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Pressurized hot water extraction of anthraquinones from *Heterophyllaea pustulata* Hook f. (Rubiaceae)

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ABSTRACT

Heterophyllaea Pustulata Hook. f. (Rubiaceae), commonly known as "cegadera", is a phototoxic native plant from South America, containing anthraquinones with important antibacterial, antiviral and anticancer activity. In this work, pressurized hot water extraction (PHWE) was applied to extract anthraquinones from the stems of the plant. The effect of temperature, pressure and water flow rate on the extraction yield of four anthraquinones (soranjidiol, rubiadin, rubiadin 1-methyl ether and 2-hydroxy-3-methyl anthraquinone) was studied. Within the experimental range explored, the optimum extraction temperature was 170 °C. Lower yields were obtained at higher temperatures, apparently due to the thermal decomposition of these anthraquinones. The experimental extraction curves were fitted with three models: the thermodynamic partitioning and the one-site and two-site kinetic models.

1. Introduction

Heterophyllaea pustulata Hook. f. (Rubiaceae), commonly known as "cegadera", is a native plant from South America [1], which has been reported as a phototoxic species [2,3]. Four anthraquinones (AQs): 1,6-dihydroxy-2-methyl-9,10-anthraquinone (soranjidiol), 1,3-dihydroxy-2-methyl-9,10-anthraquinone (rubiadin), 3-hydroxy-1-methoxy-2-methyl anthraquinone (rubiadin 1-methyl ether) and 2-hydroxy-3-methyl anthraquinone were isolated from the aerial parts of the plant and were identified by our research group [4–6]. The first three AQs, stand out as the main components of leaves and stems and have photosensitizing properties, mediated by the generation of superoxide anion (O^{2-}; Type I mechanism) and/or singlet molecular oxygen ($^1O_2$; Type II mechanism) [4–6]. AQs show important antibacterial and anticancer activity *in vitro* by means of the photosensitizing phenomenon [7,8]. In addition, we have previously established that extracts containing these compounds exhibit a significant antibacterial, antifungal and antiviral activity *in vitro* without the involvement of a photosensitizing action [4,9].

Conventionally, the isolation of these compounds involves a series of Soxhlet extractions using organic solvents of increasing polarity; starting with hexane followed by benzene, ethyl acetate and ethanol [4]. This conventional technique requires long time and large amounts of organic solvents, and has a negative impact on the environment and human health [10,11].

Pressurized hot water extraction (PHWE) is an environmentally friendly separation technique, carried out with water at high temperatures (usually from 100° to 370 °C), under a pressure sufficiently high to maintain water in the liquid state. AQs are polycyclic aromatic compounds with a melting point above 250 °C. Even though the solubility of AQs in water is very low at room temperature, the use of PHWE is attractive because generally the solubility of solid solutes increases as the temperature becomes closer to their melting points. On the other hand, the dielectric constant and molecular association of water decrease with temperature, making it a better solvent for non-polar or slightly polar compounds. Karásek et al. [12] found, for example, that the solubility of 9,10-anthraquinone in pressurized hot water increases from a molar fraction of 7.25×10^{-8} at 40 °C to 2.96×10^{-5} at 160 °C.

PHWE has been applied to recover various extracts from natural products, with applications in the food and pharmaceutical industries [10,11,13–21]. The objectives of this work were to

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evaluate the PHWE for isolation of anthraquinones from the aerial parts of *H. pustulata* and to examine the effect temperature (120, 170 and 220 °C), pressure (45, 60 and 75 bar) and water flow-rate (3, 5 and 7 ml/min) on the yield and kinetics of extraction. For this purpose, the concentration of four AQs (soranjidiol, rubiadin, rubiadin 1-methyl ether and 2-hydroxy-3-methyl anthraquinone) in the extracts was determined by high-performance liquid chromatography (HPLC) and the extraction curves were fitted with three models: thermodynamic partitioning and onsite and two-site kinetic models.

2. Experimental

2.1. Plant material

Aerial parts of *H. pustulata* were collected in La Almona, Jujuy province, Argentina, in January 2011. The vegetal specie was identified by Prof. Gloria Bardoza (Instituto Multidisciplinario de Biología Vegetal, IMBIV-CONICET), and a voucher specimen has been deposited at the Cordoba Botanical Museum as CORD 305. The plant material was air-dried at room temperature during seven days and separated into stems and leaves. Based on previous works [22] only stems were used for the study presented in this manuscript because of the AQs content in the stems is higher than the other plant parts.

