Open Access Research Journal of
Life Sciences
Journals home page: https://oarjpublication/journals/oarjls/
ISSN: 2783-025X (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Determination of antimicrobial activity of *Chlamydomonas reinhardtii* extracts obtained with different solvents

Roxana Olvera-Ramírez [1], Nora Beatriz Medina-Jaritz [1] and Margarita Pineda-López [2]

[1] *Department of Botany. National School of Biological Sciences. National Polytechnic Institute.*
[2] *Department of Microbiology. National School of Biological Sciences. National Polytechnic Institute.*

Open Access Research Journal of Life Sciences, 2025, 09(01), 027-031

Publication history: Received on 07 December 2024; revised on 02 February 2025; accepted on 05 February 2025

Article DOI: https://doi.org/10.53022/oarjls.2025.9.1.0022

Abstract

The abuse in the use of antibiotics has resulted in the selection of bacterial strains with resistance to them, making it evident that new classes of antibiotics are needed, with novel structures to combat this trend, for this reason is necessary to find alternative sources. There are natural products, such as those obtained from green algae, that have an inhibitory effect against various pathogenic bacteria; this group of algae includes the genus *Chlamydomonas*. The objective of this work was to determine if extracts of the green algae *Chlamydomonas reinhardtii* made with some solvents have antimicrobial activity. *C. reinhardtii* was propagated in BG-11 medium; under optimal light conditions (photoperiod of 16 h light: 8 h dark), constant aeration and temperature of 28 °C; the biomass was harvested and the extracts were made by adding 1g of wet biomass to a volume of 3mL of the following solvents: hexane, methane and ethyl acetate, and the determination of the antimicrobial activity was carried out by the Kirby-Bauer method. The microorganisms used were *Staphylococcus aureus* ATCC 259223, *Pseudomonas aeruginosa* ATCC 9028, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Salmonella typhi* ATCC 6534. It was found that the extracts obtained with methanol and hexane at the two concentrations used (23µg/70µL and 33µg/100µL) showed antimicrobial activity against the 5 ATCC strains used, when testing the concentration of 33 µg/100µL, a greater inhibition was found; the extract obtained with ethyl acetate did not show activity against any of the ATCC strains.

Keywords: Antibacterial activity; Green algae; *Chlamydomonas reinhardtii*; Kirby-Bauer method; Hexane; Methane and ethyl acetate extracts

1. Introduction

The emergence and spread of drug-resistant pathogens, that is, those that have acquired new mechanisms of resistance to antimicrobials, continue to compromise the ability to treat common infections. Of particular concern is the rapid global spread of multi- and pan-resistant bacteria known as superbugs that cause infections that cannot be treated with antimicrobial medicines, such as antibiotics [1].

Antibiotics are becoming increasingly ineffective as drug resistance spreads around the world, leading to more difficult-to-treat infections and increased mortality (700,000 deaths each year globally) [1]. Therefore, new antibacterials are urgently needed, for example, to treat infections due to Gram-negative bacteria resistant to carbapenem antibiotics identified on the list of priority pathogens [2].

A source of antibacterials are microalgae, photosynthetic organisms, capable of generating products such as oils, proteins, biodiesel, antioxidants and other food supplements, which are also useful in the generation of bioactive products (these compounds fulfill functions in the body that can promote good health) and can be used in the search for antimicrobial agents, capable of inhibiting the proliferation of certain bacteria [3]. The factor that has made its cultivation and industrial exploitation possible is the development and implementation of biotechnology [4].

* Corresponding author: Roxana Olvera-Ramírez

The pharmacological activities reported in compounds from cyanobacteria and microalgae include antiprotozoa, bactericidal, antiviral, cytotoxic, inhibitors of proteases or calcium channels, immunomodulatory and antioxidants [5, 6]. One of the elements associated with the great diversity of activities found is the wide spectrum of secondary metabolites present in these organisms [7], among which are lipopeptides, amino acids, proteins, fatty acids, macrolides, amides, phenols, alkaloids, low molecular weight organic compounds and carbohydrates [8]. The objective of this work was to determine if hexane, methane and ethyl acetate extracts of *Chlamydomonas reinhardtii* have an inhibitory effect on the growth of reference bacterial strains.

2. Material and methods

2.1. Microalgae and bacterial strains

The *Chlamydomonas reinhardtii* strain used in this work was provided by the Plant Physiology Laboratory of the Department of Botany of the National School of Biological Sciences of the National Polytechnic Institute.

The bacterial strains used in this work were provided by Microbiology Department of the National School of Biological Sciences of the National Polytechnic Institute, the strains used were *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhi* ATCC 6534, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633.

2.2. *Chlamydomonas reinhardtii* culture

Chlamydomonas reinhardtii strain was propagated in BG-11 medium, under optimal light conditions (photoperiod of 12 h light:12h dark) and constant aeration (Fig. 1).

Figure 1 Culture of *C. reinhardtii* strain

2.3. Bacterial strains culture

For the conservation and cultivation of the bacterial strains used in the antimicrobial activity tests, nutrient agar Petri plates with yeast extract were used.

