OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF ANTHRAQUINONES FROM HETEROPHYLLAEA PUSTULATA HOOK F. (RUBIACEAE) USING TAGUCHI L9 ORTHOGONAL DESIGN

M.F. Barrera Vázquez, L.R. Comini, R.E. Martini, A.E. Andreatta, S.C. Núñez Montoya, S. Bottini, J.L. Cabrera

(1) IDTQ- Grupo Vinculado PLAPIQUI – CONICET. Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba. Av. Vélez Sarsfield 1611, Ciudad Universitaria, Córdoba

(2) Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba - IMBIV, CONICET. Ciudad Universitaria, Córdoba

(3) UTN. Facultad Regional San Francisco. Av de la Universidad 501, San Francisco, Córdoba, Argentina.

(4) PLAPIQUI (UNS-CONICET). Cno. La Carrindanga Km 7. Bahía Blanca

E-mail: mfbarreravazquez@plapiqui.edu.ar

Abstract. In this study, ultrasound-assisted extraction (UAE) was evaluated as an alternative, easy and more efficient method for the isolation of anthraquinones (AQs) from Heterophyllae pustulata compared with conventional extraction methods. The extraction was performed using ethanol-water solution as solvent. The influence of different operating conditions, as temperature, time, solvent composition and solvent/sample ratio was analyzed. To achieve global optimization of ultrasound-assisted extraction of H. pustulata the experimental design Taguchi with a L9 orthogonal array was applied.

The results showed that the performance of the ultrasound assisted extraction is approximately twice higher than the traditional method, and Taguchi analysis demonstrated that the most influential factor and the optimal combination is solvent concentration (ethanol/water 60:40 (v:v)) followed by time (30 min), temperature (55°C) and solvent/sample ratio (20:1).
Keywords: ANTHRAQUINONE, ULTRASOUND-ASSISTED, TAGUCHI STATISCAL METHOD

1. INTRODUCTION

AQs are an important group of secondary metabolites and constitute the largest group of natural quinones. The anthraquinone derivatives are chemotaxonomic features in certain families of plants: Rubiaceae, Rhamnaceae, Polygonaceae, Fabaceae, Liliaceae and Verbenaceae [1]. To this group belongs the *Heterophyllaea pustulata* Hook. F. (Rubiaceae) species commonly known as "cegadera", "ciegadera" or "saruer" which grows spontaneously in the mountainous regions of NW Argentina and Peru between 2500 and 3000 m sea level [2]. Nine anthraquinones (AQs) with photosensitizing properties, mediated by the generation of superoxide anion (O$_2^-$; type I mechanism) and/or singlet molecular oxygen (O$_2$; type II mechanism), were isolated and identified by our research group, from the aerial parts of the plant [3-5]. Three of these AQs, soranjidiol, rubiadin and 1-methyl ether rubiadin, stand out as the main components of leaves and stems [3]. These AQs have demonstrated important antibacterial and anticancer activity by means of the photosensitization phenomenon [6,7]. In addition, we have previously established that extracts containing these compounds exhibited a significant antibacterial, antifungal and antiviral activity without the photosensitizing effect [3, 8]. Due to the multiple biological applications presented by these AQs and their wide range of applications in the pharmaceutical industry, is of great interest optimizing the processes of extraction of these compounds.

Traditional extraction methods for AQs involve using organic solvents of increasing polarity, starting with hexane, followed by bencene, ethyl acetate and ethanol used the Soxhlet apparatus [3]. Although this extraction method is faster, less laborious, and consumes less amount of solvent than other conventional methods (maceration, reflux, decoction, infusion, etc.), it presents low selectivity for certain secondary metabolites, such as AQs from *H. pustulata*. Furthermore, most of these organic solvents behave as a risk due to its toxicity, its power flammable and the waste generated. Therefore, it is important to develop a more selective and efficient technique for obtaining AQs, using less harmful solvents and with lower extraction times. In this sense, UAE is a very...
interesting alternative to the obtention and purification of these substances, because it is
a technique widely used to isolate bioactive substances in different parts of plants [9-
11]. All published studies under ultrasound indicate higher extraction yield as well as
the reduction in extraction time [12,13]. The largest extraction efficiency is mainly due
to the effects of the cavitation, which improves mass transfer and solvent penetration in
the plant material by disrupting the cell walls [14]. Particularly, some experiments have
been performed for the extraction of diverse AQs applying this method [15,16]. The
efficiency of this process (UAE) is influenced by numerous conditions, for example, the
solvent composition, temperature, time and relative solvent/sample. Therefore, it is
necessary to optimize conditions for the extraction. In this context, Taguchi
experimental design is often used in experiments on various factors and levels, because
they minimize the number of trials, the experimental time and cost [17].

