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Review

Spirulina phenolic compounds: natural food additives with antimicrobial properties

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Abstract

Food safety is a scientific discipline to ensure consumers' safety and prevent food-related harms, hazards, or risks along the entire food supply chain. Although fish and seafood products are the best means for securing food and nutrition in a population, they are also highly perishable, being vulnerable to pathogenic bacteria and fungi. This review thus aimed to provide updated scientific information on the role of the phenolic compounds of *Spirulina* as food additives, and their antimicrobial activities in fish and seafood products, in a food safety context. Recent applications of *Spirulina* phenolic compounds showed good results in contaminated fish and seafood products. Different studies have concluded that *Spirulina* phenolic compounds such as polyphenols, C-phycocyanin, γ-linolenic acid, fatty acids, and the combination of lauric and palmitoleic acids have antimicrobial activity in eliminating and/or controlling the growth of pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa*, and *Bacillus subtilis*, and fungi such as *Aspergillus flavus* and *Aspergillus niger*. Overall, *Spirulina* can be considered as an emerging natural food additive with antimicrobial activities against pathogenic fungi and bacteria.

Abbreviations:

A. flavus, Aspergillus flavus; A. niger, Aspergillus niger; A. parasiticus, Aspergillus parasiticus; ANSES, French Agency for Food, Environmental and Occupational Health and Safety; *A. sessilis, Alternanthera sessilis; B. subtilis, Bacillus subtilis; C. cibarius, Cantharellus cibarius; C. tora, Cassia tora; C. versicolor, Coriolus versicolor; E. coli, Escherichia coli*; FAO, Food and Agriculture Organization; GAC, gallic acid equivalents; *L. deliciosus, Lactarius deliciosus; L. pyrogalus, Lactarius pyrogalus; M. luteus, Micrococcus luteus; P. aeruginosa, Pseudomonas aeruginosa; P. oleracea, Portulaca oleracea*; QE, quercetin equivalents; *S. aureus, Staphylococcus aureus; S. platensis, Spirulina platensis; A. platensis, Arthrospira platensis; S. maxima, Spirulina maxima*; TPC, total phenolic content; TFC, total flavonoid content; *V. vulnificus, Vibrio vulnificus*; WHO, World Health Organization.

Introduction

Food safety is a scientific discipline to ensure consumers' safety and prevent food-related harms, hazards, or risks along the entire food supply chain, and it should be observed "from farm to fork" (WHO, 1997; FDA, 2020). Fish and seafood products are the main sources of healthy nutrition all over the world; more than 4.5 billion of the world's population get at least 15% of their animal protein intake from fish (Béné *et al.,* 2015; FAO, 2016). However, fish and seafood products are the most perishable foodstuffs. Therefore, monitoring, inspection, and quality control are the key agendas in the fishery industry.

Microbial contamination of fish and seafood products occurs after catching. During processing and postharvest, biochemical changes cause spoilage in these food products very rapidly (Møretrø *et al.,* 2016; Jia *et al.,* 2019; FDA, 2020). In aquaculture, fungal contamination such as *A. flavus* has been reported in fish feed and the fish itself (Mohamed *et al.,* 2017). Another study on smoked fish reported that fungal contamination is due to unhygienic handling, filleting, and storage (Akwuobu *et al.,* 2019). Fish contamination may arise from the culturing environment such as the water used (Hennersdorf *et al.,* 2016). For example, fungi may contaminate the fish through water, soil sediments, or plant remains.

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Aspergillus and *Penicillium* have been reported as the most common fungi related to sediments and biofilms in water bodies (Gonçalves *et al.,* 2006). Foysal and Lisa (2018) reported different *Bacillus* spp. from soil sediment, whereas other studies reported *Vibrio* spp., *Photobacterium* spp., and *Flavobacterium* spp. from different farmed fish species (Hennersdorf *et al.,* 2016). *Vibrio* spp., *Listeria monocytogenes*, *Yersinia* spp., *Salmonella* spp., and *Clostridium botulinum* have also been reported from fish and seafood products (Terentjeva *et al.,* 2015; Novoslavskij *et al.,* 2016; Don *et al.,* 2020).