2.2. Pressurized hot water extraction (PHWE)

The PHWE experiments were carried out in a high-pressure device designed and built in our group. It consists of a stainless steel high-pressure extractor cell with 10 ml internal volume, a HPLC pump (Waters 501, Dickinson, Texas, USA) having a maximum flow rate of 10 ml/min, a coiled preheater and a downstream back pressure regulator (BPR). The extraction cell is equipped with a heating system using aluminum heating jackets with two electrical resistances and connected to a temperature regulator. To maintain the set temperature, the cell is installed within a thermally insulated box. The pressure in the extractor is measured with a pressure gauge (Dynisco Dynipack 16, Franklin, Massachusetts, USA). The experimental apparatus is completed with stainless steel connecting lines and accessories. Fig. 1 shows a diagram of the PHWE experimental setup.

To begin the extraction, 1 g of air-dried stems of *H. pustulata* were triturated mechanically using a knife mill (Retsch K.G. 5657 HAAN, West-Germany) with a mesh no. 18 (sieve opening 1 mm). Then, the cell is loaded with this material and the system is closed. Later, the operating temperatures of the preheater and extraction cell are set. The preheater is operated at a temperature 20 °C below that of the extraction vessel. When the temperature reaches the set point, the extraction begins at a constant water flow-rate. The BPR is used to set the pressure at the operating value, which should be higher than the vapor pressure of water at the extraction temperature to assure a liquid state.

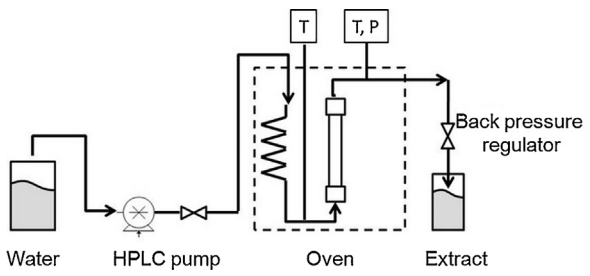

Fig. 1. Schematic diagram of PHWE apparatus.

The following operating conditions were studied: temperatures of 120, 170 and 220 °C; pressures of 45, 60 and 75 bar and flow-rates of 3, 5 and 7 ml/min. For this study, two variables were fixed in the middle point values and the third factor was modified in the range mentioned above. The total time for each run was set at 120 min, and the extracts were collected in glass flasks every 20 or 40 min. For each set of experimental conditions, the runs were duplicated.

The concentration of rubiadin, soranjidiol, rubiadin 1-methyl ether and 2-hydroxy-3-methyl anthraquinone, in the extracts was determined by high-performance liquid chromatography (HPLC, Varian Prostart 210, Walnut Creek, CA, USA). Prior to analysis, the extracts were subjected to evaporation in a rotary evaporator with controller of vacuum semiautomatic at 60 °C and 199 mbar (Buchi V-850, Postfach, Switzerland).

2.3. Chromatographic analysis

The dried extracts obtained in each experiment were dissolved in 1 ml of methanol (HPLC grade). All samples were filtered through a 0.2 mm cellulose acetate membrane (Micro Filtration System) before HPLC analysis. Qualitative and quantitative analysis was performed in a Varian Pro Star chromatograph (model 210, series 04171), equipped with a UV–vis detector and a Microsorb-MV column 100-5 C_{18} (250 × 4.6 mm i.d., Varian). The mobile phase was methanol:water (8:2, v:v) at constant flow (1 ml/min) and the injection volume was 20 μl. The detection was performed at a wavelength of 269 nm. Rubiadin (1,3-dihydroxy-2-methyl anthraquinone), soranjidiol (1,6-dihydroxy-2-methyl anthraquinone), rubiadin 1-methyl ether (3-hydroxy-1,2-methyl-anthraquinona) and 2-hydroxy-3-methyl anthraquinone were identified in each extract by comparison of the HPLC retention times with the corresponding standards previously obtained in our laboratory [3], under the same chromatographic conditions.

The external calibration method was applied to quantify the amount of each AQ in the extracts, interpolating the area under the peaks for each compound from the corresponding calibration curves of rubiadin ($Y = 2.5572 \times 10^6 X$ ($r^2 = 0.99997$, $p < 0.0001$), where Y is the peak area given by data processor and X is the amount of rubiadin expressed as molarity [23]. Therefore, the amount of each AQ was expressed as rubiadin. The extraction yield of each AQ was expressed as mg of compound/g of vegetal material and the extraction yield of the total AQs is the sum of the four analyzed AQs.

