"GREEN" SOLVENTS AS AN ALTERNATIVE FOR BIOACTIVE AND HIGH ADDED-VALUE COMPOUNDS ISOLATION FROM *CHRYSOCHROMULINA ROTALIS*

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IX SIMPOSIO de Investigación EN CIENCIAS EXPERIMENTALES

1. Introduction

Many bioactive and high added-value compounds from microalgae are of industrial and economic interest, due to their numerous applications in the food, cosmetic, or pharmaceutical industries. Traditional extraction methods for these compounds from microalgae typically involve the use of organic solvents that are often harmful to the health of workers and/or the environment. Such solvents as hexane, toluene, dichloromethane (DCM) and n-butanol, are frequently used for sequential gradient partition of microalgae biomass, in order to isolate bioactive compounds contained in it[1]. The search for new extraction methods or the improvement of current processes could help to find more sustainable and environmentally friendly processes, and, in this regard, "green" solvents are a little-explored source of viable alternatives that could solve this problem[2].

2. Objective

The main objective of this work is to propose an alternative sequential gradient partition method through the use of "green" solvents instead of other classical and hazardous solvents, and which in turn allows obtaining similar bioactive and high added-value compounds extraction yields from microalgae biomass.

3. Materials and methods

Biomass: ***Chrysochromulina rotalis***

GREEN SOLVENT ASSAYS

SOLUBILITY TEST
◊ 1:1 v/v solvent mix with different MeOH/H_2O mixtures.

SOLID-LIQUID EXTRACTION
◊ 10 mg biomass extraction with MeOH (40ºC, 2 min).

LIQUID-LIQUID EXTRACTION
◊ Methanolic extract partition with organic solvent and proper MeOH/H_2O mixture (previously determined).

ORGANIC EXTRACT VALORIZATION
◊ Photodiode-array HPLC Carotenoids analysis[3].
◊ Fatty acids determination by GC-FID[4].

SEQUENTIAL GRADIENT PARTITION

⇒ Double solid-liquid extraction with methanol (40ºC, 30 min).
⇒ Multiple liquid-liquid extraction steps with polarity gradient organic solvents (hexane, toluene, DCM and n-butanol) in different MeOH/H_2O mixtures (9:1, 7:3, 6:4 and 0:10, respectively)[1].
⇒ Antiproliferative activity assays with extracts from each extraction step.

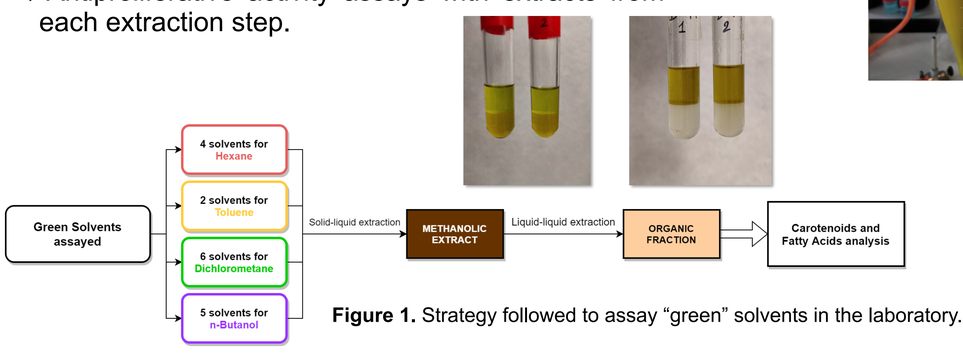


Figure 1. Strategy followed to assay "green" solvents in the laboratory.