Fish contaminated with fungi that could produce mycotoxins are harmful to human health (Mohamed *et al.,* 2017; Akwuobu *et al.,* 2019). Due to this, in many countries, fish and seafood products are traditionally processed and preserved to allow for long-term storage without spoilage. Preservation is mainly performed by reducing the water content in the fish, along with high and low temperature treatments, as well as combined measures (Nithin *et al.,* 2020; Tsironi *et al.,* 2020) to control and eliminate toxic bacteria such as *E. coli, P. aeruginosa*, and *B. subtilis*, and the growth of moulds and yeasts such as *A. niger* (El-Sheekh *et al.,* 2014).

Many studies have shown that extracted *Spirulina* phenolic compounds exhibit good results in controlling certain toxic bacteria and fungi, and could serve as an antimicrobial agent in fish and seafood products (El-Sheekh *et al.,* 2014; Pugazhendhi *et al.,* 2015; Usharani *et al.,* 2015; Pagnussatt *et al.,* 2016). Therefore, this review explores the up-to-date scientific knowledge and future perspectives regarding the antimicrobial role of *Spirulina* phenolic compounds in fish and seafood products, in a food safety context.

What is Spirulina (Arthrospira)?

Spirulina is a Gram-negative free-floating filamentous cyanobacterium capable of photosynthesis, having robust cell wall, of blue green coloration (hence the name *cyano_*), and non-toxic (Fedor, 2011; Koru, 2012; Usharani *et al.,* 2012; ANSES, 2017). Prior to 1962, they were considered a eukaryotic alga (blue green algae) until they were reclassified as a prokaryote (domain Bacteria). Taxonomically, two genera are currently accepted; *Spirulina* (various species) and *Arthrospira* (important species include *A. platensis* and *A. maxima*) (Belay, 2008; ANSES, 2017).

Spirulina can be sourced and harvested from two sources; the first one is natural warm alkaline water or lakes found in African countries (Ethiopia, Chad, and Tunisia), Latin American countries (Mexico and Peru), and Southern Asian countries (India, Sri Lanka, and Thailand) (Belay, 2008; ANSES, 2017; Assaye *et al.,* 2018; Karssa *et al.,* 2018; IOC, 2011), and the second one is artificial aquaculture ponds (Moorhead *et al.,* 2011; ANSES, 2017).

Across the world, *Spirulina* is primarily known for its high nutritional value, and considered as a miracle / super food (Moorhead *et al.,* 2011; Tidjani *et al.,* 2018; Uddin *et al.,* 2018; Jung *et al.,* 2019), functional food and dietary supplement (ANSES, 2017; Panjiar *et al.,* 2017), feed supplement (Yusuf *et al.,* 2016), technofunctional ingredient in meat products to improve meat quality (de Medeiros *et al.,* 2020), and protein and vitamin supplement for fish farming industries (Usharani *et al.,* 2012; Ragaza *et al.,* 2020). *Spirulina* is rich in micro- and macronutrients (Moorhead *et al.,* 2011; ANSES, 2017), and used as an ingredient in the production of valuable innovative foods in the food industries (Lafarga *et al.,* 2020). It is also considered as a future food for mankind around the globe (Belay, 2008; Soni *et al.,* 2017; Mathur, 2019). Throughout history, the Aztecs in Mexico and Chad communities in tropical Africa have eaten the naturally harvested *Spirulina* in a dried cake form (Ahsan *et al.,* 2008; Tidjani *et al.,* 2018; Lafarga *et al.,* 2020).

Spirulina contains desirable chemical compositions such as proteins, carbohydrates (e.g., sulphated polysaccharides), essential amino acids, minerals, essential fatty acids (e.g., γ-linoleic acid), vitamins, and pigments (e.g., C-phycocyanin) (Belay, 2008; Thomas, 2010; IOC, 2011; Lafarga *et al.,* 2020).

