Keywords: Spirulina Platensis, Antiviral, COVID-19, SARS-COV-2, calcium spirulan

Introduction

Spirulina is a multicellular filamentous, spiral-shaped blue-green microalgae derived from class Cyanophyta/ Cyanobacteria which is an ancient organisms known by its ability to make photosynthesis it is a member of the Oscillatoriaceae family that grows naturally in warm climates and are found in variety of environments; Sand, soil and marshes and different kinds of aquatic media like fresh water, sea water and brackish water (Charpy, Casareto, Langlade, & Suzuki, 2012; M. Khan et al., 2005; Sáncbez, Bernal-Castillo, Rozo, & Rodríguez, 2003).

Spirulina platensis acknowledged as a highly nutritious food with high protein content (60-70% by dry weight) fulfilling all essential amino acids (Ishimi, Sugiyama, Ezaki, Fujioka, & Wu, 2006). Its protein content resembles that of legumes and could be comparable to that of meat, egg and milk although its reduced content of cysteine, methionine and lysine (Babadzhanov et al., 2004). It contains a wide spectrum of nutrients that include essential fatty acids, gamma-linolenic acid, alpha-linolenic acid, linoleic acid, eicosapentaenoic acid, stearidonic acid, arachidonic acid and docosahexaenoic acid (Mendes, Nobre, Cardoso, Pereira, & Palavra, 2003). It also has phycocyanin and other photo chemicals like phycocyanobilin, chlorophyll and xanthophyll phytopigments (Chamorro et al., 2002; Gong, Ding, Liu, Chen, & Liu, 2005; Gong et al., 2005; Upasani & Balaraman, 2003).

Beyond nutritional value, Spirulina species possess specific therapeutic properties. Certain species of Spirulina have shown to exhibit immunomodulating and bio modulating properties. *Spirulina platensis* (*S. platensis*) has a positive and regulatory effect on immune system. Studies indicated immune enhancing properties of *S. platensis* in animals and humans. Administration of this cyanobacteria improved immunological resistance in subjects with various types of cancer, AIDS and other viral diseases (Z. Khan et al., 2005).

Relevant outlining of beneficial effects and mechanisms of action of Spirulina:

Antioxidant functions: Spirulina improves the antioxidant enzymes such as SOD, CAT, GSH, GSH-PX and reduce lipid peroxidation (MDA) as well as has scavenging activity of free radicals (., 2003; Chaiklahan, Chirasuwan, Siangdung, Paithoonrangsarid, & Bunnag, 2010; Chen & Wong, 2008; Gad et al., 2011; Hwang et al., 2011; Ibrahim & Abdel-Daim, 2015; Kurd & Samavati, 2015).

Immunomodulatory effect: Spirulina increased the phagocytic potential of macrophages, enhanced the activities of NK-cell and lysozyme and increased the production of antibodies, interferon gamma (IFN-gamma) and cytosines

Correspondence concerning this article should be addressed to Dach B, . E-mail: baruch@algae.co.il, lior@algae,co,il

(interleukins; IL-1,4 and 17) (Al-Batshan, Al-Mufarrej, Al-Homaidan, & Qureshi, 2001; Nielsen et al., 2010; Ragap2012; Soltani2012; Chu, Van Quynh, & Radhakrishnan, 2013; Jamil, Akanda, Rahman, Hossain, & Islam, 2015; Krishnaveni, Palanivelu, & Velavan, 2013; Shokri, Khosravi, & Taghavi, 2014; Şahan et al., 2015).

Anti-inflammatory effect: In addition to inhibition of colon inflammatory markers; MPO and PGE2 as well as pro inflammatory cytosines; TNF-alpha and IL-1beta, IL-6) with restoring the histological structure of colon by decreasing levels of lysosomal enzymes, tissue marker enzymes and glycoproteins (Abdel-Daim, Farouk, Madkour, & Azab, 2015; Ali, Barakat, & Hassan, 2015; BAYLAN, ÖZCAN, Oya, & Mustafa, 2012; Chu et al., 2013; Coskun et al., 2011; Gutiérrez-Rebolledo et al., 2015; Pugh et al., 2015; Rasool & Sabina, 2008; Somchit et al., 2014; Syeda Hurmatul Quader, Shoaib Ul Islam, Arm Saifullah, Md Fakhar Uddin Majumder, & Jma Hannan, 2013; Vidé et al., 2015).