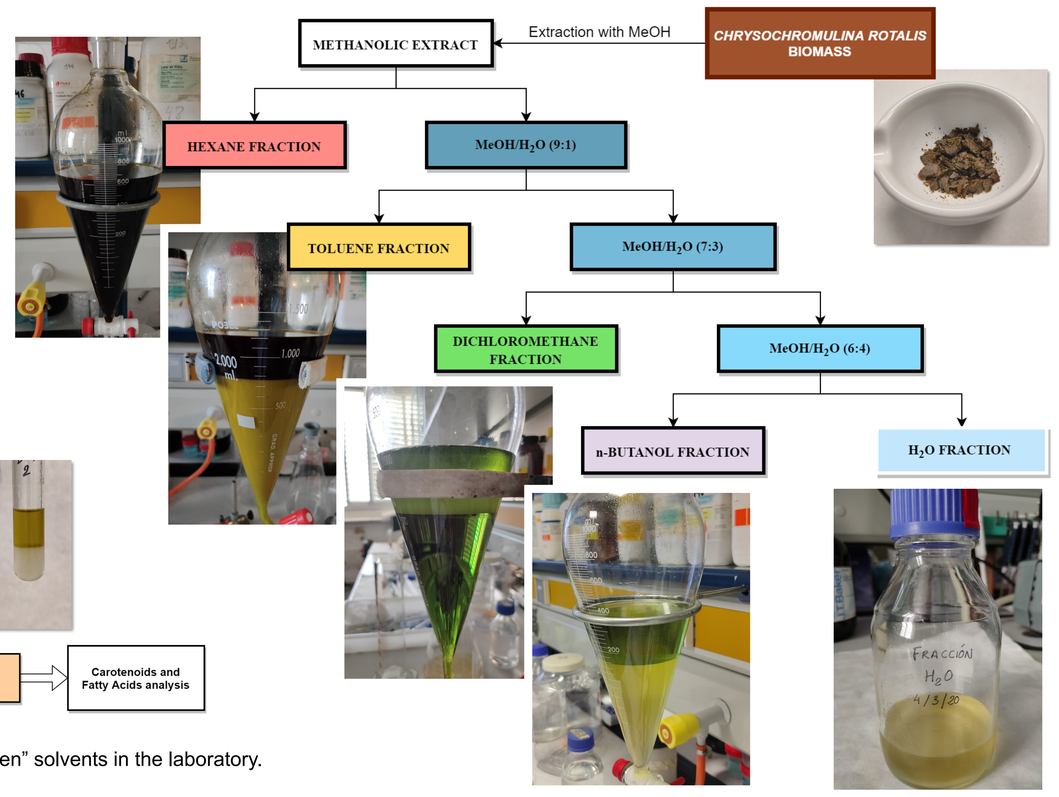


Figure 2. Diagram and pictures of the sequential liquid-liquid extraction process with polarity gradient used as control experiment for bioactive and high added-value compounds recovery yields.

4. Results and discussion

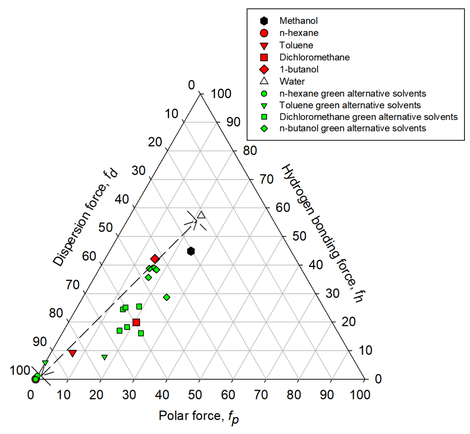


Figure 3. Ternary diagram for Hansen's solubility parameters, representing classical solvents (black, white and red symbols) and proposed solvents (green symbols).

For liquid-liquid extraction experiments, only MeOH/H_2O mixtures with **total inmiscibility** (marked as '++' in Table 1) for each solvent were selected.

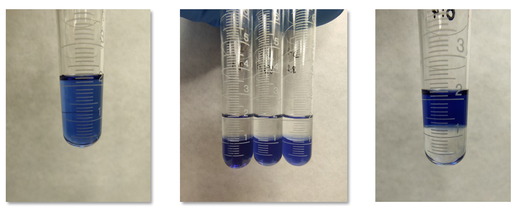


Table 1. Solubility assays for each green solvent proposed in this work (++, totally inmiscible; +, partially miscible; -, miscible).

Solvent to replace	Proposed Green solvents	MeOH/H_2O mixture	Inmiscibility
Hexane	Cyclohexane	9:1	++
	Heptane	9:1	++
	Isooctane	9:1	++
	Pentane	9:1	++
Toluene	Chlorobenzene	7:3	++
	Methylcyclohexane	7:3	++
DCM	t-amyl methyl ether	6:4	++
	n-butyl acetate	6:4	++
	Cyclopentyl methyl ether	6:4	++
	Ethyl acetate	6:4	-
		5:5	-
		4.5:5.5	-
		4:6	++
	Isobutyl acetate	6:4	+
		5.5:4.5	++
		5:5	+
	Methylisobuthyl ketone	6:4	-
		5:5	+
		4.5:5.5	++
n-butanol	t-amyl alcohol	0:10	++
	Benzyl alcohol	0:10	++
	Dimethyl carbonate	0:10	++
	Isoamyl alcohol	0:10	++
	1-pentanol	0:10	++