Phenols and polyphenols of Spirulina

In organic chemistry, phenols or polyphenols are defined as compounds that have one or more hydroxy substituents in an aromatic ring, such as structural compounds (e.g., esters, methyl ethers, glycosides) (Lattanzio, 2013). Together, these phenolic compounds range from simple phenols to polyphenols and can be derived from plants and microorganisms including *Spirulina* (Al-Dhabi and Valan Arasu, 2016; Hussain *et al.,* 2019; Olszewska *et al.,* 2020). Most phenolics have two or more classes of hydroxyl, and are biologically active, having antimicrobial and/or antioxidant properties. Phenolics occur widely in plant sources, and are consumed daily by the global population, as illustrated in Table 1. Polyphenols have different categories based on their food sources, which include flavonoids, phenolic acids, lignans, and stilbenes.

Table 1. Phenolic and flavonoid contents from selected foods.

Author	Article title	Studied food	Total phenolic content (mg GAE/g)	Total flavonoid content (µg QE/g)
El-Baky *et al.* (2009)	Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects	*Spirulina maxima*	12.94 ± 0.93	4.65 ± 0.14
Gurnani *et al.* (2016)	Chemical composition, total phenolic and flavonoid contents, and *in vitro* antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens* L.)	Chili seed	7.95 - 26.15	4.64 - 12.84
Jang *et al.* (2018)	Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents	Garlic	47.58 ± 5.27	338.04 ±1.60[a]
Alexandre *et al.* (2019)	Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction	Pomegranate	219 ± 2.6[b] and 211 ± 0.7	No report
Ayvaz *et al.* (2019)	Phenolic profile of three wild edible mushroom extracts from Ordu, Turkey and their antioxidant properties, enzyme inhibitory activities	Mushroom	2.27 to 5.34 ± 0.55	5.02 to 17.54 ± 1.45
Papalia *et al.* (2019)	Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass	*Arthrospira platensis*	15.77 ±1.10[b]	20.82 ± 0.08[c]
Seghiri *et al.* (2019)	Functional composition, nutritional properties, and biological activities of Moroccan *Spirulina* microalga	*Spirulina*	4.19 ± 0.21	15.60 ± 2.74

[a]aged garlic powder extracted with distilled water; [b]high pressure extraction condition; and [c]total phenol and flavonoid of fresh *A. platensis*.

