Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

THERAPEUTIC APPLICATIONS OF *Spirulina* AGAINST HUMAN PATHOGENIC VIRUSES

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Received – July 18, 2020; Revision – September 17, 2020; Accepted – January 03, 2021
Available Online – March 25, 2021

DOI: http://dx.doi.org/10.18006/2021.9(Spl-1-GCSGD_2020).S38.S42

KEYWORDS

Spirulina

Cyanobacteria

Antiviral

Immunostimulant

ABSTRACT

Viruses can spread worldwide and the early detection of emerging infectious diseases and outbreaks in humans and animals is important for effective surveillance and prevention. Viruses such as human immunodeficiency virus (HIV), swine flu, and influenza virus are some of the viruses that spread diseases worldwide. However, the non-availability of effective antiviral drugs and the drug-resistance among the virus and host have become the major problems in controlling viral infections. The natural products from microalgae can be an alternative therapeutic agent to control viral infections in humans. *Spirulina* is a well-known cyanobacterium that has been consumed by humans as a food supplement for more than centuries without side-effects. *Spirulina* possesses high nutritional values and provides numerous health benefits to the consumers. *Spirulina* can be an alternative natural therapeutic agent for numerous virus infections as it contains several bioactive compounds with proven antiviral effect on enveloped viruses (Herpes simplex virus, measles virus, mumps virus) and non-enveloped viruses (astrovirus, rotavirus) by preventing the spread of the virus in the host cells. *Spirulina* also serves as a natural supplement that strengthens the immune system. This review focuses on the antiviral properties and immunostimulant effects of *Spirulina* as a potential therapeutic supplement on human health.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Viruses are cellular and obligate intracellular parasites that can cause viral infection in host cells and are harmful to all living organisms including humans, animals, and plants (Koonin et al., 2006). Viruses can spread disease worldwide through many factors such as contaminated water and food, environmental factors such as air and soil, person to person, insects, and vectors. Early detection of emerging infectious diseases and outbreaks in humans and animals is important for effective surveillance and prevention (Ramakrishnan, 2013). There are around 219 human infecting virus species that are known, and the first human infecting virus discovered was yellow fever in 1901 (Woolhouse et al., 2012). Carcinogenic viruses and other disease causative viruses including Chikungunya virus, dengue virus, acute respiratory syndrome coronavirus (SARS-CoV), human immunodeficiency virus (HIV), influenza virus, Herpes simplex virus (HSV), severe swine flu, human papilloma virus (HPV), and human T-lymphotropic viruses (HTLV-I, HTLV-II) are the most important viruses spreading diseases worldwide (Ramakrishnan, 2013). Drug-resistance of the virus and the host have made the treatment of viral infection using chemical drugs difficult. One alternative way to overcome the drug-resistant issue is to substitute chemical drugs with natural biological compounds (Ramakrishnan, 2013). The natural products from microalgae can be an alternative therapeutic agent to effectively control the viral infections in humans.

Cyanobacteria are one of the oldest photosynthetic organisms that are found in different aquatic environments (Nuhu, 2013). *Spirulina* is known to be a "superfood", named by the World Health Organization (WHO), and is recommended to be a compact food during space travel for astronauts of National Aeronautics and Space Administration (NASA) (Koyande et al., 2019). *Spirulina* has been consumed for more than centuries as food and supplements for humans, traditionally by the native population at Lake Chad area and the Aztec population of Mexico (Djeramane et al., 2018; Koyande et al., 2019). The species of *Spirulina* includes *S. platensis*, *S. laxissima, S. subsalsa, S. fusiformis, S. maxima*, and *S. lonar*. However, between all the species, *S. platensis* and *S. maxima* are more popular and mostly used for nutritional and therapeutic purposes (Kameshwari et al., 2020).

Spirulina contains a high level of nutrients such as protein, lipid, carbohydrate, amino acids, vitamins, minerals, pigments, and others (Khan et al., 2005). Generally, *Spirulina* contains 55-70% protein, 15-25% carbohydrate, 6-13% nucleic acids, 5-6% lipid, and 2.2-4.8% minerals (Reboleira et al., 2019). Vitamins such as B_1, B_2, B_3 and B_{12}, photosynthetic pigments, and minerals like calcium, copper, iron, magnesium, phosphate, sodium, and zinc are rich in *Spirulina* species (Wan et al., 2016). *Spirulina* is a natural immunity booster, detoxifier, appetite suppressant, and antioxidant that provide a wide range of benefits upon consumption (Singh et al., 2020). The antibacterial, anticancer, antiviral, and anti-parasitic properties of *Spirulina* are being widely studied by researchers (Martínez-Galero et al., 2016; Fayyad et al., 2019; Abd El-Baky & El-Baroty, 2020; Joseph et al., 2020). Besides, *Spirulina* is also being used in clinical studies due to its high functional and nutritional properties in treating a range of pathogenic conditions such as allergies, inflammatory diseases, hypercholesterolemia, heavy-metal poisoning, cardiovascular diseases and radiation poisoning (Reboleira et al., 2019; Kameshwari et al., 2020).