Effect of spirulina on innate immunity: Spirulina showed specific positive effects on innate immune functions and can affect the nonspecific immunity in several ways. A sulphated polysaccharide isolated from water extract of Spirulina, Ca-SP showed immunomodulatory and anti-viral activities (Luescher-Mattli, 2005; Pugh, Ross, Eisohly, Eisohly, & Pasco, 2001).

Polysaccharides and phycocyanin from Spirulina increased immunity in mice by enhancing bone marrow reproduction, thymus growth, and spleen activity (Hayashi et al., 1998; Hirahashi et al., 2002; Hong-Quan, An-Ping, Yun, & Yang-Mei, 2001; Qishen, 1988; Qureshi, Garlich, & Kidd, 1996). It was reported that Spirulina up-regulates key cells and organs of the immune system improving their ability to function in spite of stress from environmental toxins and infectious agents. Studies on animal models documented that phycocyanin of Spirulina stimulates hematopoiesis, especially erythropoiesis by inducing erythropoietin hormone (EPO). There is also evidence that c-phycocyanin and polysaccharides of Spirulina enhance white blood cell production (Qureshi et al., 1996). The percentage of phagocytic macrophages increased when cats were administered water-soluble extract of S. platensis (Qureshi, Kidd, & Ali, 1995). Increased phagocytic activity was also observed in other animals such as mice and chicken (Al-Batshan et al., 2001; Hayashi & Katoh, 1994; Qureshi et al., 1996). The water-soluble extract of S. platensis induces secretion of interleukins such as IL-1 from peritoneal macrophages (Hayashi & Katoh, 1994). The activity of NK cells was also enhanced significantly (Hirahashi et al., 2002). Studies on chicken model showed increased tumoricidal activity of NK cells (Qureshi et al., 1996, 1995). Further studies are needed to establish the exact biochemical mechanisms involved.

Effect of spirulina on specific immunity: Experimental studies indicated that Spirulina products buildup both the humoral and cellular arms of the immune system (Qureshi et al., 1996). Lymphocytes are key players of specific immunity. Spirulina stimulates mobilization of lymphocytes and other immune cells into the blood (Luescher-Mattli, 2005). It was found that when mice were fed with Spirulina there was a significant increase in splenic cells producing IgM antibody (K. Hyashi et al., 1996; Hyashi & Hayashi, 1996). Addition of water extract of Spirulina also increased proliferation of spleen cells in culture. Several studies on animal model indicated increased production of specific classes of antibodies such as IgA and IgE (Hayashi & Katoh, 1994; Nichols1968; Qureshi et al., 1996). It was observed that Spirulina possess anti-allergic properties by inducing IgA antibody against food allergens. Studies on rats suggested mast cell inhibiting functions of Spirulina [Kim, Lee, Cho, and Moon (1998); Yang1997]. Further studies revealed that phycocyanin of Spirulina inhibit release of histamine and functions as anti-inflammatory compound (Qureshi et al., 1995). In addition, it was observed that phycocyanin enhances mucosal immunity (Nemoto-Kawamura et al., 2004).

Spirulina Inhibitory effects in case of- viral infection: Sulfated polysaccharides, such as dextran sulfate and heparin, have proved to be potent inhibitors of human HIV-1 *in-vitro* (Hayashi, Hayashi, & Kojima, 1996). As to their mechanism of action, these agents have been shown to inhibit the binding of the virions to CD4 molecules on target cells and virus-induced syncytium formation (Hayashi et al., 1996). However, *in-vivo* effectiveness has not been established due to poor absorbability, short half-life and unfavorable anticoagulant activity in blood (Hayashi et al., 1996). A study on water-soluble extract of *Spirulina platensis*, exhibited an inhibitory effect against HSV-1 replication (Hayashi et al., 1996). From that extract the polysaccharide Calcium Spirulan (Ca-SP) was extracted as an antiviral component. Ca-SP was found to be active against not only HSV-1 but also HIV-1 components that are more likely to be active *in-vivo*. The study indicates that Ca-SP has an anti-HIV activity almost equal to dextran sulfate and is a much more potent inhibitor of HSV-1 (Hayashi et al., 1996). Moreover, Ca-SP is also active against human cytomegalovirus replication (Hayashi et al., 1996). The same compound may be used to suppress opportunistic passengers such as human cytomegalovirus and HSV-1 (Azabji-Kenfack et al., 2011). The mechanism of virus replication inhibition is suggested due to inhibition of virus binding to host cells and subsequent virus-cell fusion step (Hayashi et al., 1996). The team achieved better Ca-SP purification later in their works and detailed its composition and Ca-SP was found to be composed of rhamnose, 3-O-methylrhamnose (acofriose), 2,3-di-O-methylrhamnose, 3-O-methylxylose, uronic acids, and sulfate (Lee et al., 1998). Ayehunie, Belay, Baba, and Ruprecht (1998) performed a