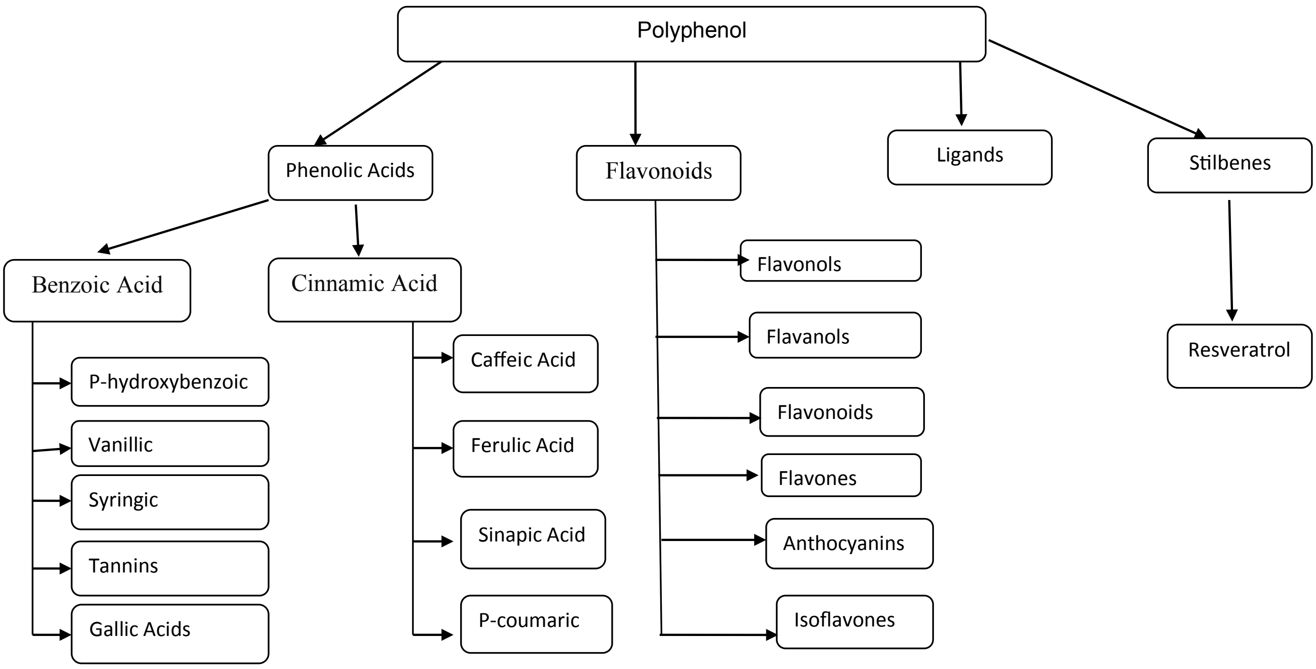

Figure 1. Categories of polyphenols.

Figure 1 illustrates the categories of polyphenols (Becerra-Herrera *et al.,* 2014; de Medina *et al.,* 2015; Papalia *et al.,* 2019).

The functions of *Spirulina* polyphenols are not only limited to antimicrobial and antimutagenic activities, but also anticancer, antiaging, anti-inflammation, lung defensive; neuroprotective, cardioprotective, hepatoprotective, and many more as illustrated in Figure 2. Furthermore, different studies have indicated their antimicrobial roles against different foodborne pathogens (Ghomari *et al.,* 2019; Papalia *et al.,* 2019; Polverari *et al.,* 2019), antioxidative roles (Salamatullah, 2014; Irakli *et al.,* 2018; Papalia *et al.,* 2019; Kukharenko *et al.,* 2020), antifungal and antimycotoxin roles (Shishido *et al.,* 2015; Christ-Ribeiro *et al.,* 2019), cardiovascular health promoting properties (Huang *et al.,* 2018), control and elimination of life-threatening viruses (Carrera-González *et al.,* 2013; Martínez-Martos *et al.,* 2014; Mathur, 2019), neural protection and boosting the immune system (Martínez-Martos *et al.,* 2014; Kumar *et al.,* 2017), antidiabetes, antihypertensive, and anti-obesity roles (Hu *et al.,* 2019; Lafarga *et al.,* 2020), anticancer properties (Jung *et al.,* 2019; Marková *et al.,* 2020), and the prevention of retinal neurodegeneration and improvement of eyesight (Okamoto *et al.,* 2019). Another study showed the chemopreventive role of *Spirulina* water extract solution which revealed good results in a human lung cancer A549 cell line with a considerable reduction of cancer cell viability and multiplication (Czerwonka *et al.,* 2018).

The quantity and efficiency of phenols, polyphenols, C-phycocyanin, and other bioactive compounds like γ-linolenic acid and carotenoids depend on the growth medium and condition of *Spirulina* (Sassano *et al.,* 2014; Gupta *et al.,* 2018; Papalia *et al.,* 2019), light intensity (Kepekçi and Saygideger, 2012), storage (Papalia *et al.,* 2019), processing and extraction methods, and the solvents used (Salamatullah, 2014; Papalia *et al.,* 2019). According to Papalia *et al.* (2019), the highest quantity of phenols, phytocyanin-C, and ascorbic acid was found in fresh *Spirulina* biomass, and the highest total flavonoid content was found in freeze-dried *Spirulina* biomass. On the other hand, the highest antimicrobial activity was found in both fresh and freeze-dried biomass due to the high carotenoid concentration, thus indicating the high concentration of phenolic compounds present in *Spirulina*.

