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TESTING THE ANTIBACTERIAL POTENTIAL OF *SPIRULINA PLATENSIS* ETHANOLIC EXTRACT

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ABSTRACT

Spirulina platensis produces a wide range of potentially bioactive compounds for the pharmaceutical industry (secondary or primary metabolites), making spirulina a source for various types of medications. Five concentrations of ethanolic spirulina extract were tested in vitro for their potential antimicrobial effects against Gram+ (positive) bacterial strains, Staphylococcus aureus and Streptococcus pyogenes, and Gram- (negative) strains Shigella flexneri, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, and Haemophilus influenza type B. Bacterial sensitivity to the ethanolic spirulina extract was determined using the diffusion method, measuring the values of inhibition zones obtained when the extract was applied. The ethanolic spirulina extract at the tested concentrations did not exhibit antibacterial effects against the Gram+ bacterial strains studied. In the case of Gram- bacterial strains, the ethanolic spirulina extract at the tested concentrations showed antibacterial effects only against the S. typhimurium and E. coli strains.

KEY WORDS: *spirulina, antibacterian, ethanolic extract*

INTRODUCTION

Spirulina platensis (Arthrospira platensis) is a blue-green alga belonging to the Oscillatoriaceae family, found in spiral shapes in the oceans as well as in freshwater (Fujisava et *al.*, 2010). It contains a wide range of essential nutrients, including proteins (55-70%), carbohydrates (15-25%), essential fatty acids, primarily α-linolenic and sulfolipid (18%), vitamins (B12, provitamin A), minerals, and pigments such as

carotenoids (β-carotene), chlorophyll a, phycocyanin, and phycoerythrin (Mendes *et al.*, 2003).

Spirulina is a term commonly used for dietary supplements produced primarily from two species: *Arthrospira platensis* and *Arthrospira maxima*. *Spirulina platensis* is a valuable source of gamma-linolenic acid, an essential polyunsaturated fatty acid with significant economic importance (Sajilata *et al.*, 2008). It is widely used as a dietary supplement due to its immune-boosting properties and its ability to combat viruses. Studies have shown that it activates various immune cells, such as macrophages, natural killer cells, T cells, and B cells, while also promoting the production of gamma interferon and cytokines. Compounds naturally derived from *S. platensis* have been observed to act as inhibitors of certain encapsulated viruses by preventing viral attachment and entry into host cells (Karkos *et al.*, 2011; Yakoot *et al.*, 2012).

Certain algal species contain naturally-occurring bioactive compounds that exhibit potential as antimicrobial, antiviral, and antioxidant agents (Ozdemir *et al.*, 2004). These compounds include heptadecane and tetradecane in spirulina extracts, phenolic compounds in *Nostoc muscorum*, and lipopeptides in *Anabaena spp*., which are found in significant concentrations and contribute to the antimicrobial activity of these extracts (Fujita *et al*., 2002; Ozdemir *et al.*, 2004; El-Sheekh *et al.*, 2006).

Research has revealed that a range of algal components serves as a valuable source of safe and often more efficient alternatives to synthetic antimicrobial agents. Medications derived from algae are widely adopted because they are relatively safer and more cost-effective compared to their synthetic counterparts. The initial generation of medicinal products comprised raw botanical materials. With the advancement of the pharmaceutical industry, a second generation of algal medicinal products has emerged, driven by the processes of isolating and extracting algal content. These products are well-documented in scientific literature as a result of extensive research and studies. The utilization of algal extracts and phytochemicals with established antimicrobial properties holds the potential for significant contributions to therapeutic treatments.

It is well-known that products obtained naturally generally exhibit much easier and faster biodegradation compared to artificial ones, and from an ecological perspective, they are relatively more accepted in the market. These assumptions are based on the diversity and adaptability of microorganisms, which are capable of degrading most natural substances. To date, hundreds of extracts from various plant species have been studied to assess their impact on pathogenic microorganisms, yet relatively few have proven to be both highly active and non-toxic to human beings (Constantino *et al*., 2004; Newman *et al.*, 2007).

Based on the information presented above, this paper presents the results of tests aimed at identifying potential antimicrobial effects of various concentrations derived from an ethanolic extract of spirulina on standardized bacterial strains (Gram+ and Gram-). The novelty of this study lies in the examination of lower concentrations of the ethanolic extract in comparison to the existing literature in the field.

MATERIALS AND METHODS

The compounds tested

Extracts of *S. platensis* were carried out in ethyl alcohol (95%). Five concentrations of ethanolic spirulina extract: c1 - 512 µg/ml, c2 - 256 µg/ml, c3 - 128 µg/ml, c4 - 64 µg/ml, c5 - 32 µg/ml were administered to the chosen bacterial strains.

Bacterial strains

Antimicrobial activity was studied on standardized bacterial strains, Gram+ bacteria: *Staphilococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615) and Gram- bacteria: *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* tipe B (ATCC 10211).

