In vitro evaluation of hemolytic properties of *Raphidiopsis raciborskii* strains: Geographic and toxicological variations

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Introduction

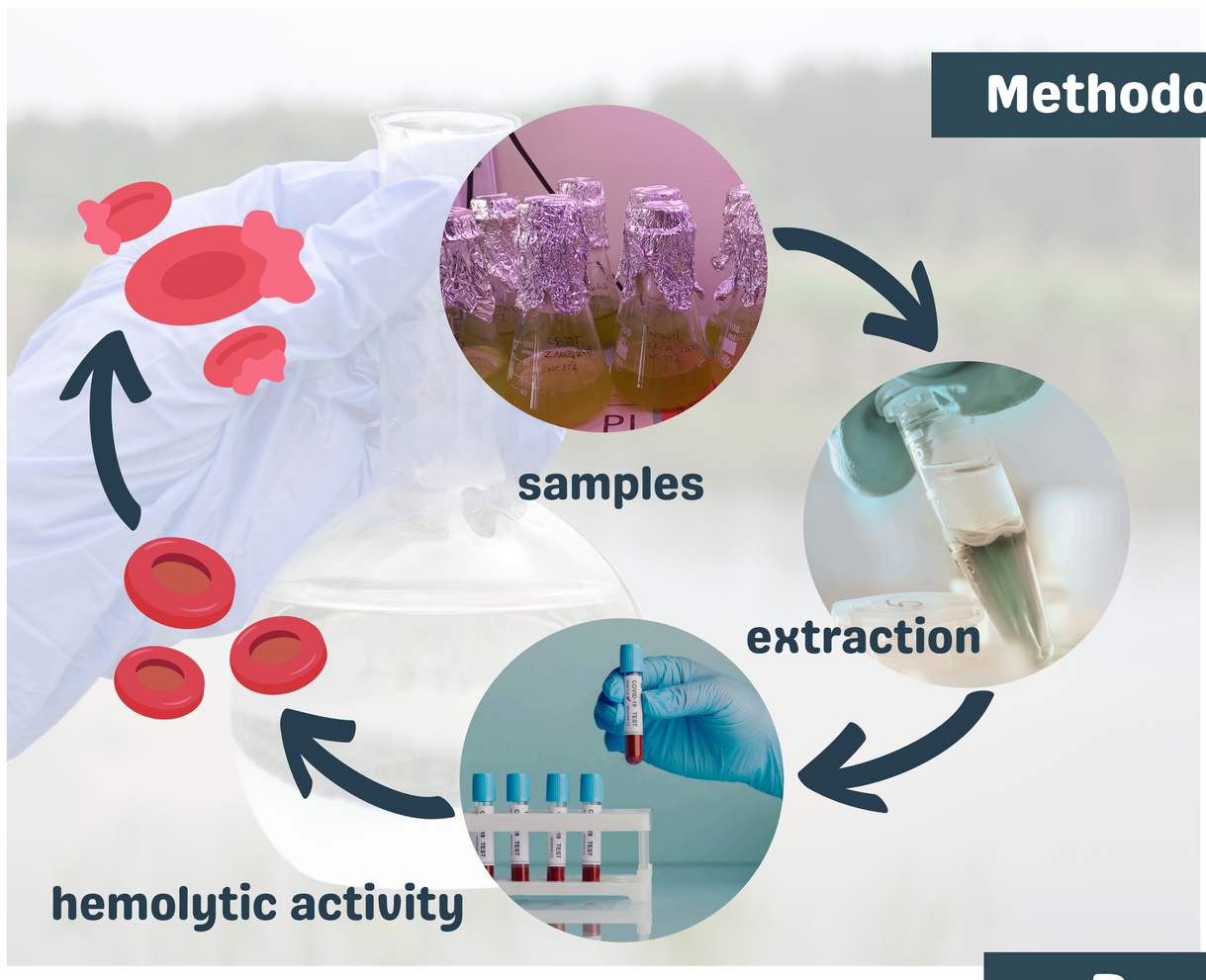
Raphidiopsis raciborskii (Wołoszyńska) Aguilera & al. 2018 is a freshwater cyanobacterial species widely distributed in aquatic environments. Certain strains of this species have the potential to produce a range of harmful metabolites, including cylindrospermopsin and saxitoxin. These toxins are known to have harmful effects on humans and other organisms, posing a significant risk to both human health and the surrounding ecosystem. Therefore, the investigation of the toxic effects of *R. raciborskii* metabolites is of great importance, as they can be present in drinking water, recreational waters, or contaminate food intended for both animals and humans.

Samples

R. raciborskii strains from different geographical origin and toxicity

NON-TOXIC STRAINS		CYLINDROSPERMOPSIN (CYN) PRODUCTION		MICROCYSTIN PRODUCTION
POLAND		ISRAEL	AUSTRALIA	FROM THE PASTEUR CULTURE COLLECTION OF CYANOBACTERIA (PCC)
Lakes	Strains	Lakes / Strains	Lakes	Strains	*Microcystis aeruginosa*
Kierskie Male	UAM/DH-KmRr	Kinneret / KLL07	Solomon Dam, Palm Island	CS-505, CS-506	PCC 7813
Miejskie	UAM/DH-MRr				
Biskupinskie	UAM/DH-BiRr				
Pniewskie	UAM/DH-PnRr				
Boczowskie	UAM/DH-BoRr				
Żnińskie Małe	UAM/DH-ZmRr				