similar study concerning *Spirulina platensis* water extract inhibition of HIV-1 replication. In their research, hot water extract of *Spirulina platensis* demonstrated inhibition of HIV-1 replication and syncytium formation in human T-cell lines, Jurkat cells (PBMC) and human Langerhans cells (LC) (Ayehunie et al., 1998). T-cell-tropic viruses (HIV-IIIB and HIVRF) and a primary patient isolate (11G) were inhibited (Ayehunie et al., 1998). A polysaccharide-containing fraction as well as a tannin-free fraction were responsible for this anti-HIV-1 activity (Ayehunie et al., 1998).

Mader et al. (2016) presented greater evidence for Ca-SP and spirulina extract antiviral potential against HSV-1 and Kaposi sarcoma-associated herpes virus/human herpes virus 8 (KSHV/HHV-8). Mader et al. (2016) compared Ca-SP and with Acyclovir (ACV), a standard treatment for lip herpes, in a standard plaque reduction assay. Ca-SP showed strong inhibition of HSV-1 infection with an IC50 of 0.04 µg/ml, comparable with ACV, while spirulina extract showed lower inhibition (IC50, ~30 µg/ml) when added 2h before infection of Vero cells (Mader et al., 2016). To further examine Ca-SP and spirulina extract potential, the team tested inhibitory effects on keratinocytes, a relevant cellular target of human HSV-1 infection. The inhibitory potency of Ca-SP stayed similar to that of ACV (IC50, 0.07 µg/ml) for the same cell culture

(Mader et al., 2016). Spirulina extract showed stronger antiviral effect in keratinocytes than in Vero cells (IC50, 8.2 µg/ml), while addition of spirulina extract to Ca-SP (ratio, 1:1.5) had no synergetic inhibitory activity (Mader et al., 2016). Moreover, cell viability showed no toxic effects of the active substances and solvents in the experiments (Mader et al., 2016).

The team (Mader et al., 2016) extrapolated the mechanism of action for Ca-SP HSV-1 inhibitory effect on human keratinocytes and determined by different approaches that Ca-SP inhibits the infection of human keratinocytes by HSV-1 through interference with the attachment phase of viral entry. A similar mechanism of heparin, a homologue of heparan sulfate, which has been shown to inhibit HSV-1 infection of human keratinocytes (Mader et al., 2016).

Ca-SP was also found to inhibit KSHV/HHV-8, which cause cutaneous Kaposi sarcoma, a disease accessible to topical therapy. Inhibitory dose-dependent KSHV/HHV-8 antiviral activity was shown to reduce KSHV/HHV-8 titers and viral DNA copy numbers with IC50 of 1.5 µg/ml, which is well below the IC50 of the currently used treatment (Foscarnet, 40-100 µg/ml) (Mader et al., 2016). Further, Mader et al. (2016) performed a clinical trial for Ca-SP/Spirulina extract cream concatenation (ratio, 1:1.5). The trial revealed that though less potent than systemic antivirals, the cream proved more potent that topical ACV cream in preventing herpes labialis exacerbation. Moreover, if herpes occurred, crusts and dryness of the lips were reported less frequently by patients using the novel cream, whereas the duration of herpes labialis was similar in both topical treatment groups (Mader et al., 2016). Coupling of Ca-SP with spirulina extract was performed in order to capture the spirulina extract antiviral activities not related to Ca-SP and due to its regenerative and antibacterial properties (Mader et al., 2016).

A different research conducted on Vero cells administrating 2 mg/ml hot water extract (HWT) of *Spirulina maxima* by Hernández-Corona, Nieves, Meckes, Chamorro, and Barron (2002), showed inhibitory effects for the infection of HSV-2, PRV, HSV-2, PRV, HCMV, and HSV-1, and the highest antiviral activity was against HSV-2, without affecting VSV, SA-11, and poliovirus type 1 (Hernández-Corona et al., 2002). Results revealed that the extract competed with the virus and inhibited the viral infection in a dose dependent manner, 2 h after viral infection, suggesting that S. maxima could interfere with the initial events of the viral infectious cycle, adsorption and penetration. Events which take place on the first 2 h after virus infection of the cells (Hernández-Corona et al., 2002). These results suggest that S. maxima inhibited from the beginning the herpesvirus infection by blocking virus adsorption and penetration to Vero cells without a direct viricidal effects [COVID191].