2.4. Mathematical modeling

Various kinetic models have been proposed in the literature to describe high-pressure, high-temperature extraction processes [24–29]. These models try to represent the effect of different mechanisms on the extraction kinetics: (a) the transport of the solute through the solid matrix (intra-particle diffusion); (b) the partition of the compound between the matrix and the solvent (thermodynamic equilibrium); (c) the diffusion of the solute through a liquid film around the matrix (external diffusion).

The thermodynamic partitioning model assumes that the extraction process is controlled by thermodynamic equilibrium. In this case, the ratio between the concentrations of the solute in the matrix and in the solvent is determined by the partition coefficient K_p. If a and b represent two consecutive points of a given extraction curve, the model establishes the following relation between the masses m_a and m_b of solute being extracted by the volumes V_a and V_b of solvent [28]:

$$\frac{m_b}{m_o} = \frac{m_a}{m_o} + \frac{1 - \left(m_a/m_o\right)}{1 + \left(k_p m/\left(V_b - V_a\right)\rho\right)} \quad (1)$$

In this equation m represents the mass of sample being extracted, m_0 the initial mass of solute in the sample and ρ is the density of the extraction solvent.

The so called one-site and two-site kinetic models [20] describe the extraction rate through first-order kinetics. In the one-site model, the ratio between the mass m_t of solute removed by water after time t is given by:

$$\frac{m_t}{m_0} = 1 - \exp(-kt) \quad (2)$$

where m_0 is the initial mass of the solute in the matrix and k is a first-order rate constant.

The two-site model makes a distinction between a certain fraction f of solute that desorbs at a fast rate, defined by constant k_1, and a remaining fraction $(1-f)$ which is extracted at a lower rate, with constant k_2:

$$\frac{m_t}{m_0} = 1 - f\ \exp(-k_1 t) - (1-f)\exp(-k_2 t) \quad (3)$$

3. Results and discussion

3.1. Effect of pressure on the extraction

Liquids are nearly incompressible in the subcritical region; therefore the change in solvating power that accompanies density variation is several orders of magnitude lower, compared to the effect of temperature. For this reason, it is expected that the effect of pressure on the PHWE performance would be minor, as shown in previous work from the literature [30–32].

Extractions carried out two variables were fixed in the middle point values (temperature, 170 °C and water flow rate, 5 ml/min) and the third factor (pressure) was modified in the range of 45 to 60 bar. Fig. 2 showed only a slight increment on total AQs recovery when the pressure was increased from 45 to 60 bar. However, extractions carried out at 75 bar showed a lower total yield. This decrease in yield of AQs could be attributed to a channeling process due to a high compaction of the solid matrix at this pressure [33], but not due to thermal degradation, since the experiments carried out at the two pressures (60 and 75 bar) show the same chromatographic profile (Fig. 3).

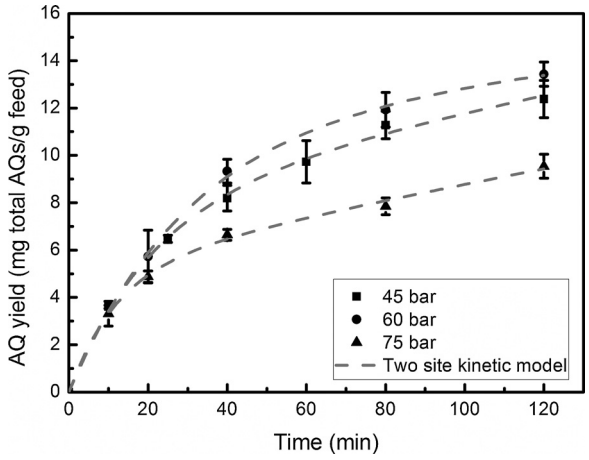

Fig. 2. Effect of the pressure of extraction on yield of total AQS (flow rate 5 ml/min at temperature of 170 °C). * Expressed as rubiadin.