Antimicrobial effect of Spirulina phenolic compounds

Antimicrobials are natural or man-made substances that can be used to control, kill, and/or inhibit the development and growth of microorganisms including viruses, bacteria, fungi, and protozoa (de Medina *et al.,* 2015; Sherrard *et al.,* 2014). *Spirulina* is known globally as a dietary supplement for human consumption, but in recent times, it has also been recognised as a good antimicrobial compound due to its ability to control and inhibit the development or growth of foodborne pathogens and

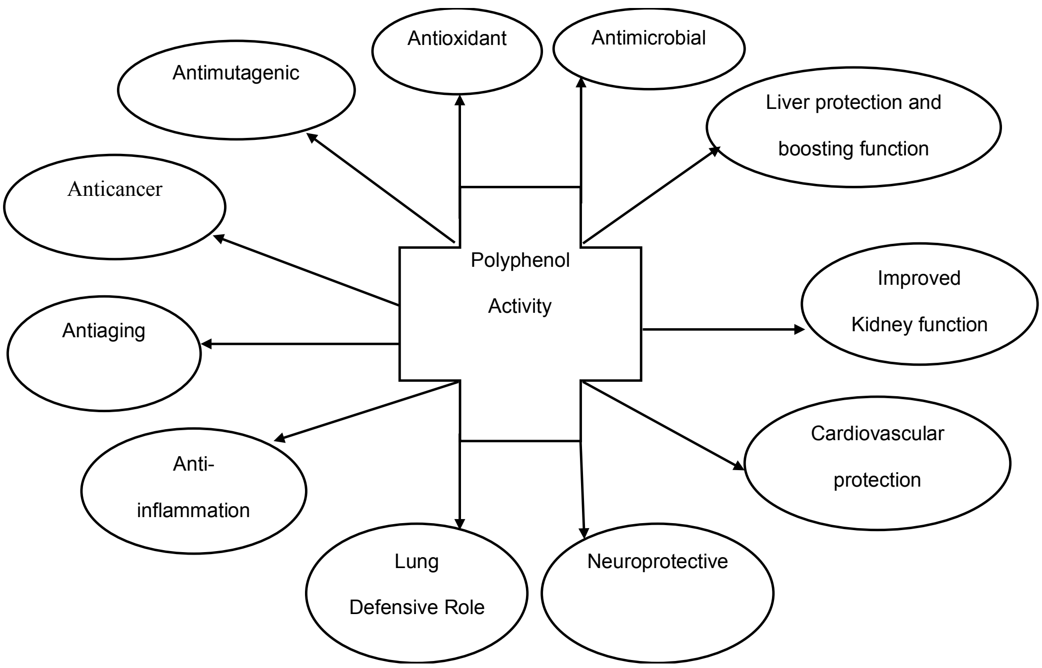

Figure 2. Polyphenol activities of *Spirulina* spp.

food spoilage microorganisms in the food industry (Duda-Chodak, 2013; Seghiri *et al.,* 2019; Elshouny *et al.,* 2020; Martelli *et al.,* 2020). Different studies reported the antimicrobial effect of *Spirulina* on the growth of different bacteria, yeasts, and moulds including *Alicyclobacillus acidoterrestris, Aspergillus flavus, Aspergillus niger, Bacillus subtilis, Cladosporium, Geotrichum, Micrococcus luteus, Penicillium*, and *Rhodotorula* (Souza *et al.,* 2011; Duda-Chodak, 2013).