Antioxidative properties reducing viral propagation Phycocyanobilin: Lethal strains of influenza are characterized by increased capacity to induce proinflammatory cytokines in human macrophages. Thus, measures which dampen the influx and activation of leukocytes during influenza infestation may aid survival (McCarty, Barroso-Aranda, & Contreras, 2010).

One potential target is NF-kappaB a family of transcription factors that is activated in influenza-infected pulmonary epithelial cell. This activation appears to contribute to the life cycle of the virus by aiding the Ca-SP-mediated nuclear export of viral nucleoproteins. NF-kappaB also protects the virus by inhibiting the ability of type-1 interferons to induce antiviral proteins. Moreover, NF-kappaB activation evidently plays a role in the inflammatory response to influenza infection, as it promotes the transcription of a number or proinflammatory cytokines; thus, inhibitors of IkappaB kinase suppress the production of interleukin-8 in human epithelial cells infected with influenza (McCarty et al., 2010).

Another Potential target in treatment of influenza is NADPH oxidase. Influenza has been shown to induce oxidant stress in the cells it infects, an effect mediated at least in part by the viral hemaglutinin protein; this evoked oxidant stress may contribute to activation of NF-kappaB.

The increased oxidant stress evoked by influenza infection may contribute to the efficient viral propagation, while also playing a key role in inflammatory lung damage (McCarty

et al., 2010). Lung epithelial cells express several different isoforms of NADPH oxidase and it seems likely that this enzyme complex is a major source of excess oxidant stress in influenza-infected cells and decrease in intracellular glutathione levels exacerbates the impact of oxidants in infected cells. The phagocytic form of NADPH oxidase expressed by leukocytes infiltrating infected lung tissue also evidently contributes to total oxidative stress in infected lung parenchyma [COVID192]. The increased oxidant stress evoked by influenza infection bay contribute to the efficient viral propagation, while also playing a key role in inflammatory lung damage (McCarty et al., 2010).

Demonstrated in influenza-infected mice, adenovirus mediated transfer of heme oxygenase-1 (HO-1) cDNA reduces the lung damage and influx of leukocytes, while markedly boosting survival (60% vs 0% in controls). HO-1 generates bilirubin, which recently has been shown to be a potent physiological inhibitor of NADPH oxidase activity. It is reasonable to suspect that the potent antioxidant activity of bilirubin contributed prominently to the protection afforded by HO-1 transfection. This conclusion is of particular interest in light of suggestions that oral administration of phycocyanobilin (PCB) – richly supplied by spirulina, a biliverdin homolog, can mimic the NADPH oxidase-inhibitory activity of endogenous bilirubin. It would thus be of great interest to examine the impact or oral dietery spirulina or PCB-enriched spirulina extracts on the course of viral infections (McCarty et al., 2010). Reasonably, jointly targeting both NADPH oxidase and NF-kappaB would produce a more substantial impact on the survivability of lethal influenza strains. Joint administration of ample doses of spirulina and salicylate initiated as soon as feasible during infection might be expected to have life-saving potential. This would be a reasonably feasible and inexpensive strategy (McCarty et al., 2010).

Conclusions:

Several scientific findings suggested that Spirulina proved to be a potential and ideal candidate for conjugative therapy due to the possible synergetic effect of many phytochemicals in whole cell. It has been demonstrated that the use of Spirulina and its extracts may reduce viral diseases as demonstrated by Hayashi et al. (1996); Ayehunie et al. (1998), Hernández-Coronaetal. (2002), Maderetal. (2016); the sulfated polysaccharide Ca-SP has a potential in inhibiting virus attachment, in some cases (Hayashi et al., 1996) better than known substances such as the case with HSV-1. Ayehunie et al. (1998) demonstrated a polysaccharide spirulina extract fraction inhibition on HIV-1 replication while Hernández-Corona et al. (2002) showed inhibitory effects for HSV-2, PRV, HCMV and HSV-1, via hot water spirulina extract (S. maxima), which might owe to the antiviral activity of Ca-SP. Mader et al. (2016) presented even greater evidence for Ca-SP inhibition of HSV-1 and KSHV/HHV.

As suggested by McCarty et al. (2010), phycobilin proteins (such as phycocyanin) can mimic NADPH oxidase inhibitory activity of bilirubin and ease oxidative stress and reduce viral proliferation. Furthermore, Ngo-Matip et al. (2015) revealed how dietary supplementation with *Spirulina platensis* can aid in lowering viral load and prevent further exogenous infections by aiding the immune system function. *Spirulina platensis* is readily available and sustainably produced, it merits a myriad of characteristics that prove it an interesting potential source for new natural products.