3.2. Effect of temperature on the extraction

Temperature is one of the most important variables in PHWE processes. As mentioned before, a high extraction temperature would increase solubility. Additionally, increasing temperature decreases surface tension and viscosity and, therefore, improves wetting and penetration of the solvent in the sample. These phenomena increase the diffusion flux and the extraction yield [33]. However, AQs could decompose at high temperatures. Teixeira Sousa et al. [34] studied the thermal behavior of quinones and found that the thermal stability of these compounds is strongly influenced by their molecular structure. These authors found that 9,10-antraquinone starts to decompose above 215 °C. On the other hand, working on the PHWE of *Morinda citrifolia* roots, Shotipruk et al. [18] found alizarin (1,2-dihydroxy anthraquinone) to be stable up to 220 °C, while Anekpankul et al. [20] reported the decomposition of damnacanthal (3-hydroxy-1-methoxy anthraquinone-2-aldehyde) above 170 °C.

In this work, the effect of temperature on the PHWE of *H. pustulata* was studied in the range 120–220 °C, using the middle point

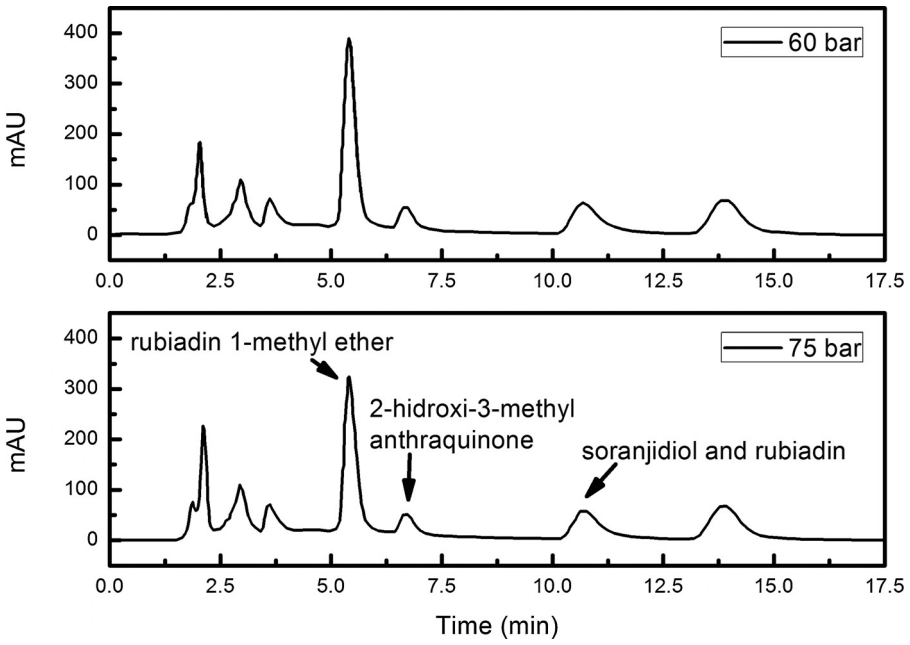

Fig. 3. Qualitative HPLC analysis of extract of AQs at different presures.

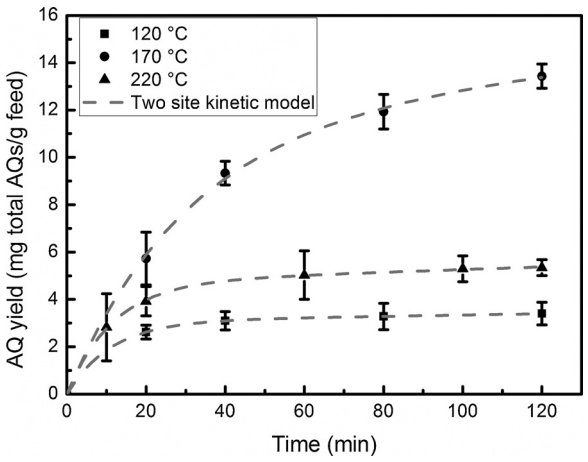

Fig. 4. Effect of the temperature of extraction on yield of total AQS (flow rate 5 ml/min at pressure of 60 bar). * Expressed as rubiadin.

values of the parameters: extraction pressure (60 bar) and water flow rate (5 ml/min), in concordance with Shotipruk et al. [18] and a total extraction time of 2 h.