2.4. Extract preparation

In three tubes, 1 g of the harvested biomass of *C. reinhardtii* was added, and to each one were added 10 mL of methanol, 10 mL of hexane and 10 mL of ethyl acetate respectively, and left to stand for 24 hours. Each of the solvents used (methanol, hexane and ethyl acetate) was evaporated to dryness, the extract was diluted with 3 mL of the solvent used for the extraction. Finally, the extract was sterilized by filtration using a Millipore filter with a pore size of 0.22 µm and was kept refrigerated until use.

2.5. Antimicrobial testing

The Kirby-Bauer method was used, bacterial strains were streaked onto Mueller-Hinton agar. Subsequently, 5 sterile filter paper discs with a diameter of 6 mm were placed, 3 of these discs impregnated with a concentration of 23 µg/70 µL or 33 µg/100 µL of the extract, plus a disc impregnated with the solvent used (methanol, hexane and ethyl acetate as a negative control, in the fifth disc 20 µL of chloramphenicol was used as a positive control. Five replicates were made for each strain and each concentration of the extract used; they were incubated for 24 h at 37 °C, then the plates were checked and the diameters of the halos formed were measured fig. 2.

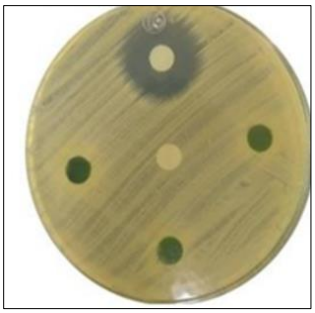

Figure 2 Distribution of disks in the agar diffusion method.

The three-way ANOVA statistical analysis was applied to the results of the antimicrobial effect produced by the extracts obtained on the ATCC microorganisms used, using the Sigmaplot 12.0 program.

3. Results and discussion

The table 1 shows the averages of the halos obtained with the extracts made with the different solvents, and for the strains of bacteria used.

Table 1 Antimicrobial effect shown by *Chlamydomonas reinhardtii* extracts with different solvents (methanol, ethyl acetate and hexane).

Strain ATCC	**Average halos obtained with the extract of *Chlamydomonas reinhardtii* with methanol in mm**		**Average halos obtained with the extract of *Chlamydomonas reinhardtii* with hexane in mm**		**Average halos obtained with the extract of *Chlamydomonas reinhardtii* with ethyl acetate in mm**	
	33µg/100 µL	**23µg /70 µL**	**33µg /100 µL**	**23µg/70 µL**	**33µg /100 µL**	**23µg/70 µL**
Escherichia coli ATCC 8739	10	6	6	2	0	0
Bacillus subtilis ATCC 6633	5	6	6	2	0	0
Pseudomonas aeruginosa ATCC 9027	5	8	8	2	0	0
Salmonella Typhi ATCC 6534	4	6	6	2	0	0
Staphylococcus aureus ATCC 25923	7	5	5	2	0	0
Negative control	0	0	0	0	0	0
Positive control	14	14	14	18	14	18

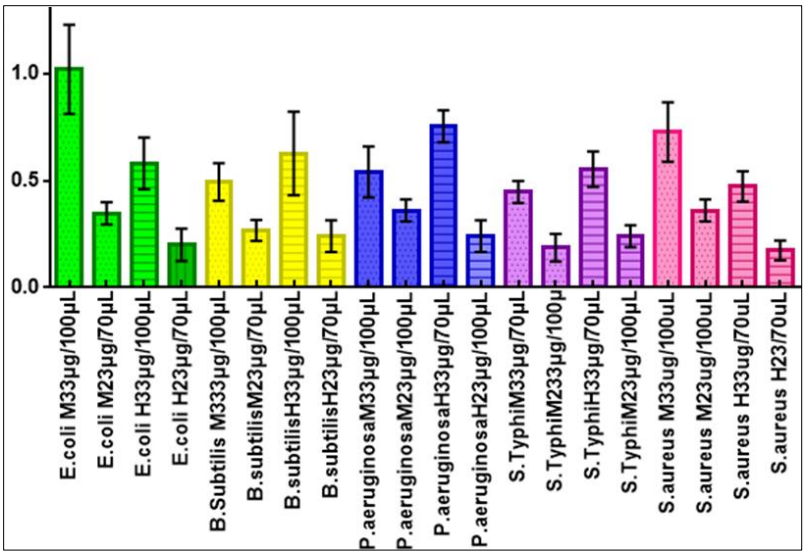

Figure 3 Antimicrobial effect observed in *Chlamydomonas reinhardtii* extracts obtained with methanol and hexane at different concentrations (halo diameter)