Due to the unique composition of spirulina, with emphasis on minimal processing to maintain native structure and potency of heat-sensitive and oxygen-sensitive components such as Ca-SP and Phycocyanin (Oliveira, Duarte, Moraes, Crexi, & Pinto, 2010; Tiburcio, Galvez, Cruz, & Gavino, 2007), it can serve as a multi-functional nutritional treatment and support and have a therapeutic potential that can aid in mitigating damage and suffering in the recent COVID-19 eruption. In light of these evidence, and due to the fact that Spirulina is considered a food substance and not a pharmaceutical, current circumstances call for an expedited trial runway to estimate its efficacy in this current epidemic. Additional enhancements are also possible in the form of functional enhancement during cultivation, in the form of adding Zinc, Selenium or other effective anti-viral agents.

References

.H. H. A. E.-B. (2003). Over Production of Phycocyanin Pigment in Blue Green Alga Spirulina sp. and It‘s Inhibitory Effect on Growth of Ehrlich Ascites Carcinoma Cells. *J. Med. Sci.*, *3*(4), 314–324. https://doi.org/10.3923/jms.2003.314.324

Abdel-Daim, M. M., Farouk, S. M., Madkour, F. F., & Azab, S. S. (2015). Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to Dunaliella salina in acetic acid-induced rat experimental colitis. *Immunopharmacol. Immunotoxicol.*, *37*(2), 126–139. https://doi.org/10.3109/08923973.2014.998368

Al-Batshan, H. A., Al-Mufarrej, S. I., Al-Homaidan, A. A., & Qureshi, M. A. (2001). Enhancement of chicken macrophage phagocytic function and nitrite production by dietary Spirulina platensis. *Immunopharmacol. Immunotoxicol.*, *23*(2), 281–289. https://doi.org/10.1081/IPH-100103866

Ali, E. A. I., Barakat, B. M., & Hassan, R. (2015). Antioxidant and angiostatic effect of spirulina platensis suspension in complete freund's adjuvant-Induced arthritis in rats. *PLoS One*, *10*(4). https://doi.org/10.1371/journal.pone.0121523

Ayehunie, S., Belay, A., Baba, T. W., & Ruprecht, R. M. (1998). Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis). *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology*, *18*(1), 7–12. https://doi.org/10.1097/00042560-199805010-00002

Azabji-Kenfack, M., Dikosso, S. E., Loni, E. G., Onana, E. A., Sobngwi, E., Gbaguidi, E., … Ngogang, J. (2011). Potential of Spirulina Platensis as a Nutritional Supplement in Malnourished HIV-Infected Adults in Sub-Saharan Africa: A Randomised, Single-Blind Study. *Nutr. Metab. Insights*, *4*, NMI.S5862. https://doi.org/10.4137/nmi.s5862

Babadzhanov, A. S., Abdusamatova, N., Yusupova, F. M., Faizullaeva, N., Mezhlumyan, L. G., & Malikova, M. K. (2004). Chemical composition of Spirulina platensis cultivated in Uzbekistan. *Chem. Nat. Compd.*, *40*(3), 276–279. https://doi.org/10.1023/B:CONC.0000039141.98247.e8

BAYLAN, M., ÖZCAN, B. D., Oya, I., & Mustafa, A. (2012). A mini review on Spirulina. *Türk Bilim. Derlemeler Derg.*, *5*(1), 31–34.

Chaiklahan, R., Chirasuwan, N., Siangdung, W., Paithoonrangsarid, K., & Bunnag, B. (2010). Cultivation of spirulina platensis using pig wastewater in a semi-continuous process. *J. Microbiol. Biotechnol.*, *20*(3), 609–614. https://doi.org/10.4014/jmb.0907.07026

Chamorro, G., Salazar, M., Araújo, K. G. de L., Santos, C. P. dos, Ceballos, G., & Castillo, L. F. (2002). [Update on the pharmacology of Spirulina (Arthrospira), an unconventional food]. *Arch. Latinoam. Nutr.*, *52*(3), 232–240. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12448336

Charpy, L., Casareto, B. E., Langlade, M. J., & Suzuki, Y. (2012). Cyanobacteria in Coral Reef Ecosystems: A Review. *J. Mar. Biol.*, *2012*, 1–9. https://doi.org/10.1155/2012/259571