Thus, in this work the technique UAE was applied to extract AQs (soranjidiol,
rubiadin and 1-methyl ether rubiadin) of H. pustulata. Since ethanol is widely used in
the extraction of active principles plants, being non-toxic and economical [18], different
ethanol-water solutions were considered as solvent for this extraction. Four factors
(ethanol-water solvent composition, relative solvent/sample, temperature and time) were
evaluated to determine the optimal conditions of AQs extraction by using L9 Taguchi
orthogonal design. This study was complemented with an analysis of variance ANOVA
to determine which is the statistically significant factor. The results obtained by UAE,
were compared with those obtained with the conventional Soxhlet extraction

2. MATERIALS AND METHODS
2.1. Plant material
Aerial parts of Heterophyllae pustulata were collected in La Almona, Jujuy
province, Argentina, in January 2011. The material was identified by Prof. Dr. Gloria
Bardoza (Instituto Multidisciplinario de Biologia Vegetal, IMBIV-CONICET), and a
voucher specimen has been deposited at the Cordoba Botanical Museum as CORD 305.

2.2. Solvents
The solvents used in the extractions were: ethanol (Porta, 96 % v/v) and distilled
water.

2.3. Conventional Soxhlet extraction
Air-dried aerial plant material was separated into stems and leaves. An amount of 29 grams of stems were mechanically triturated and treated with an ethanol-water solution 60% v/v of ethanol. This concentration was selected in function of results obtained in UAE, explained later (3.1). The extraction was performed using 435 mL of solvent during 9 hours. This length of time ensures the exhaustion of the vegetable material. The amount of solvent and sample used in the extractions was determined by the dimensions of the Soxhlet apparatus [19]. The obtained extracts were dried under vacuum. The concentration of rubiadin, soranjidiol and 1-methyl ether rubiadin in the extracts was determined by High-performance liquid chromatography (HPLC).

2.4. Ultrasonic-assisted extraction.

The ultrasonic irradiation experiments were carried out in a TESTLAB SRL sonomatic cleaning bath (model- TB02TACF) operating at 80 W power and 40 kHz frequency. The dimensions of the tank were 150 x 140 x 100 mm.

For the extraction with ultrasound, according to the experimental design, was used the same kind of solvent (ethanol-water solution) used for the conventional extraction. Air-dried aerial plant material was separated into stems and leaves. An amount of 0.25 g of stems of *H. pustulata*, mechanically triturated, was extracted in this apparatus. The extraction of the plant material was evaluated with different factors including the solvent composition (60, 80 and 96% v/v of ethanol), temperatures (35, 45 and 55°C), extraction time (15, 30, 45 min), and solvent / sample ratio (10:1, 20:1 and 30:1). These experiments were performed by triplicate. Finally, the extracts were filtered and dried under vacuum. The concentration of rubiadin, soranjidiol and 1-methyl ether rubiadin in the extracts was determined by High-performance liquid chromatography (HPLC).

2.4.1. The effect solvent composition on the extraction of anthraquinones by ultrasound-assisted extraction

In order to study the effect of solvent composition on the extraction yield AQs, 0.5 g stems of *H. pustulata* were extracted in a ultrasonic bath, following the procedure explained above, and using the optimal conditions determined by Taguchi method (extraction time 30 min, solvent / sample ratio 20:1 and 55 °C) for different solvent compositions (50, 60, 70, 80 and 96% v/v of ethanol).