Determination of Minimum Inhibitory Concentration (MIC)

Antimicrobial tests (antibiogram and other compounds testing) for microorganisms studied were carried out using the disc diffusion method (CLSI, 2017). A small quantity of each microbial culture was diluted in a sterile 0.9% sodium chloride solution until their turbidity matched the 0.5 McFarland standard. These suspensions were further diluted 1:10 in medium CHROM agar for bacteria (Oxoid) and then spread on sterile Petri plates.

Sterile micro compresses were applied on the agar surface in Petri dishes, and 10µL of each sample was added. Commercially available discs containing antibiotics were used as positive controls. The plates were incubated at 37° C for 24 h.

The inhibition zones were assessed by comparatively testing compound vs. amikacin (Ak), cloramphenicol (C) and gentamicin (Gn) for Gram+ bacteria. The diameters of the corresponding zones of inhibition were contained between varied: 11.2-12.5 mm (for Ak), 11.2-13 mm (for C) and 7-12 (for Gn). The inhibition zones were assessed by comparatively testing compound vs. amikacin (Ak), cloramphenicol (C), eritromicin (E), ampicilin (Amp) and gentamicin (Gn) for Gram- bacteria. The diameters of the corresponding zones of inhibition were contained between varied: 10.3-18.8 mm (for Ak), 7.7-17.7 mm (for C), 6-19.5 mm (for E), 10-22 mm (for Amp) and 8.7- 16.3 (for Gn).

All experiments were conducted in triplicate.

RESULTS AND DISCUSSIONS

When analyzing the effect of the ethanolic extract on Gram+ bacterial strains *S. pyogenes* and *S. aureus*, we observed relatively small inhibition zone sizes, measuring less than 2 mm, indicating the absence of a bacteriolytic effect on these two strains (figure 1). The five concentrations established for antibacterial potential testing proved to be ineffective in countering the growth and development of the two Gram+ bacterial strains. When comparing the inhibition zone sizes of the five concentrations of ethanolic spirulina extract with those determined for the control antibiotics (amikacin, chloramphenicol, gentamicin) after 48 hours of incubation, significantly lower values were observed ($p<0.05$).

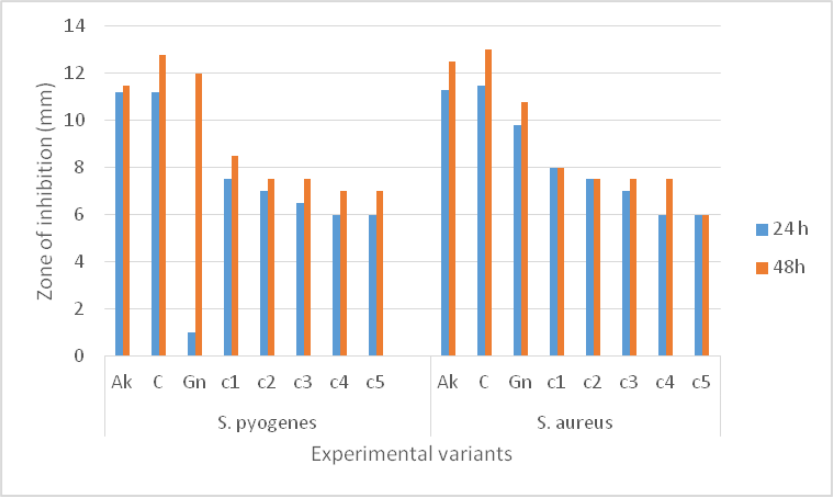

FIG. 1. Evolution of the values of the zones of inhibition to the action of extracts in ethanol on Gram+ bacterial strains

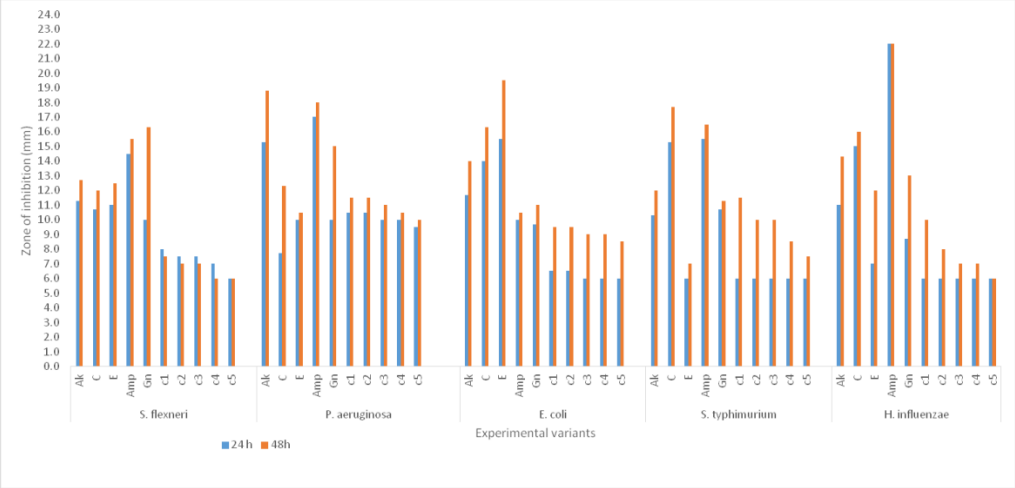

FIG. 2. Evolution of the values of the zones of inhibition to the action of extracts in ethanol on Gram- bacterial strains

