

Optimizing the Water-based Extraction of *Rubia tinctorum* L. Pigments

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Abstract. *Water is one of the greenest solvents, but it is often limited to the extraction of polar compounds. Microwave assisted extraction (MAE) was used to extend the range of polarity of extracted compounds from madder. The main pigments in madder are anthraquinones, generally extracted with hydroalcoholic solvents due to their medium polarity. However, water-MAE was as efficient as reference hydroalcoholic MAE and soxhlet.*

Keywords. Microwave-assisted extraction, water-based extraction, *Rubia tinctorum*, response-surface methodology, HRMS/MS, anthraquinones

1 Introduction

In the current context of ecological crisis, the use of natural products and the development of green extraction methods to retrieve them are of great interest. Amongst these, plant pigments are gaining attention after having been replaced by cheaper synthetic pigments during the XIXth century [1].

Madder pigment (*Rubia tinctorum* L.) contains mostly anthraquinones in their free and glycosylated forms [2]. Anthraquinones are orange-reddish compounds resistant to washing, UV and temperature making them a good ingredient in cosmetics [1]. Because of their medium polarity, they are generally extracted by hydroalcoholic solvents [2].

Water is one of the greenest solvents. It is accessible, non-toxic, non-pollutant and easily disposable compared to organic solvents. However, its high polarity makes it selective towards very polar compounds and less effective for the extraction of other compounds of interest [3]. Some methods, like microwaves can help lower the polarity of water and improve the extraction of less polar molecules [3].

In this paper, water-based microwave-assisted extraction (MAE) was developed to extract pigments from madder. The extraction was optimized using an experimental design. The optimized extract was compared to reference hydroalcoholic extracts.

2 Development of the water-based microwave assisted extraction

2.1 Experimental design

Microwaves cause dipole rotation and ionic conduction both in the solvent and in the sample, which helps to generate heat faster and to increase solubility and diffusivity of compounds. Water is particularly able to absorb microwaves and to generate heat due to its high dielectric constant (78.3) and dielectric loss factor (1.87) [4].

Table 1. Coded and uncoded variables used in the experimental design

X_1 (cycles)	X_2 (power in W)	X_3 (ratio in mg/20 mL)	Coded variables
1	200	100	-1
3	600	550	0
5	1000	1000	1

To develop an efficient extraction method with no degradation of the extracts, some parameters must be optimized. We used response surface methodology (RSM) with a Box-Behnken experimental design conducted and analyzed on the Ellistat software. It allowed us to analyze together the influence of the number of cycles, power and plant to water ratio on the yield (table 1) in a limited number of assays. To prevent overheating, each cycle only lasted 30 seconds and the extracts were cooled down in icy water between each cycle.

The experimental design involved 17 extractions including 3 central points. The number of cycles, as well as its interactions with the other two factors were significant. Quadratic terms X_1X_1 and X_3X_3 were also significant. The maximum yield was obtained after 5 cycles at 1000 W and 550 mg/20 mL (extract 16).

All extracts displayed a yellowish to bright orange color that was not correlated to the yield (Fig.1). Indeed, extract 16 was not the most orange extract despite having the best yield. The best combination of yield and color was extract 13 (1 cycle, 1000 W, 100 mg/mL). Like extract 16, it was obtained with a high power that allowed a higher extraction temperature, which is generally correlated with higher yields [4]. However, the lower plant to water ratio increased the surface exchange and facilitated the extraction of more compounds of interest [5]. Only two extracts (8 and 16) had a higher yield than extract 13 but their colors were paler. Both were obtained at 1000 W but in 5 cycles, implying that a longer extraction only recovered non-colored compounds. These results were confirmed by UV-visible spectrophotometry.

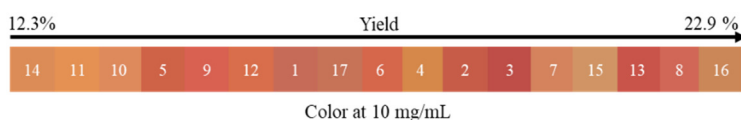


Figure 1. Representation of the color of all extracts on a yield scale.

2.2 Comparison to hydroalcoholic extracts

Extracts 13 and 16 were compared to reference hydroalcoholic (EtOH/water 50/50) extractions (Table 3). A microwave assisted extraction was used to assess the impact of the solvent. It was carried out using the parameters of extract 16. A Soxhlet was also performed as it is the most frequently used extraction method for madder pigments [2]. 2.5 g of madder were extracted for 4 h by 100 mL of EtOH/water (50/50) at boiling temperature. All MAE extracts were prepared in triplicates for the comparison, but only one Soxhlet extraction was done as it consumes more energy and water and did not perform well compared to MAE.

Table 2. Comparison of water-based and hydroalcoholic extracts

Extract	13 n = 3	16 n = 3	16 EtOH/W n = 3	Soxhlet n=1
Yield (%)	21.5 ± 0.5%	21.6 ± 1.1%	20.7 ± 0.4 %	14.1
Abs at 500 nm (ua)	0.35 ± 0.05	0.16 ± 0.01	0.31 ± 0.09	0.17
Total phenolics (mg gallic acid eq/g)	21 ± 4 ^a	15 ± 1 ^b	21 ± 2 ^a	-
Anthraquinones (mg alizarin eq/g)	49.4 ± 9.3 ^a	25.2 ± 1.7 ^b	48.0 ± 11.5 ^a	-

^{a,b,c}: significantly different results for each test using Tukey's range test ($p < 0.05$)

In terms of yield, all MAE extracts were comparable at approximately 21% (table 3). Soxhlet however, yielded lower at 14.1%. In fact, its yield was on the lower side compared to the range obtained in the experimental design (12.6 – 22.9%). The Soxhlet extract also had a lower absorbance at 500 nm, comparable to the one of extract 16. Extract 13 and the hydroalcoholic microwave extract had an absorbance about twice higher (table 3).

The composition of all microwave extracts was then compared. The total polyphenolic content was assessed using the Folin-Ciocalteu method in gallic acid equivalent. Anthraquinone content was also assessed by a colorimetric method in alizarin equivalent. Once again, extract 13 and the hydroalcoholic extract gave similar results that were superior to extract 16. The lower anthraquinone content in extract 16 explains its paler color. Extract 13 was further characterized by UHPLC-HRMS/MS leading to the putative identification of 11 anthraquinones and 7 flavonoids. The main pigments of the extract were pseudopurpurin, purpurin, lucidin and lucidin primveroside.

3 Conclusion

We developed the first water-based microwave extraction of madder pigments. The number of cycles was the most significant parameter, as well as its interactions with power and plant to water ratio. During the optimization we noticed that color and yield were not correlated, due to the parallel extraction of non-colored compounds like sugars. The best combination of yield and color was obtained after 1 cycle (30 s) at 1000 W and 100 mg/20 mL. The optimized extraction was as efficient as hydroalcoholic MAE and better than Soxhlet in terms of yield, color and content. This method is eco-responsible thanks to the use of water, a green solvent, in low quantities and the economy of energy allowed by a 30 seconds extraction. It is also fast, accessible and easy to develop, contrary to other green extraction techniques. Thus, water-based MAE is a good alternative to other methods when extracting non-thermolabile compounds with medium polarity.

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