2.4.2. The effect time on the extraction of anthraquinones by ultrasound-assisted extraction
In order to study the effect of time on the extraction yield AQs, 0.5 g stems of *H. pustulata*, were extracted in a ultrasonic bath, following the procedure explained above, and using the optimal conditions determined by Taguchi method (solvent composition 60% v/v of ethanol, solvent / sample ratio 20:1 and temperature 55 °C) at different times (15, 30, 45, 60, 75 and 90 min).

### 2.5 Experimental Design

The experiments were designed to examine the effect of extraction factors and optimize operation conditions of the AQs extraction with ethanol-water solutions. In order to optimise the extraction conditions, the Taguchi-based optimization technique was adapted for the process optimization of UAE of *H. pustulata*. Taguchi-based optimization technique is a unique and powerful optimization discipline that allows optimization with minimum number of experiments [17]. Thus by this method, it is possible to reduce the time and cost for experimental investigations and improve the performance characteristics. In this study, four control factors (solvent composition [A], solvent / sample ratio [B], temperature [C] and the time of ultrasonic irradiation [D]), with three levels setting, were considered to be the independent variables and they are summarized in Table 1.

**Table 1.** Factors and levels for the orthogonal design (A–D are the respective codes for each factor)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Factors</th>
<th>[A] Concentration of solvent</th>
<th>[B] Ratio solvent / sample</th>
<th>[C] Temperature (°C)</th>
<th>[D] Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96</td>
<td>10</td>
<td>35</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>20</td>
<td>45</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>30</td>
<td>55</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

For the process optimization was adapted L9 orthogonal matrix, the rows of the matrix represent 9 experimental runs which were carried out in a random sequence [17]. The extraction results performed under orthogonal design conditions are shown in Table 2.

**Table 2.** The results of orthogonal test L9 (3^4)

<table>
<thead>
<tr>
<th>Test</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>(mg AQs/g of vegetal)</th>
<th>S/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.13</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1.56</td>
<td>3.58</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1.66</td>
<td>4.19</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2.97</td>
<td>8.09</td>
</tr>
</tbody>
</table>
Each experiment was repeated three times under the same conditions at different times to observe the effects of noise sources in the extraction process. All the results at each step of the design are expressed as the mean of three experiments. Mean value of these replications is the response of this treatment.

After conducting the experiments, the results were converted into a statistical measure of performance called (S / N), using the category "the biggest-the-best" and the equation can be expressed as:

\[
\text{ratio } S/N=-10 \log \left( \text{M.S.D.} \right)
\]
\[
\text{M . S . D} = \frac{1}{m} \sum_{i=1}^{m} \frac{1}{T_i^2}
\]

where \( m \) is the number of tests and \( T_i \) is the value of yield and the \( i \) the test. The importance of each factor is studied by signal to noise (S/N) ratio considering both mean (signal) and the standard deviation (noise) [20-21].

### 2.6. Statistical analysis

The purpose of the analysis of variance (ANOVA) is to investigate the design factors that significantly affect the quality characteristic. This is accomplished by first measuring the sum of the square deviation for each factor (SSj) that shows the influence of factor j in the experimental results [21,22]. SSj can be calculated by the following equation:

\[
SSj = \frac{1}{3} \sum_{j=1}^{3} K_j \sum_{i=1}^{9} Y_i^2 - \left( \frac{\sum_{i=1}^{9} Y_i}{9} \right)^2 \quad (j=A,B,C,D)
\]

Secondly, the degree of freedom of each factor is equal to each factor level minus 1, so that the degree of freedom A: \( \text{g.fA} = 3-1 = 2 \). The degree of freedom of the error is
equal to the total degrees of freedom (TFG) minus the degree of freedom of each factor, so (g.f.e = 8-2-2-2-2), in this case the error term is zero and the value of F, can not be calculated. In this case to prevent the error degrees of freedom are non-zero can take account of repetitions of the experiment, therefore g.f.T are equal to the number of replications multiplied by the number of runs, minus 1 (g.f.T = 3x9-1 = 26) and thus the g.f.e = 18 [21]. Thirdly, the variance for each factor ($V_j$) can be calculated:

$$V_j = \frac{SS_j}{df_j} \quad (j=A,B,C,D)$$

The variance ratio, is commonly called the F statistic is the ratio of the variance due to the effect of a factor and the variance due to the error term, $F_j = V_j / Ve$. This ratio is used to measure the importance of a research factor with respect to the variance of all factors including the error term. Fj value obtained is compared with a value of F-standard tables (Fα) for a given statistical level of significance. When the calculated value is less than the value determined from the F-table for the significance level selected, the factor does not contribute to the sum of the squares within the confidence levels. Finally, the percentage contribution of each factor can also indicate the relative power of a factor to reflect each factor’s influence [21,22]

$$P_j = \frac{SS_j}{SST} \times 100$$


The dried extracts obtained in each experiment were dissolved in methanol (MeOH, HPLC grade). All samples were filtered through a 0.2 mm cellulose acetate membrane filter (Micro Filtration System) before HPLC analysis. HPLC analysis (qualitative and quantitative) 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 x 4.6 mm i.d., Varian). The mobile phase was MeOH–H$_2$O (8:2) at constant flow (1 mL/min) and the injection volume was 20 µl. The detection was performed at the

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wavelength of 269 nm. The AQs were identified by comparison of the HPLC retention times with the corresponding standards (rubiadin, soranjidiol y rubiadin 1-methyl ether) under the same chromatographic conditions.

The external calibration method was carried out to quantify each AQ in every extract, by interpolating the area under each peak for each compound from the calibration curves [23]. The seven-points calibration curves (n=3) were linear (correlation coefficients >0.99).

3. RESULTS AND DISCUSSION

3.1. Optimisation of extraction conditions. S/N ratio analysis
The effect of different process conditions was analyzed by Taguchi method as explained above. The experimental results, shown in Table 2, are transformed into a signal-to-noise (S/N) to analyze the effects of each factor and level. Taguchi recommends using a statistical measure of performance (S/N) to measure the quality characteristic that deviate from the desired values. Generally, there are three categories of quality characteristic analysis S:N ratio, ie the lower the better, the higher-the-better and the nominal-the [21]. In this work, the quality characteristic under study the highest yield of AQs, therefore, the selected quality characteristic to determine the S/N is "bigger is better".

The whatever the type of quality characteristic, the transformation is such that S/N are always interpreted in the same way: the biggest S/N is the best. [21].

To analyze the data using S/N, Taguchi suggests making a table of responses, to identify and isolate the effects of each factor on the S/N. The average of the S/N for each level of each control factor is shown in Table 3. The relative difference indicates that the factor solvent composition (A) has an important influence on the yield of AQ, followed by the time (D), temperature (C) and solvent / sample ratio (B) in decreasing order. The optimal level for each factor corresponds to the average maximum, therefore the optimal condition for the extraction of AQs is A3 B2 C3 D2 (solvent composition ethanol/water 60:40 (v:v), solvent / material ratio 20:1, temperature 55 ºC and time 30 min).

<table>
<thead>
<tr>
<th>Table 3. S/N Response Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Levels 1</td>
</tr>
</tbody>
</table>

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The contributions of the different levels of the factors in the yield of AQs, also can be visualized clearly in Figure 1.

![Graph showing the effect of different levels of L9 orthogonal array (Taguchi’s experimental design) for each parameter on the S/N ratio.](image)

**Fig. 1.** Effect of different levels of L9 orthogonal array (Taguchi’s experimental design) for each parameter on the S/N ratio.

This optimal configuration is not found in the array L9, this is one of the properties of the Taguchi, and which method detects untested configurations. Thus the predicted S/N ratio using the optimal level of the design factors can be calculated as:

\[
[S/N]_{predicted} = [S/N]_m + \sum_{i=1}^{m} ([S/N]_i - [S/N]_m)
\]

where \([S/N]_m\) is the total mean S/N ratio, \([S/N]_i\) is the mean S/N ratio at the optimal level, and the number of the main design factors that affect the quality characteristic [21,24] Using equation 6, the predicted results is S/N of 10.11, this indicates that decreased noise.
With the same equation (6) and replacing S/N by yield AQs can predict the amount AQ (mg) present in the plant. Thereby, the yield AQs was predicted as 3.07 mg AQ/g plant material. The validation experiments were repeated 3 times under the optimised conditions and the result obtained for yield of AQs is 3.28 mg AQ/g plant material (S/N=10.90). This indicates that the optimal condition, A_3B_2C_3D_2, is reliable for the extraction process.