Chen, T., & Wong, Y. S. (2008). In vitro antioxidant and antiproliferative activities of selenium-containing phycocyanin from selenium-enriched Spirulina platensis. *J. Agric. Food Chem.*, *56*(12), 4352–4358. https://doi.org/10.1021/jf073399k

Chu, W. L., Van Quynh, L., & Radhakrishnan, A. K. (2013). Effect of spirulina (arthrospira) supplementation on the immune response to tetanus toxoid vaccination in a mouse model. *J. Diet. Suppl.*, *10*(3), 229–240. https://doi.org/10.3109/19390211.2013.822452

Coskun, Z. K., Kerem, M., Gurbuz, N., Omeroglu, S., Pasaoglu, H., Demirtas, C., … Turgut, H. B. (2011). The study of biochemical and histopathological effects of spirulina in rats with TNBS-induced colitis. *Bratislava Med. J.*, *112*(5), 235–243.

Gad, A. S., Khadrawy, Y. A., El-Nekeety, A. A., Mohamed, S. R., Hassan, N. S., & Abdel-Wahhab, M. A. (2011). Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. *Nutrition*, *27*(5), 582–589. https://doi.org/10.1016/j.nut.2010.04.002

Gong, R., Ding, Y., Liu, H., Chen, Q., & Liu, Z. (2005). Lead biosorption and desorption by intact and pretreated spirulina maxima biomass. *Chemosphere*, *58*(1), 125–130. https://doi.org/10.1016/j.chemosphere.2004.08.055

Gutiérrez-Rebolledo, G. A., Galar-Martínez, M., García-Rodríguez, R. V., Chamorro-Cevallos, G. A., Hernández-Reyes, A. G., & Martínez-Galero, E. (2015). Antioxidant Effect of Spirulina (Arthrospira) maxima on Chronic Inflammation Induced by Freund's Complete Adjuvant in Rats. *J. Med. Food*, *18*(8), 865–871. https://doi.org/10.1089/jmf.2014.0117

Hayashi, K., Hayashi, T., & Kojima, I. (1996). A natural sulfated polysaccharide, calcium spirulan, iso-

lated from Spirulina platensis: In vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. *AIDS Res. Hum. Retroviruses*, *12*(15), 1463–1471. https://doi.org/10.1089/aid.1996.12.1463

Hayashi, O., Hirahashi, T., Katoh, T., Miyajima, H., Hirano, T., & Okuwaki, Y. (1998). Class specific influence of dietary Spirulina platensis on antibody production in mice. *J. Nutr. Sci. Vitaminol. (Tokyo).*, *44*(6), 841–851. https://doi.org/10.3177/jnsv.44.841

Hayashi, O., & Katoh, T. (1994). Enhancement of Antibody Production in Mice by Dietary Spirulina platensis. *Nutr. Sci. Vitaminol*, *40*, 431–441.

Hernández-Corona, A., Nieves, I., Meckes, M., Chamorro, G., & Barron, B. L. (2002). Antiviral activity of Spirulina maxima against herpes simplex virus type 2. *Antiviral Res.*, *56*(3), 279–285. https://doi.org/10.1016/S0166-3542(02)00132-8

Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M., & Seya, T. (2002). Activation of the human innate immune system by Spirulina: Augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis. *Int. Immunopharmacol.*, *2*(4), 423–434. https://doi.org/10.1016/S1567-5769(01)00166-7

Hong-Quan, Z., An-Ping, L., Yun, S., & Yang-Mei, D. (2001). Chemo- and radio-protective effects of polysaccharide of spirulina platensis on hemopoietic system of mice and dogs. *Acta Pharmacol. Sin.*, *22*(12), 1121–1124.

Hwang, J. H., Lee, I. T., Jeng, K. C., Wang, M. F., Hou, R. C. W., Wu, S. M., & Chan, Y. C. (2011). Spirulina prevents memory dysfunction, reduces oxidative stress damage and augments antioxidant activity in senescence-accelerated mice. *J. Nutr. Sci. Vitaminol. (Tokyo).*, *57*(2), 186–191. https://doi.org/10.3177/jnsv.57.186

Hyashi, K., Hyashi, T., & Kojma, J. (1996). No Title. *AIDS Res. Hum. Retrovir*, *12*(15), 1463–1471.

Hyashi, T., & Hayashi, K. (1996). No Title. *J. Nat. Prod.*, *59*, 8387.