As shown in Table 1 and Fig. 4, increasing the temperature from 120° to 170 °C increases the yield of total AQs. However, at 220 °C this yield is sharply reduced. Table 1 reports the results obtained at all the experimental conditions studied in this work, by HPLC. The sum of rubiadin and soranjidiol was the anthraquinones with the highest concentration in all extracts (more than double the concentration of 2-hydroxy-3-methyl anthraquinone). It is necessary to explain that it was not possible to separate rubiadin and soranjidiol because they are positional isomers: 1,3-dihydroxy-2-methyl anthraquinone (rubiadin) and 1,6-dihydroxy-2-methyl anthraquinone (soranjidiol). In the same table, the experiments conducted at 170 °C and 220 °C gave similar total yields. However the mass of AQs in the 220 °C extract was less than half that measured at 170 °C. This may be attributed to a AQs decomposition above 170 °C in accordance with the literature [18,20,34] and considering the difference found in the chromatographic profiles at two temperatures (170 and 220 °C) (data not shown). Furthermore, considering the relative percentages of each anthraquinone in the extracts, 2-hydroxy-3-methyl anthraquinone appears to have the lower thermal stability.

3.3. Effect of water flow rate on the extraction

The changes of the extraction yield with the solvent flow rate can give an insight on the extraction mechanism. If an extraction process is exclusively controlled by intra-particle diffusion, an increase in water flow rate will have no effect on the extraction rate. On the contrary, the extraction rate will increase with water flow rate if the process is controlled by external mass transfer. When thermodynamic partitioning is the limiting step, the extraction curves obtained at different solvent flow rates for the same process conditions, will overlap completely if the cumulative mass of solvent is plotted against the volume of water used. This is because, for a given unit of time, the amount of extracted solute is directly proportional to the volume of water passing through the matrix and, hence, to the water flow rate. In general the extraction kinetics will be determined by a combination of these factors.

Fig. 5 shows the effect of water flow rate on the total AQs extraction yield, for experiments carried out at fixed values of temperature (170 °C) and extraction pressure (60 bar). For the three flow rates studied (3, 5 and 7 ml/min), Fig. 5a. illustrates the change of AQs yield with time, while Fig. 5b shows the AQs yield as a function of water volume. From Fig. 5a it can be seen that the extraction rate increases with the increase of water flow rate. Fig. 5b, on the other hand, shows that the three extraction curves do not overlap, which indicates that the extraction kinetics is not controlled by thermodynamic partition.

Thus, the higher AQs yield (18.9 mg/g) is then obtained at 7 ml/min (170 °C and 60 bar). It is important to note that PHWE extractions showed an AQs recovery enhancement of up to 600% compared with traditional Soxhlet process with solvents of increasing polarity. As it was demonstrated in previous work [22], only a AQs yield of about 3–4 mg total AQs/g vegetable matrix was obtained using Soxhlet extraction for 16 h. On the other hand, using PHWE the extraction time is reduced from 16 h to 2 h. This high yield of AQ obtained by PHWE, is mainly due to the ability of the water to reduce the dielectric constant with increasing temperature, by modifying its polarity is possible to extract non-polar organic compounds. Furthermore, the temperature used in the high pressure process is higher than in Soxhlet extraction, favoring the extraction of solid compounds as AQs.

3.4. Modeling of extraction curves

The thermodynamic partitioning and the one- and two-sites kinetic models were used to represent the experimental extraction curves.

The value of the initial mass of AQs available in the vegetable matrix (m_0) was estimated at 24 mg AQs/g of sample. This value was the maximum yield of AQs obtained in previous experiments, using a combination of ultrasound and microwave assisted processes [22].

The partition coefficient K_p in the thermodynamic partitioning model was considered to be independent of pressure. The value of K_p at 120, 170 and 220 °C was calculated from the experimental data on the solubility of alizarin in pressurized hot water reported by Pongnaravane et al. [19] and assuming a uniform concentration of AQs in the vegetable matrix equal to 24 mg/g. The extraction curves predicted by this model at 170 °C show about 100% yield of AQs at 120 min, for the three values of water flows studied in this work. The 79% maximum yield achieved experimentally at 7 ml/min indicates the presence of mass transfer control.

Table 1
Results of experimental extractions of anthraquinones (AQs) by PHWE.