Statistical analysis of variance (ANOVA) was performed to investigate whether the process factors are statistically significant. The $F_\alpha$ is a constant and defined as the critical value of $F$-value for different inspection factors, and can be found from the distribution table of $F$-values [25]. As for inspection level, $\alpha=0.10$, the critical $F$-value can be found out ($F_{0.1}(2,18) = 2.64$). The factor effect for results is prominent when $F_j$ is larger than $F_\alpha$, otherwise on the contrary. As seen in Table 4, comparing the values of $F_j$, it is evident that the factor A is statistically significant for the extraction of AQs ($F_A > F_\alpha$), while the factor B, C and D have no significant effect ($F_B$, $F_C$ and, $F_D < F_\alpha$).

Furthermore, from the rate of contribution (Table 4), may also be deduced that the most important factor which contributes to the extraction yield of AQs is the factor A (solvent concentration, 22.56%), followed by factor D (extraction time, 1.95%) and the factor C (temperature, 0.32%) and finally, the factor B (ratio solvent / sample, 0.15%).

**Table 4. ANOVA**

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>V</th>
<th>F</th>
<th>$F_\alpha$</th>
<th>P(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.741</td>
<td>2</td>
<td>1.870</td>
<td>2.707</td>
<td>&gt;</td>
<td>22.56</td>
</tr>
<tr>
<td>B</td>
<td>0.025</td>
<td>2</td>
<td>0.012</td>
<td>0.018</td>
<td>&lt;</td>
<td>0.151</td>
</tr>
<tr>
<td>C</td>
<td>0.053</td>
<td>2</td>
<td>0.026</td>
<td>0.038</td>
<td>&lt;</td>
<td>0.324</td>
</tr>
<tr>
<td>D</td>
<td>0.324</td>
<td>2</td>
<td>0.162</td>
<td>0.235</td>
<td>&lt;</td>
<td>1.959</td>
</tr>
<tr>
<td>E</td>
<td>12.435</td>
<td>18</td>
<td>0.690</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>16.580</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS, sum of square deviation; df, degree of freedom; V, variance; F, $F$-ratio; $e$, experimental error; P(%), percentage of contribution

**3.2. The effect of solvent composition on extraction of anthraquinones by ultrasound-assisted extraction**

After determining the optimal condition for the extraction of these AQs, the effect of solvent composition on the AQ yield was analyzed. For this the process variable was maintained according to optimal conditions determined by Taguchi analysis, and modifying the composition of ethanol-water solutions.
As it can be seen in Figure 2, the extraction efficiency increases with the water content up to a content of 40 % v/v (60% v/v of ethanol). This was probably due to the relative polarity of the solvent according to the composition; the increase of water in the solution favors the lipophilic species extraction [26]. In addition, the increase in effective swelling of the plant by water, helped increase the surface area for solute solvent contact [26]. Furthermore, the presence of water lowers the mixture viscosity. This viscosity decreasing is accompanied with density decreasing, diffusivity increasing, and thus mass transfer [27-30]. A water content higher than 40%, produces recovery decreasing because high content of water increased the mixture polarity, which is not favorable for extracting AQ (nonpolar). The same effect was reported in literature for other AQS [15].

In addition, these results are in accordance with the condition of optimal solvent composition determined by the Taguchi method.

![Graph showing the effect of ethanol concentration on the UAE](image)

**Fig. 2.** Effect of ethanol concentration on the UAE. Extraction condition: time 30 min; ratio solvent/sample 20:1; temperature 55ºC.