Ibrahim, A. E., & Abdel-Daim, M. M. (2015). Modulating effects of spirulina platensis against tilmicosin-induced cardiotoxicity in mice. *Cell J.*, *17*(1), 137–144. https://doi.org/10.22074/cellj.2015.520

Ishimi, Y., Sugiyama, F., Ezaki, J., Fujioka, M., & Wu, J. (2006). Effects of spirulina, a blue-green alga, on bone metabolism in ovariectomized rats and hindlimb-unloaded mice. *Biosci. Biotechnol. Biochem.*, *70*(2), 363–368. https://doi.org/10.1271/bbb.70.363

Jamil, A. B. M., Akanda, M. R., Rahman, M. M., Hossain, M. A., & Islam, M. S. (2015). Prebiotic competence of spirulina on the production performance of broiler chickens. *J. Adv. Vet. Anim. Res.*, *2*(3), 304–309. https://doi.org/10.5455/javar.2015.b94

Khan, M., Shobha, J. C., Mohan, I. K., Naidu, M. U. R., Sundaram, C., Singh, S., … Kutala, V. K. (2005). Protective effect of Spirulina against doxorubicin-induced cardiotoxicity. *Phyther. Res.*, *19*(12), 1030–1037. https://doi.org/10.1002/ptr.1783

Khan, Z., Bhadouria, P., & Bisen, P. (2005). Nutritional and Therapeutic Potential of Spirulina. *Curr. Pharm. Biotechnol.*, *6*(5), 373–379. https://doi.org/10.2174/138920105774370607

Kim, H. M., Lee, K. H., Cho, H. H., & Moon, Y. H. (1998). No Title. *Biochem. Pharmacol*, *55*, 71071–77107.

Krishnaveni, R., Palanivelu, K., & Velavan, S. (2013). Effects of probiotics and spirulina supplementation on haemato-immunological function of catla catla. *Int. J. Fish. Aquac. Res.*, 176–181.

Kurd, F., & Samavati, V. (2015). Water soluble polysaccharides from Spirulina platensis: Extraction and in vitro anti-cancer activity. *Int. J. Biol. Macromol.*, *74*, 498–506. https://doi.org/10.1016/j.ijbiomac.2015.01.005

Lee, J. B., Hayashi, T., Hayashi, K., Sankawa, U., Maeda, M., Nemoto, T., & Nakanishi, H. (1998). Further purification and structural analysis of calcium spirulan from Spirulina platensis. *J. Nat. Prod.*, *61*(9), 1101–1104. https://doi.org/10.1021/np980143n

Luescher-Mattli, M. (2005). Algae, A Possible Source for New Drugs in the Treatment of HIV and Other Viral Diseases. *Curr. Med. Chem. -Anti-Infective Agents*, *2*(3), 219–225. https://doi.org/10.2174/1568012033483051

Mader, J., Gallo, A., Schommartz, T., Handke, W., Nagel, C. H., Günther, P., … Reich, K. (2016). Calcium spirulan derived from Spirulina platensis inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. *J. Allergy Clin. Immunol.*, *137*(1), 197–203.e3. https://doi.org/10.1016/j.jaci.2015.07.027

McCarty, M. F., Barroso-Aranda, J., & Contreras, F. (2010). Practical strategies for targeting NF-kappaB and NADPH oxidase may improve survival during lethal influenza epidemics. *Med. Hypotheses*,

74(1), 18–20. https://doi.org/10.1016/j.mehy.2009.04.052

Mendes, R. L., Nobre, B. P., Cardoso, M. T., Pereira, A. P., & Palavra, A. F. (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chim. Acta*, *356*, 328–334. https://doi.org/10.1016/S0020-1693(03)00363-3

Nemoto-Kawamura, C., Hirahashi, T., Nagai, T., Yamada, H., Katoh, T., & Hayashi, O. (2004). Phycocyanin enhances secretary IgA antibody response and suppresses allergic IgE antibody response in mice immunized with antigen-entrapped biodegradable microparticles. *J. Nutr. Sci. Vitaminol. (Tokyo).*, *50*(2), 129–136. https://doi.org/10.3177/jnsv.50.129

Nielsen, C. H., Balachandran, P., Christensen, O., Pugh, N. D., Tamta, H., Sufka, K. J., … Pasco, D. S. (2010). Enhancement of natural killer cell activity in healthy subjects by Immulina, a Spirulina extract enriched for Braun-type lipoproteins. *Planta Med.*, *76*(16), 1802–1808. https://doi.org/10.1055/s-0030-1250043