2 Antiviral properties

The National Cancer Institute (NCI) research group screened the lipophilic and hydrophilic extracts obtained from 300 species of cyanobacteria to examine their antiviral properties and reported that about 10% of the cultures were shown to reduce the cytopathic effects induced by a viral infection (Patterson et al., 1993). The bioactive compounds of microalgae such as nostoflan from *Nostoc flagelliforme* and fucoidan from the sporophyll of *Undaria pinnatifida* exhibit an antiviral effect on several enveloped viruses (Hayashi, 2008) and, ichthyopeptins A and B, the two novel depsipeptides from *Microcystis ichthyoblabe* inhibit the viral protein of influenza A virus (Zainuddin et al., 2007). *Spirulina* naturally can inhibit the activity of viruses as it has all the biomolecules which can build a strong immune system that can scavenge free radicals (Ali & Saleh, 2012). The extracts isolated from *S. platensis* and *S. maxima* were reported to possess antiviral properties (Hernandez-corona et al., 2002; El-Baz et al., 2013; Kameshwari et al., 2020). The antiviral activity of *Spirulina* mostly depends on the richness of proteins (phycocyanin), sulphated polysaccharide fragments, fatty acids (sulpholipids), minerals, and some other constituents (Nuhu, 2013; Kameshwari et al., 2020).

The antiviral effect of *S. platensis* against enveloped viruses such as human cytomegalovirus (HCMV), HSV-1, measles virus, mumps virus, HIV-1, and influenza virus is mainly contributed by the acidic or sulphated polysaccharides, calcium spirulan (Ca-SP) isolated from hot water extract of *S. platensis* that acts through inhibiting the replication of the viruses (Nuhu, 2013; Ramakrishnan, 2013). The aqueous extract of *S. platensis* is found to significantly inhibit the *in vitro* replication of HIV-1in human T-cell lines, Langerhans cells, and peripheral blood mononuclear cells (PBMCs) (Hoseini et al., 2013; Ramakrishnan, 2013), with up to 50% viral reduction on PBMCs using extract concentration between 0.3 to 1.2 µg/mL (Ayehunie et al., 1998). Further, the water extract of *S. platensis* inhibited HSV-1 replication in HeLa cells by interfering with the entry of the virus into the host cells, although the extract is not virucidal (Hayashi et al., 1993). Besides the enveloped viruses, *S. platensis* also demonstrated the antiviral effects on non-enveloped RNA and DNA enteric viruses. The

ethanol extract of *S. platensis* caused a reduction of 76.7% on astrovirus type 1, 66.7% on Coxsackievirus, 53.3% on adenovirus type 7, 56.7% on rotavirus Wa strain, and 50% on adenovirus type 40. The virucidal effect of *S. platensis* extract using ethanol showed a higher reduction on RNA viruses than DNA viruses (El-Baz et al., 2013). Allophycocyanin isolated from *S. platensis* is proven to inhibit 50% of viral-induced cytopathic effect in African green monkey kidney cells and human rhabdomyosarcoma cells at concentrations of 0.056-0.101 µM on enterovirus 71 by interfering with viral RNA synthesis in infected cells. The treatment of allophycocyanin to the host cells before viral infection showed higher antiviral activity than treatment after infection (Shih et al., 2003). Other than *S. platensis*, Hernandez-corona et al., (2002) reported that methanol-water (3:1) extract of *S. maxima* exhibited the highest antiviral activity on HSV-2 among hexane, chloroform, methanol, and hot water extracts. *S. maxima* extract prepared using hot water showed less than 20% inhibition on adenovirus type 3 with IC_{50} 5.2 mg/mL and no inhibition was observed at concentrations below 2 mg/mL (Hernandez-corona et al., 2002; Rahman et al., 2006).