To assess the antibacterial potential of the ethanolic extract of *S. platensis* on Gram- bacterial strains, the determination of inhibition zone sizes after 48 hours of incubation was carried out and subsequently compared with values recorded for the five control antibiotics (amikacin, chloramphenicol, erythromycin, ampicillin, gentamicin). The bacterial strain *Shigella flexneri* exhibited resistance to all five concentrations of the ethanolic extract of *S. platensis* applied, and the established concentrations proved to be ineffective against bacterial activity in this strain.

In the case of the *Pseudomonas aeruginosa* bacterial strain, the determined inhibition zone values indicated intermediate sensitivity to the application of c1-c2 of the ethanolic spirulina extract, while c3-c5 proved to be ineffective as potential antibacterial agents. The five concentrations of the ethanolic spirulina extract had different effects on the *E. coli* bacterial strain; c1 exhibited a bacteriolytic effect, while c2-c3 showed intermediate sensitivity, with the extract becoming inefficient at c4-c5. Decreasing the concentration of the ethanolic spirulina extract resulted in a decrease in antibacterial effectiveness against the Gram- *E. coli* bacterial strain.

When examining the Gram- bacteria strain *S. typhimurium*, a bacteriolytic effect was observed with the c1-c2 testing of the ethanolic spirulina extract, while for c3-c5 from the same extract, the bacterial strain exhibited intermediate sensitivity. In the case

of *S. typhimurium*, there was a decline in the antibacterial efficacy of the ethanolic spirulina extract as the tested concentrations decreased.

For the *H. influenzae* bacterial strain, after applying the five concentrations of the ethanolic spirulina extract and 48 hours of incubation, we observed insignificant inhibition in terms of pathogen growth. The strain demonstrated resistance to the tested concentrations of the ethanolic extract.

Upon comparative analysis of the inhibition zone sizes resulting from the five concentrations of the ethanolic spirulina extract with the values obtained for the selected antibiotics, it is evident that significantly lower values were observed ($p<0.05$), except for one case in the Gram- bacterial strain *S. typhimurium*.

Spirulina platensis stands as one of the most promising candidates for synthesizing potential therapeutic compounds. It is known for producing both intracellular and extracellular metabolites with various biological activities, including antifungal, antiviral, and antibacterial properties (MacMillan *et al.*, 2002; Kaushik & Chauhan, 2008; Kumar *et al*., 2011).

Extracts of *S. platensis* prepared using various solvents were tested on microbial strains, and their antimicrobial effects varied significantly. Among the extracts most studied for their antimicrobial properties are the ethanolic and methanolic extracts, which exhibit a wide spectrum of antibacterial and antifungal activities.

The methanolic extract of *S. platensis* was analyzed for its antimicrobial potential against bacterial strains *S. aureus*, *E. coli*, and *P. aeruginosa* (Parisi *et al.*, 2009). Studies focusing on the antibacterial potential of the ethanolic extract were conducted on bacterial strains, including *S. thyphi*, *S. flexneri*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *Klebsiella sp.* (Parekh & Chanda, 2007; Sudha *et al.*, 2011; Pannindriya *et al.*, 2020). The antifungal potential of the methanolic extract of S. platensis was demonstrated against the *A. flavus* species, while the ethanolic extract exhibited antifungal activity against *A. niger* and *C. albicans* species (Souza *et al.*, 2011; Sudha *et al.*, 2011; Pannindriya et al., 2020).

The varying response of Gram+ and Gram- bacterial strains to the tested concentrations of the ethanolic extract, along with the absence of antibacterial effects for most strains, leads us to consider further research, including testing higher concentrations of spirulina.

CONCLUSIONS

The concentrations of the ethanolic spirulina extract applied in this study resulted in insignificant inhibition of growth for the two Gram+ bacterial strains, which

exhibited resistance to the tested concentrations. In the case of the Gram- bacterial strains, the effects of the five concentrations of the ethanolic extract varied significantly depending on the strain and concentration applied. Thus, the antibacterial effect of the tested extract was only evident in the case of the *S. typhimurium* strain at all five concentrations, while for the *E. coli* strain, the effect was observed only at c1-c3.

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