Oliveira, E. G., Duarte, J. H., Moraes, K., Crexi, V. T., & Pinto, L. A. A. (2010). Optimisation of Spirulina platensis convective drying: Evaluation of phycocyanin loss and lipid oxidation. *Int. J. Food Sci. Technol.*, *45*(8), 1572–1578. https://doi.org/10.1111/j.1365-2621.2010.02299.x

Pugh, N. D., Edwall, D., Lindmark, L., Kousoulas, K. G., Iyer, A. V., Haron, M. H., & Pasco, D. S. (2015). Oral administration of a Spirulina extract enriched for Braun-type lipoproteins protects mice against influenza A (H1N1) virus infection. *Phytomedicine*, *22*(2), 271–276. https://doi.org/10.1016/j.phymed.2014.12.006

Pugh, N., Ross, S. A., Eisohly, H. N., Eisohly, M. A., & Pasco, D. S. (2001). Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from Spirulina platensis, Aphanizomenon flos-aquae and Chlorella pyrenoidosa. *Planta Med.*, *67*(8), 737–742. https://doi.org/10.1055/s-2001-18358

Qishen, P. (1988). No Title. *Chinese J. Genet.*, *15*(5), 374–381.

Qureshi, M. A., Garlich, J. D., & Kidd, M. T. (1996). Dietary spirulina platensis enhances humoral and cell-mediated immune functions in chickens. *Immunopharmacol. Immunotoxicol.*, *18*(3), 465–476. https://doi.org/10.3109/08923979609052748

Qureshi, M. A., Kidd, M. T., & Ali, A. R. (1995). No Title. *Nutr. Immun*, *3*(4), 35–45.

Rasool, M., & Sabina, E. P. (2008). Evaluation of the protective potential of Spirulina fusiformis on lipid peroxidation and antioxidant status in adjuvant-induced arthritic mice. *Pharmacologyonline*, *1*, 300–310.

Sáncbez, M., Bernal-Castillo, J., Rozo, C., & Rodríguez, I. (2003). Spirulina (Arthrospira): An Edible Microorganism: A Review. *Univ. Sci. Rev. La Fac. Ciencias, Pontif. Univ. Javeriana*, *8*(1), 7–24.

Shokri, H., Khosravi, A., & Taghavi, M. (2014). Efficacy of Spirulina platensis on immune functions in cancer mice with systemic candidiasis. *J. Mycol. Res.*, *1*(11), 7–13.

Somchit, M. N., Mohamed, N. A., Ahmad, Z., Zakaria, Z. A., Shamsuddin, L., Fauzee, M. S. O., & Kadir, A. A. (2014). Anti-inflammatory and anti-pyretic properties of Spirulina platensis and Spirulina lonar: A comparative study. *Pak. J. Pharm. Sci.*, *27*(5), 1277–1280.

Syeda Hurmatul Quader, Shoaib Ul Islam, Arm Saifullah, Md Fakhar Uddin Majumder, & Jma Hannan. (2013). In-vivo Studies of the Anti-inflammatory Effects of Spirulina platensis. *Pharma Innov. J.*, *2*(4), 70–80. Retrieved from http://www.thepharmajournal.com/vol2Issue4/Issue%7B/_%7Djune%7B/_%7D2013/21.1.pdf

Şahan, A., Taşbozan, O., Aydın, F., Özütok, S., Erbaş, C., Duman, S., & Özcan, F. (2015). DETERMINATION OF SOME HAEMATOLOGICAL AND NON-SPECIFIC IMMUNE PARAMETERS IN NILE TILAPIA (Oreochromis niloticus L., 1758) FED WITH SPIRULINA (Spirulina platensis) ADDED DIETS. *J. Aquac. Eng. Fish. Res.*, 133–139. https://doi.org/10.3153/jaefr15014

Tiburcio, P. C., Galvez, F. C. F., Cruz, L. J., & Gavino, V. C. (2007). Determination of shelf life of Spirulina platensis (MI2) grown in the Philippines. *J. Appl. Phycol.*, *19*(6), 727–731. https://doi.org/10.1007/s10811-007-9227-y

Upasani, C. D., & Balaraman, R. (2003). Protective effect of Spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. *Phyther. Res.*, *17*(4), 330–334. https://doi.org/10.1002/ptr.1135

Vidé, J., Virsolvy, A., Romain, C., Ramos, J., Jouy, N., Richard, S., … Rouanet, J. M. (2015). Dietary silicon-enriched spirulina improves early atherosclerosis markers in hamsters on a high-fat

diet. *Nutrition*, *31*(9), 1148–1154. https://doi.org/10.1016/j.nut.2015.03.014