Abd El-Baky & El-Baroty (2020), reported that L-asparaginase (L-AsnA) purified from *S. maxima* showed a dose-dependent antiviral effect on Coxsackie B3 (CSB3) virus where 17.03 µg/mL of L-AsnA inhibited 50% of CSB3 virus and suggested that inhibition of viral replication cycle might be the mechanism behind the antiviral effect. Similarly, Sharaf et al. (2010) reported that the crude extracts of *S. fusiformis* caused inhibition of HSV replication in host cells on both pre and post-infection stages.

Further, *Spirulina* contains about 2-5% of sulpholipids, which are effective against the enzymatic activity of HIV-1 reverse transcriptase (RT). A minimum concentration of 24 nM of sulpholipids can exhibit 50% inhibition by selectively acting on DNA polymerase of HIV-1 RT (Hoseini et al., 2013). A study on carbohydrate-binding agents that demonstrated the inhibition of HIV-1 and other enveloped viral particles suggested that cyanovirin-N (CV-N) isolated from *Spirulina* has the potential as an anti-HIV therapeutic agent in the future (Balzarini, 2007). Table 1 and 2 shows the antiviral properties of *S. platensis* and *S. maxima*, respectively.

Table 1 Antiviral properties of *S. platensis*

Compound name	Virus	Antiviral property	Authors
Ca-SP from hot water extract	HSV-1, HCMV, Measles, Mumps, HIV-1, Influenza	Inhibit viral replication *in vitro*.	Nuhu, 2013; Ramakrishnan, 2013
Ethanol extract	Astrovirus type 1, Coxsackievirus, Rotavirus Wa strain, Adenovirus type 7, Adenovirus type 40	Higher virucidal effect on RNA viruses than DNA viruses (*in vitro)*.	El-Baz et al., 2013
Aqueous extract	HIV-1	Inhibit viral replication in PBMCs, human T-cell lines, and Langerhans cells (*in vitro)*.	Ayehunie et al., 1998
Water extract	HSV-1	Inhibit viral replication and prolong the survival time of virally infected hamster.	Hayashi et al., 1993
Allophycocyanin	Enterovirus 71	Delay viral RNA synthesis and activate apoptosis (*in vitro)*.	Shih et al., 2003

Table 2 Antiviral properties of *S. maxima*

Compound name	Virus	Antiviral property	Authors
Hot water extract	HSV-1, HSV-2	Inhibit viral infection in Vero cells by interfering viral infectious cycle, adsorption and penetration.	Hernandez-corona et al., 2002
Methanol-water (3:1) extract	HSV-2	Block viral infectious cycle at adsorption and penetration stages.	Hernandez-corona et al., 2002
L-AsnA	CSB3 virus	Inhibit viral replication cycle.	Abd El-Baky & El-Baroty, 2020

3 Immunostimulant effects

Numerous *Spirulina* supplements can be found in the market nowadays as they contain high nutritional values and are reported to exhibit immune-stimulating properties (Jung et al., 2019; Singh et al., 2020). *Spirulina* was found to activate macrophages, natural killer (NK) cells, T-cells, and B-cells (Nuhu, 2013; Singh et al., 2020), and further enhance immunity by increasing the production of antibodies, interferon-gamma (IFN-γ), and cytokines (Nuhu, 2013; Banakar et al., 2020).

Natural substances isolated from *Spirulina* are reported to be effective inhibitors against enveloped and non-enveloped viruses by interfering or blocking the adsorption and penetration of virus, and also inhibit viral replication in the host cells (Singh et al., 2020). Populations in Japan, Korea, and Africans at Chad area that consume *Spirulina* daily with an average of 3-13 g, reported to have lower cases of HIV and acquired immune deficiency syndrome (AIDS) as compared to the other populations that do not take *Spirulina* diet (Teas et al., 2004).

The HIV/AIDS prevalence of algae-consuming populations in Eastern Asia (Japan and Korea) is about 1/10,000 adults, as compared to Africa which has a high prevalence of 1/10 adults (Teas et al., 2004).

Conclusion and recommendation for future research

It is evident from the literature that *Spirulina* has high nutritional values and provides a wide range of nutritional and health benefits. Numerous research studies have proven that the extracts from *S. platensis* and *S. maxima* can inhibit the spreading of enveloped and non-enveloped viruses in host cells. Besides, the immune-stimulating effects of *Spirulina* products on human health offer *Spirulina* to be a potential therapeutic supplement. Further research is needed to determine its' usefulness against different viruses and unlock its potential. The multifunctional role of *Spirulina* makes it as an ideal natural drug with immense prophylactic and therapeutic properties.

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