Methodology



Cyanobacterial biomass was harvested after five weeks of growth and extracted with 75% methanol. The obtained dry extracts, dissolved in PBS, were tested in three different concentrations on erythrocytes.
Three different concentrations of extract samples (75, 150, and 300 mg/mL) prepared in PBS (1 mL) were mixed with 1 mL of a 5% erythrocyte suspension in PBS. Following incubation at 37 °C for 1 h, the samples were centrifuged at 1200 rpm for 15 minutes. The absorbance of the obtained supernatant was measured at 540 nm. PBS was used as the negative control, while a 1% sodium dodecyl sulfate (SDS) solution served as the positive control. The following formula was used to calculate hemolytic activity:

$$\% \text{ hemolysis} = ((A_s - A_0) - A_k / (A_{SDS} - A_k)) \; x \; 100$$

A_S is the absorbance of samples with extract
A_0 is the absorbance of the corresponding concentration of extract in PBS
A_{SDS} is positive control with SDS solution
A_k is negative control (only RBCs in PBS)

Results

The results of the hemolytic properties of *R. raciborskii* extracts at three different concentrations (75 µg/mL, 150 µg/mL, and 300 µg/mL) showed that strains from Poland exhibited the highest hemolytic activity among examined samples. The hemolysis increased with concentration, with 300 µg/mL consistently causing the highest percentage of hemolysis across all tested strains. Strains from Israel and Australia showed minimal or no hemolytic activity. The reference strain *M. aeruginosa* exhibited negligible hemolysis at all tested concentrations. Although strains from Poland localities are refer as non-toxic, one of them, isolated from Żnińskie Małe locality, caused approximately 45% of homolyses at the concentration of 300 µg/mL. In contrast, two *R. raciborskii* strains from Australia with confirmed production of cylindrospermopsin cyanotoxin and *M. aeruginosa*, known to produce microcystin, did not show hemolytic effects. This suggests that the known cyanotoxins are not main compounds involved in the hemolysis. Moreover, the variability in responses across hemolytic tests indicates that each strain may produce a unique set of compounds with distinct effects.

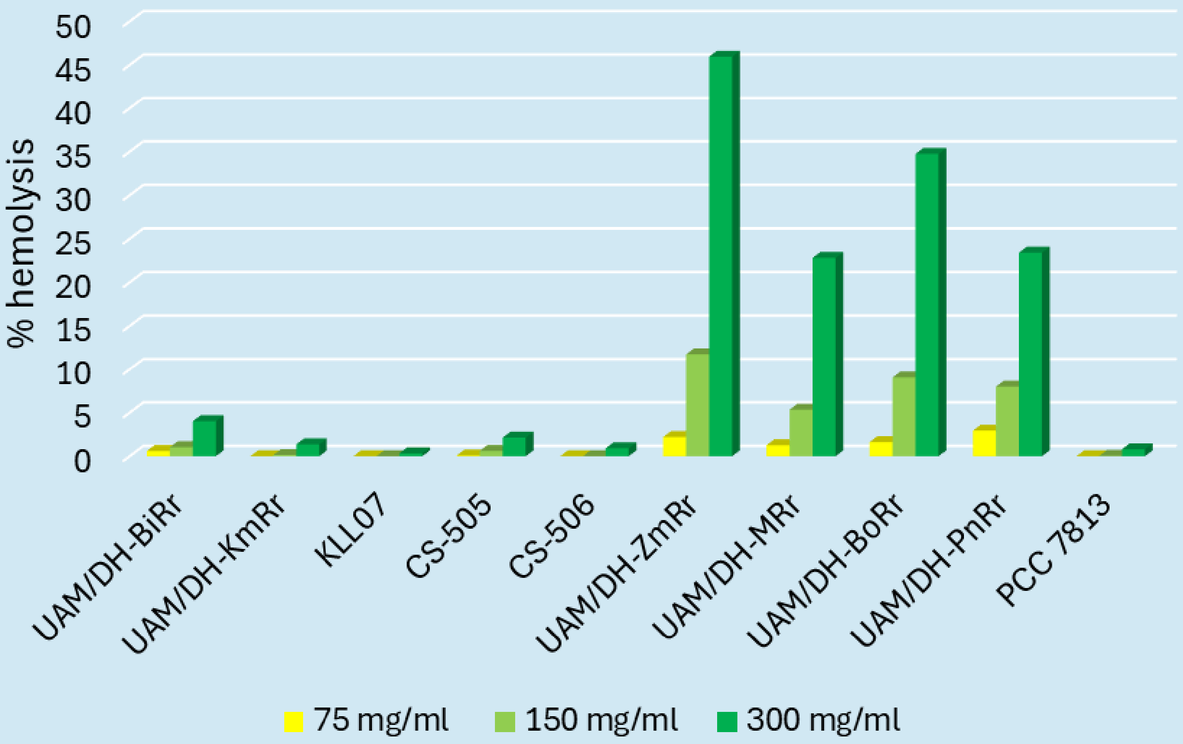


Figure 1. Hemolytic activity of *R. raciborskii* extracts

Conclusion

These findings highlight the potential ecological and health risks not only associated with known cyanotoxin-producing *R. raciborskii* strains but also with strains producing unidentified hemolytic metabolites, emphasizing the need for further research to identify and assess the impact of undefined and undescribed metabolites.

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