T (°C)	P (bar)	Flow (ml/min)	mg extract/g vegetal	mgAQs totals/g vegetal*	2-Hidroxi-3-methyl anthraquinone (%)	Rubiadin and Soranjidiol (%)	Rubiadin 1-methyl ether (%)
120	60	5	195 ± 15	3.44 ± 0.48	19.14 ± 0.48	74.10 ± 0.27	6.64 ± 0.26
170	60	5	520 ± 23	13.43 ± 0.51	14.61 ± 0.65	82.50 ± 0.38	2.93 ± 0.18
220	60	5	520 ± 31	5.23 ± 0.34	5.04 ± 0.41	93.84 ± 0.44	1.12 ± 0.56
170	45	5	570 ± 42	12.38 ± 0.79	17.29 ± 0.39	79.31 ± 0.65	3.40 ± 0.17
170	75	5	320 ± 18	9.54 ± 0.51	20.92 ± 0.22	73.68 ± 0.26	5.42 ± 0.23
170	60	3	390 ± 24	9.78 ± 0.16	25.08 ± 0.19	95.79 ± 0.19	4.30 ± 0.47
170	60	7	850 ± 57	18.59 ± 0.52	13.83 ± 0.53	83.57 ± 0.39	2.60 ± 0.51

(%) Mass percentage.
* Expressed as rubiadin.

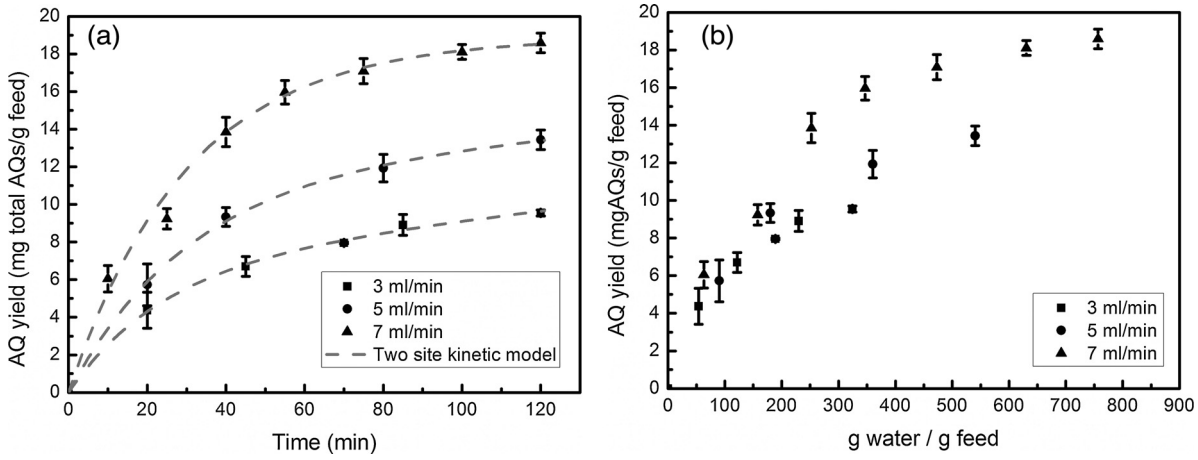

Fig. 5. (a) Effect of the flow rate of extraction on yield of total AQS (temperature 170 °C at pressure of 60 bar). * Expressed as rubiadin. (b) Effect of the water volume on yield of total AQS (temperature 170 °C at pressure of 60 bar). * Expressed as rubiadin.

Table 2
Extraction kinetic models.

Experimental conditions			One site-kinetic desorption model		Two site-kinetic desorption model				Thermodynamic partitioning	
T (°C)	P (bar)	Flow (ml/min)	k_d (min^{-1})	AARD%	F	k_{d1} (min^{-1})	k_{d2} (min^{-1})	AARD%	Kp	AARD%
120	60	5	0.0014	31.71	0.13	0.0902	0.0001	0.11	167	80
170	60	5	0.0087	14.83	0.47	0.0333	0.0017	1.35	46	53.67
220	60	5	0.0027	34.27	0.20	0.0865	0.0003	0.75	28	78.95
170	45	5	0.0081	18.04	0.31	0.0497	0.0031	1.92	46	59.57
170	75	5	0.0053	28.00	0.21	0.0855	0.0022	1.42	46	68.10
170	60	3	0.0054	15.57	0.27	0.0427	0.0017	1.49	46	63.38
170	60	7	0.0182	11.40	0.78	0.0334	0.0003	3.81	46	34.34

The parameters required to apply the one-site and two-site kinetic models were obtained by adjusting the experimental extraction curves using the least squares method from Microsoft Excel Solver regression routine. Table 2 reports the values of these parameters and the average absolute relative deviation (AARD) between the predicted and experimental extraction yields. The results show that only the two-site kinetic desorption model is able to correlate the experimental data with reasonable accuracy. The continuous curves in Figs. 2–4 represent the extraction paths predicted by the two-site kinetic desorption model.