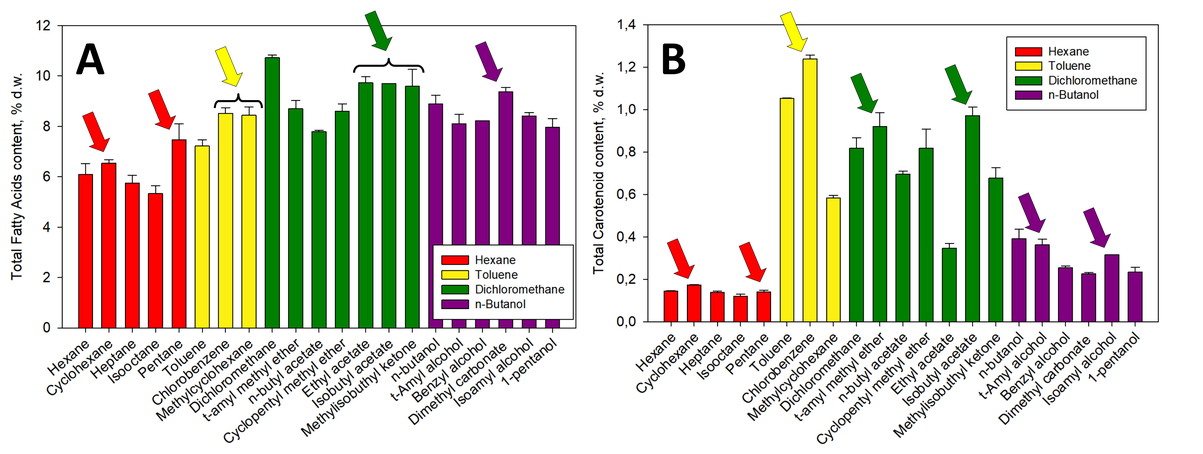


Figure 4. Total Fatty Acids (A) and Carotenoids (B) content determined from each green solvent extract (hexane, toluene, DCM and n-butanol as control, followed by its respective alternative assayed solvents). With arrows those solvents that extracted the highest amount of fatty acids and carotenoids, or an amount similar to that extracted by the control, have been marked.

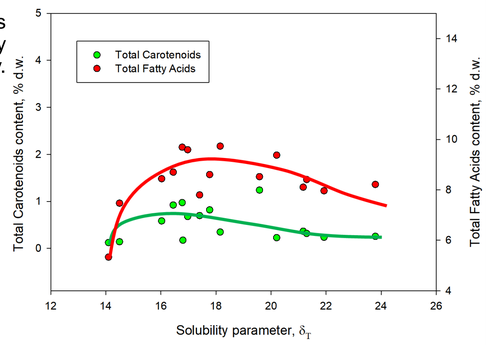
In order to propose an alternative sequential gradient partition, only one green solvent was selected for each liquid-liquid extraction step. Thus, the selection criterion followed was the one extracting the **most total amount of compounds** from the methanolic extract. Also, other criteria such as **green score** or **additional operational costs** derived from the use of these solvents were taken into account. Finally, the solvents selected were **cyclohexane** as a substitute for hexane, **chlorobenzene** instead of toluene, **isobutyl acetate** for DCM, and **isoamyl alcohol**, for replacing n-butanol.

Table 2. Antiproliferative activity found from the different sequential gradient partition fractions (++, high activity; +, little activity; -, no activity).

Antiproliferative activity				
Hexane fraction	Toluene fraction	Dichloromethane fraction	n-butanol fraction	H_2O fraction
-	+	++	-	-

Toluene and DCM fractions were shown to have **antiproliferative activity** against different tumor cell lines, being higher for the DCM fraction.

Figure 5. Total Carotenoids and Fatty Acids extracted as a function of the solubility parameter δ_T, related with solvent polarity.



5. Conclusions

◊ **Seventeen "green" solvents** have been assayed for fatty acids and carotenoids extraction performance, against control solvents. At least five of them showed **higher fatty acids content** extracted than in the control, and another five solvents did the same for **carotenoids content**.
◊ A sustainable and more **environmentally friendly** method for bioactive and high added-value compounds isolation is proposed.
◊ Sequential polarity gradient fractionation showed potential to **isolate bioactive compounds**, mainly in the DCM fraction and, to a lesser extent, in toluene.

6. References

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7. Acknowledgements

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