The need to produce safe foods has encouraged the exploration of natural ingredients that possess antimicrobial activity (Souza *et al.,* 2011; Jung *et al.,* 2019; Martelli *et al.,* 2020). Some of these natural ingredients can be obtained from *Spirulina*. *Spirulina* phenolic compound extracts have shown excellent antimicrobial activities (Sun *et al.,* 2016; Swain *et al.,* 2017; Jung *et al.,* 2019). *Spirulina platensis* methanolic extract (1.15 mg phenolic compound per gram of *S. platensis* biomass) has shown antifungal effect against *A. flavus* at 54 µg/mL minimum inhibitory concentration (MIC), four times lower than the control (Souza *et al.,* 2011). Parisi *et al.* (2009) reported that *S. platensis* phenolic compound extract inhibited *S. aureus* at 47.5 mg/mL MIC. Scaglioni *et al.* (2019) reported 50% inhibition using 33.9 µg/mL *S. platensis* phenolic compound extract against *Fusarium*. Other studies reported good inhibition results of *S. platensis* phenolic compound extract against *E. coli, P. aeruginosa, B. subtilis, V. vulnificus, A. flavus*, and *A. niger* (El-Sheekh *et al.,* 2014; Rajasekar *et al.,* 2019; Seghiri *et al.,* 2019). C-phycocyanin from *S. platensis* inhibited drug-resistant bacteria such as *E. coli, K. pneumonia, P. aeruginosa*, and *S. aureus* (Sarada *et al.,* 2011).

Another study regarding the inhibition success of *Spirulina* phenolic compound extract against fungus was carried out, and the application of 8% liposomal phenolic compound was found more effective, with 90% inhibition, than the free extract solution, with 74% inhibition (Pugazhendhi *et al.,* 2015). Seghiri *et al.* (2019) reported that the methanolic extract of *Spirulina* phenolic compound exhibited a good result against pathogenic bacteria and fungi. It was assumed that the cell concentrate of *S. platensis* has the suitable ability to control the development and growth of pathogenic bacteria and fungi including *B. subtilis, S. aureus, E. coli, M. luteus, A. flavus, A. niger*, and *C. versicolor* (Usharani *et al.,* 2015; Pugazhendhi *et al.,* 2015; Seghiri *et al.,* 2019).

Other researchers reported that the extract of *S. platensis* is active for the control and inhibition of fungi and bacteria, and serves as a good source of antimicrobial activity due to the bioactive compounds like γ-linolenic acid, fatty acids, and interaction of lauric and palmitoleic acids (Mendiola *et al.,* 2007; Ramadan *et al.,* 2008; El-Sheekh *et al.,* 2014; Yang *et al.,* 2019). Other studies reported that the antibacterial activity of C-phycocyanin from S. platensis was amplified at higher concentrations, specifically 400 µL/disk and above (Safari *et al.,* 2019). This is possibly due to the resistance of those bacterial strains to the extract of C-phycocyanin differing from one species to another. As concluded in a study conducted by Safari *et al.* (2019), *Listeria* and *Streptococcus* were the most susceptible and resistant bacterial isolates to C-phycocyanin, respectively. The researchers' finding also highlighted that C-phycocyanin from *S. platensis* has excellent antimicrobial activity, and it can be used as a natural antimicrobial in different food commodities, mainly foods with high lipids, as well as in cosmetic products. Both antioxidant and antibacterial activities of C-phycocyanin decrease in long-term storage at -18°C, most likely due to the effect of the freezing temperature on the protein structure of C-phycocyanin (Safari *et al.,* 2019; Usharani *et al.,* 2015).

Conclusion

Fish and seafood products are the best means of food and nutrition security. At the same time, however, they are highly perishable, being vulnerable to pathogenic microorganisms including *E. coli, P. aeruginosa, B. subtilis, A. flavus,* and *A. niger*. Currently, *Spirulina* is known as a dietary supplement, and its bioactive compound extracts are known for their antimicrobial activity towards pathogenic bacteria and fungi. Promoting this emerging natural compound in fish and fish products will save both our food and the public from food-related risks. *Spirulina* bioactive compounds include polyphenols, phycocyanins, and fatty acids. Flavonoids and phenolic acids from the polyphenol category could be promising. Future perspectives of *Spirulina* should include issues such as multifunctional study of polyphenols including antimicrobial agent development, drug development, and application in animal and fish feed production. Implications for climate mitigation measures should also be further elucidated and studied.

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