4. Conclusions

In this work the PHWE process was applied to extract AQs from *H. pustulata*. The effect of temperature, pressure and water flow rate on the extraction of soranjidiol, rubiadin, rubiadin 1-methyl ether and 2-hydroxy-3-methyl anthraquinone was studied. Working at 170 °C, 60 bar and 7 ml/min, a AQs extraction yield of 18.9 mg of total AQs/g of vegetal was obtained in 2 h. Extractions at higher temperature (220 °C) gave lower yields of AQs, presumably due to thermal degradation. The two-sites kinetic desorption model was able to correlate adequately the experimental extraction curves

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References

[1] N.M. Bacigalupo, in: A.L. Cabrera (Ed.), Flora de la Provincia de Jujuy, Colección Científica INTA, tomo III, parte IX, INTA, Buenos Aires, 1993, pp. 375–380.
[2] E.W. Hansen, C.A. Martiarena, Contribución al estudio de la toxicidad de *Heterophyllaea pustulata* Hook "cegaderaën el ganado. Dermatitis. Queratoconjuntivitis tóxica experimental en especies animales receptivas, Rev. Inv. Agropec. Patol. Anim. (INTA) 4 (1967) 11–81.
[3] D.H. Aguirre, R.A. Neumann, Intoxicación por "cegadera(*Heterophyllaea pustulata*) en caprinos del noroeste argentino, Medicinae Veterinariae 18 (2001) 487–490.
[4] S.C.N. Nuñez Montoya, A.M. Agnese, C. Pérez, I.N. Tiraboschi, J.L. Cabrera, Pharmacological and toxicological activity of *Heterophyllaea pustulata* anthraquinone extracts, Phytomedicine 10 (2003) 569–574.
[5] S.C.N. Núñez Montoya, L.R. Comini, M. Sarmiento, C. Becerra, I. Albesa, A.J. Argüello, L. Cabrera, Natural anthraquinones probed as Type I and Type II photosensitizers: singlet oxygen and superoxide anion production, J. Photochemistry and Photobiology B: Biology 78 (2005) 77–83.
[6] L.R. Comini, S.C. Núñez Montoya, M. Sarmiento, J.L. Cabrera, G.A. Argüello, Characterizing some photophysical, photochemical and photobiological properties of photosensitizing anthraquinones, J. Photochemistry and Photobiology A: Chemistry 188 (2007) 185–191.
[7] L.R. Comini, S.C. Núñez Montoya, P.L. Paéz, G.A. Argüello, I. Albesa, J.L. Cabrera, Antibacterial activity of anthraquinone derivatives from *Heterophyllaea pustulata* (Rubiaceae), J. Photochemistry and Photobiology B: Biology 102 (2011) 108–114.
[8] L.R. Comini, I.M. Fernandez, N.B. Rumie Vittar, S.C. Núñez Montoya, J.L. Cabrera J.L., V.A. Rivarola, Photodynamic activity of anthraquinones isolated from *Heterophyllaea pustulata* Hook f. (Rubiaceae) on MCF-7c3 breast cancer cells, Phytomedicine 18 (2011) 1093–1095.
[9] B.S. Konigheim, M. Beranekb, L.R. Comini, J.J. Aguilar, J. Marioni, J.L. Cabrera, M.S. Contigiani, S.C. Núñez Montoya, In vitro antiviral activity of *Heterophyllaea pustulata* extract, Natural Products Communications 6 (2012) 1–3.
[10] L. Ramos, E.M. Kristenson, U.A.T. Brinkman, Current use of pressurized liquid extraction and subcritical water extraction in environmental analysis, J. Chromatography A 975 (2002) 3–29.
[11] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: a review, Analytica Chimica Acta 703 (2011) 8–18.
[12] P. Karásek, J. Planeta, M. Roth, Solubilities of oxygenated aromatic solids in pressurized hot water, J. Chemical and Engineering Data 54 (2009) 1457–1461.
[13] D.J. Miller, S.B. Hawthorne, Solubility of liquid organic flavor and fragrance compounds in subcritical (hot/liquid) water from 298 to 473 K, J. Chemical and Engineering Data 45 (2000) 315–318.
[14] A. Basilea, M.M. Jimenez-Carmona, A.A. Clifford, Extraction of rosemary by superheated water, J. Agricultural and Food Chemistry 46 (1998) 5205–5209.