### 3.3. The effect of time on extraction of anthraquinones by ultrasound-assisted extraction

In the same way, the effect of time was analyzed performing the UAE at different times (0, 15, 45, 60, 75 and 90 min), and maintaining the other extraction factors according to the optimal conditions (solvent composition 60% v/v of ethanol, solvent /
sample ratio 20:1 and temperature 55 °C). All tests were carried out in triplicate and the results are shown in Figure 3. As it can be observed the yield increases with increasing contact time between the plant material and solvent, as expected. The figure shows that the higher yield is obtained at 30 min (3.28 mg AQ/g plant material). After this time, the yield of AQ is characterized by a constant tendency, indicating that in this case the plant material is exhausted. Also, these results are in accordance with the condition of optimal time determined by the Taguchi method.

Fig. 3. Effect of time on the UAE. Extraction condition: solvent composition 60% ethanol; ratio solvent/sample 20:1; temperature 55°C

3.4 Comparison of UAE with other conventional technique

In this study, UAE was compared with the conventional extraction technique (Soxhlet) for the extraction of these AQs. For UAE the experiment were carried out under the optimal conditions. To compare the two techniques, in the case of soxhlet, the only factor that can be kept equal to the UAE is the composition of the solvent, which is precisely the factor that most influences the extraction. The conditions of the different techniques and their results are shown in Table 5.

This Table shows that the total AQs yield obtained by UAE is almost double that obtained with Soxhlet. In addition, it can be noted that in both techniques the majority AQ in the extracts is soranjidiol. With respect to extraction time, UAE is also the fastest
extraction method with only 30 min. So, efficiency (yield of total AQs obtained from the plant / time of extraction) is greater in UAE than in Soxhlet method.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Ethanol (%v/v)</th>
<th>Time (h)</th>
<th>AQs Content</th>
<th>Yield total AQs (mg/g of vegetal matrix)</th>
<th>Efficiency (mg AQs/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE</td>
<td>60</td>
<td>0.5</td>
<td>0.77</td>
<td>2.32</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.56</td>
</tr>
<tr>
<td>Soxhlet</td>
<td>60</td>
<td>9</td>
<td>0.55</td>
<td>0.96</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

In this paper, the effect of several factors on AQs extraction from *H. pustulata* by UAE was investigated using Taguchi method, which was adapted into an orthogonal array L9. This process optimization was carried out effectively for the extraction of these AQs. UAE optimal conditions for AQs are solvent composition 60% v/v of ethanol, solvent/sample ratio 20:1, extraction time of 30 min, and extraction temperature of 55 °C. The yield predicted by Taguchi in the optimal conditions was validated experimentally demonstrating that the method was efficient because since experimental yields were higher than predicted yields.

Furthermore the Taguchi analysis determined that the most influential factor in the extraction of AQs is the solvent composition, followed by time, temperature and relative solvent/sample. This study was completed by the analysis of variance (ANOVA), also indicating that the most statistically significant factor in the extraction of AQs is the composition solvent.

The experimental results presented in this work proved that UAE is an easiest, faster and more efficient method than traditional technique (Soxhlet) to extract AQs from *H. pustulata*.

Acknowledgements

The authors are grateful to CONICET, ANPCyT and Universidad Nacional de Córdoba (Argentina) and Universidad Tecnológica Nacional for financial support.

References


[8] L. R. Comini et. al., 2012, In Vitro Antiviral Activity of Heterophyllaea pustulata Extract, Natural Products Communications 6 1-3


[18] L. Galvan d’Alessandro et. al., 2012, K. Kriaa, I. Nikov, K. Dimitrov, Ultrasound assisted extraction of polyphenols from black chokeberry, Separation and Purification Technology 93 42–47.


[24] Sh. Rouhani et. al., 2009, Ultrasonic Assisted Extraction of Natural Pigments from Rhizomes of Curcuma Longa L, Prog. Color Colorants Coat. 2 103-113


[30] J. M. Roldán-Gutiérrez et. al. 2008, Ultrasound-assisted dynamic extraction of valuable compounds from aromatic plants and flowers as compared with steam distillation and superheated liquid extraction. Talanta. 75, 1369-1375.