Note: *E. coli* M33 (methanol extract 33µg/µL) *E.coli* M23 (methanol extract 23µg/70 µL), *E. coli* H33 (hexane extract 33µg/ 100µL), *E coli* H23 (hexane extract 23 µg/70µL); *B. subtilis* M33 (methanol extract 33 µg/100 µL), *B. subtilis* M23 (methanol extract 23 µg/70µL), *B. subtilis* H33 (hexane 33 µg/100µL), *B. subtilis* H23 (hexane 23 µg/70µL); *P. aeruginosa* M33 (methanol extract 33 µg/100 µL), *P. aeruginosa* M23 (methanol extract 23 µg/70µL), *P. aeruginosa* H33 (hexane 33 µg/100µL), *P. aeruginosa* H23 (hexane 23 µg/70µL), *S. typhi* M33 (methanol extract 33µg/100µL), *S. typhi* M23 (methanol extract 23µg/70µL), *S. tiphy* H33 (hexane 33 µg/100µL*), S. tiphy* H23 (hexane 23 µg/70µL); *S. aureus* M33 (methanol extract 33 µg/100 µL), *S. aureus* M23 (methanol extract 23 µg/70 µL), *S. aureus* H33 (hexane 33 µg/100µL), *S. aureus* (hexane 23 µg/70µL).

The antimicrobial activity of the *Chlamydomonas reinhardtii* extract obtained with the different solvents (methanol, hexane and ethyl acetate) was observed with the formation of inhibition halos with each of the extracts obtained when challenging them against the ATCC strains used (Figure 3).

Of the three solvents used, only two of them showed antimicrobial activity against all the microorganisms used in the test; methanol and hexane were efficient solvents for obtaining extracts with this activity, since an inhibitory effect was observed in each ATCC strain used at the two concentrations used (23 µg/70 µL and 33 µg/100 µL); however, the extract obtained with ethyl acetate at both concentrations did not have an inhibitory effect against any of the challenged ATCC strains. In addition, the statistical analysis indicates that there are significant differences between the two concentrations of the extract (33 µg / 100 µL and 23 µg / 70 µL) for both hexane and methane. When comparing the two solvents, it was found that there is no significant difference between the antimicrobial activity between the two solvents on the strains used, except for *E. coli* where the methanolic extract at the highest concentration presented a greater inhibition halo than the others.

The inhibitory effect present with the extracts obtained with methane and hexane could be attributed to the fact that some compound of *Chlamydomonas reinharditti* was extracted with these solvents that inhibited the growth of the bacterial strains used; the molecules that have frequently been reported in cyanobacteria and microalgae as responsible for the inhibitory activity are fatty acids, such as: palmitic acid, oleic acid, linoleic acid [9]. In *Dunaliella salina* antimicrobial activity was detected in several extracts and may be explained not only by several fatty acids, but also by such compounds as α and β-ionone, β-cyclocitral,neophytadiene and phytol [10].

4. Conclusion

The extracts made with methanol and hexane showed antimicrobial activity against the 5 ATCC strains used: *E. coli, B. subtilis, P. aeruginosa and S. typhi*, the extract made with ethyl extract did not show activity against any of the strains. Only for the *E. coli* strain there was a significant difference in diameter when the extraction was performed with methanol.

Compliance with ethical standards

Acknowledgments

This work was supported by the Commission for the Operation and Promotion of Academic Activities (COFAA) and the Program of Incentives for Teaching Performance (EDD) from the National Polytechnic Institute (IPN).

Disclosure of conflict of interest

The authors declared that they have no competing interests. The authors have participated on completing this manuscript.

References

[1] Giono-Cerezo S, Santos-Preciado J I, Rayo M, Torres-López F J, Alcántar-Curiel M.D. Antimicrobial resistance. Importance and efforts to contain it. Gaceta médica de México, 2020, 156(2), 172-180.

[2] World Health Organization (2015). Global action plan on antimicrobial resistance. World Health Organization.

[3] Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E. Metabolites from algae with economic impact. Comp Biochem Physiol C Toxicol Pharmacol. 2007. Jul-Aug;146(1-2):60-78.

[4] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Applied Microbiol Biotechnology. 2004. 65, 635–648.

[5] Volk RB, Furkert FH. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiol Research. 2006;161(2):180-6.

[6] de Morais M.G, Vaz Bda S, de Morais EG, Costa JA. Biologically Active Metabolites Synthesized by Microalgae. Biomedical Research International. 2015:835761.

[7] Singh RK, Tiwari SP, Rai AK, Mohapatra TM. Cyanobacteria: an emerging source for drug discovery. Journal of Antibiotics (Tokyo). 2011. Jun;64(6):401-12.

[8] Babadzhanov, A.S., Abdusamatova, N., Yusupova, F.M. et al. Chemical Composition of *Spirulina platensis* Cultivated in Uzbekistan. Chemistry of Natural Compounds, 2004, 40, 276–279

[9] Ouattara B, Simard RE, Holley RA, Piette GJ, Bégin A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms.. International Journal of Food Microbiology. 1997.Jul 22;37(2-3):155-62.

[10] Herrero M, Ibañez E, Cifuentes A, Reglero G, Santoyo S. *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. 2006. Journal of Food Protection, 69:2471-2477.