[15] G. Gmiz, M.D. Luque de Castro, Continuous sub-critical water extraction of medicinal plant essential oil. Comparison with conventional techniques, Talanta 51 (2000) 1179–1185.
[16] X. Liang, Q. Fan, Application of sub-critical water extraction in pharmaceutical industry, J. Materials Science and Chemical Engineering 1 (2013) 1–6.
[17] L.H. Chang, Y.C. Cheng, C.M. Chang, Extracting and purifying isoflavones from defatted soybean flakes using superheated water at elevated pressures, Food Chemistry 84 (2004) 279–285.
[18] A. Shotipruk, J. Kiatsongserm, P. Pavasant, M. Goto, M. Sasaki, Pressurized hot water extraction of anthraquinones from the roots of *Morinda citrifolia*, Biotechnology Progress 20 (2004) 1872–1875.
[19] B. Pongnaravane, M. Goto, M. Sasaki, T. Anekpankul, P. Pavasant, A. Shotipruk, Extraction of anthraquinones from roots of *Morinda citrifolia* by pressurized hot water: antioxidant activity of extracts, J. of Supercritical Fluids 37 (2006) 390–396.
[20] T. Anekpankul, M. Goto, M. Sasaki, P. Pavasant, A. Shotipruk, Extraction of anti-cancer damnacanthal from roots of *Morinda citrifolia* by subcritical water, Separation and Purification Technology 55 (2007) 343–349.
[21] K. Kiathevest, M. Goto, M. Sasaki, P. Pavasant, A. Shotipruk, Extraction and concentration of anthraquinones from roots of *Morinda citrifolia* by non-ionic surfactant solution, Separation and Purification Technology 66 (2009) 111–117.
[22] M.F. Barrera Vázquez, L. Comini, R. Martini, S. Núñez Montoya, S. Bottini, J.L. Cabrera, Ultrasonics Sonochemistry 21 (2014) 478–484.
[23] S.C. Núñez Montoya, L.R. Comini, B.R. Vittar, I.M. Fernandez, V.A. Rivarola, J.L. Cabrera, Phototoxic effects of *Heterophyllaea pustulata* (Rubiaceae), Toxicon 51 (2008) 1409–1415.
[24] Z. Huang, X.H. Shi, W.J. Jiang, Theoretical models for supercritical fluid extraction, J. Chromatography A 1250 (2012) 2–26.
[25] H. Sovová, Steps of supercritical fluid extraction of natural products and their characteristic times, J. Supercritical Fluids 66 (2012) 73–79.
[26] H. Ohira, N. Torii, T.M. Aida, M. Watanabe, R.L. Smith, Rapid separation of shikimic acid from Chinese star anise (*Illicium verum* Hookf.) with hot water extraction, Separation and Purification Technology 69 (2009) 102–108.
[27] I. Papamichail, V. Louli, K. Magoulas, Supercritical fluid extraction of celery seed oil, J. Supercritical Fluids 18 (2000) 213–226.
[28] A. Kubátová, B. Jansen, J.F. Vaudoisot, S.B. Hawthorne, Thermodynamic and kinetic models for the extraction of essential oil from savory and polycyclic aromatic hydrocarbons from soil with hot (subcritical) water and supercritical CO_2, J. Chromatography A 975 (2002) 175–188.
[29] T. Veress, Sample preparation by supercritical fluid extraction for quantification a model based on the diffusion-layer theory for determination of extraction time, J. Chromatography A 668 (1994) 285–291.
[30] S.B. Hawthorne, Y. Yang, D.J. Miller, Extraction of organic pollutants from environmental solids with sub- and supercritical water, Analytical Chemistry 66 (1994) 2912–2920.
[31] S. Rovio, K. Hartonen, Y. Holm, R. Hiltunen, M.L. Riekkola, Extraction of clove using pressurized hot water, Flavour and Fragance J. 14 (1999) 399–404.
[32] M.P.K. Choi, K.K.C. Chan, H.W. Leung, C.W. Huie, Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions, J. Chromatography A 983 (2003) 153–162.
[33] J. Kronholm, K. Hartonen, M. Riekkola, Analytical extractions with water at elevated temperatures and pressures, Trends in Analytical Chemistry 26 (2007) 396–412.
[34] E. Teixeira Sousa, M. Melo da Silva, S.J. de Andrade, M.P. Cardoso, L. Almeida Silva, J.B. de Andrade, Evaluation of thermal stability of quinones by thermal analysis techniques, Thermochimica Acta 529 (2012) 1–5.