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Original Article

# Dynamic maceration of *Matricaria chamomilla* inflorescences: optimal conditions for flavonoids and antioxidant activity

Simone Vieira Pereira [a], Rayssa A.S.P. Reis [a], Danielle Cristina Garbuio [b,c], Luis Alexandre Pedro de Freitas [a,*]

[a] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[b] Escola de Enfermagem de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[c] Faculdades Anhanguera, Valinhos, SP, Brazil

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ABSTRACT

The aim of this paper was to study and optimize the dynamic maceration process to obtain *Matricaria chamomilla* L., Asteraceae, inflorescences extracts with optimum flavonoid content and antioxidant activity using a multivariate approach. Hydroalcoholic extracts were obtained by dynamic maceration in lab scale and the influence of extraction temperature, ratio of plant to solvent, ethanol strength; extraction time and stirring speed on the flavonoid content and antioxidant activity were unveiled using a fractional factorial design. The ethanol strength, ratio of plant to solvent and temperature were the three factors that influenced most the extract properties and were studied by a central composite design. Total flavonoid content and antioxidant activity were affected by the ethanol strength and ranged from 1.49 to 3.95% and 13.3 to 36.2 μg/ml, respectively. The desirability functions resulted in an optimal dynamic maceration condition using 1 h extraction at stirring speed of 900 rpm, ethanol 74.7%, temperature of 69 °C and using 36.8% of plant in solvent (w/v). Under this set of conditions, the extract had total flavonoid content of 4.11 ± 0.07%, *in vitro* antioxidant activity with $IC_{50}$ of 18.19 μg/ml and apigenin and apigenin-7-glycoside contents of 2.0 ± 0.1 mg/g and 20.1 ± 0.9 mg/g, respectively. The results showed a low solvent consumption compared to previous works. The model was able to predict extract properties with maximum deviation of 12% and the extraction process developed herein showed to be reliable, efficient and scalable for *M. chamomilla* inflorescences, enriched with flavonoids, apigenin and apigenin-7-glycoside and high antioxidant activity.



## Introduction

Plant extracts usually present several therapeutic applications because of their richness in bioactive and have been used to treat man's health problems since ancient times. Recognizing this fact, the World Health Organization (WHO) has also been working to promote the use of these in health systems and recently published guidelines for the quality, safety and efficacy of herbal medicines. The sustainable production and efficacy of medicinal plants have made their extracts a focus of world attention (Ansel et al., 2000; Srivastava et al., 2010; Cortés-Rojas et al., 2015).

Among the worldwide known medicinal plants there is the *Matricaria chamomilla* L., Asteraceae, which is one of the oldest and best documented plants in the world (Srivastava et al., 2010) and its inflorescences are widely used to obtain infusions, extracts and essential oils. Due to *M. chamomilla* diverse therapeutic properties, it stands out as the most cultivated and consumed medicinal plant in the world today, with estimated more than one million cups of its tea consumed daily (Borsato et al., 2005; Srivastava et al., 2010).

The extract of *M. chamomilla* has a wide variety of constituents, which can provide antioxidant, anti-inflammatory, moisturizing and emollient properties that could be used in formulations for topical application. Also because of the numerous benefits to the skin the use of extracts and essential oils in pharmaceutical and cosmetic formulations is increasing. Despite this, there are still few studies on topical formulations containing the extracts of chamomile (Dal'Belo et al., 2006; Nóbrega et al. (2013)). Chamomile extraction can be performed by different processes and conditions that can influence extract characteristics. Harbourne et al. (2009) studied chamomile extraction by water infusion at 90 °C during

* Corresponding author.
*E-mail:* lapdfrei@usp.br (L.A. Freitas).

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20 min comparing fresh and dried flowers and found total phenolic content of 19.7 ± 0.5 mg/g and 13.0 ± 1.0 mg/g for fresh and oven-dried inflorescences, respectively. The *M. chamomilla* soxhlet, microwave-assisted (MAE), ultrasound-assisted (UAE) and subcritical water (SCW) extractions were compared (Cvetanovic et al., 2014) using the same solvent, drug-to-solvent proportion, time and temperature. SCW at 200 °C and 1.6 bar for 40 min gave the best results with 49.70% extraction yield, phenolic content from 117.31 to 151.45 mg/g and flavonoid content from 51.6 to 64.3 mg/g. Dynamic maceration of *M. chamomilla* (Srivastava and Gupta, 2009a,b) using methanol, ethanol, water and methanol–water mixtures as solvent at 200 rpm, 37 °C, drug/solvent ratio of 1:20 for 4 h demonstrated the water extract had no apigenin but high contents of apigenin-7-glycoside. Solid liquid extraction using aqueous 0.5 N HCl, 0.5 N NaOH and water at 80 °C and drug/solvent proportion of 1:20 for 3 h resulted in extracts with higher phenolic content in alkaline conditions, but flavonoid content and antioxidation activity was higher in acidic solvent (Osman et al., 2016).

Recently, the International Conference on Harmonization (Freitas et al., 2017) had established the guidelines for the quality of pharmaceutical products. The guideline Q8 is devoted to the development of pharmaceutical products and indicates the need for a deep understanding of the process and formulation influences on final product quality. Among the considerations, ICH-Q8 recommends the application of multivariate analysis to fully understand the processes involved. Therefore, the design of experiments, DOE, is an increasingly important tool that allows obtaining a greater number of information using a smaller number of experiments (Peralta-Zamora et al., 2005; Ferreira et al., 2007; Rodrigues and Iemma, 2009). The DOE became a very powerful tool to generate, interpret and apply scientific experiments in most efficient way (Ferreira et al., 2007) with optimization of product and process.

The aim of this work was to study the extraction of *M. chamomilla* inflorescences by dynamic maceration applying multivariate analysis to obtain an optimized hydroalcoholic extract with maximum flavonoids content, antioxidant activity (DPPH) and apigenin and apigenin-7-glycoside.

## Materials and methods

### *Materials*

*Matricaria chamomilla* L., Asteraceae, dried inflorescences were purchased from Flores e Ervas Ltda (Piracicaba, SP, Brazil). Analytical-grade apigenin and apigenin-7-glucoside were purchased from Sigma–Aldrich Brasil Ltda (São Paulo, SP, Brazil). Ethanol and phosphoric acid were acquired from Synth Ltda (Diadema, SP, Brazil). Methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA).

### *Drug (Chamomile) characterization*

Samples of the inflorescences were deposited in the herbariums of the Faculdade de Filosofia, Ciência e Letras of Ribeirão Preto, University of São Paulo with number SPFR 15116 and at Herboteca Silvio Sarti (LADIFARP/FCFRP/USP) with number HSS 0012/15.

The dried inflorescences were grounded in a knives mill SL31 (Solab Ltda, Piracicaba, São Paulo, Brazil). The powder size distribution of grounded inflorescences was measured using a set of sieves with mesh openings from 45 to 1400 μm and a sieve shaker model 01/02 (Bertel Ltda, São Paulo, Brazil).

The plant solvent uptake was determined as follows: 25 ml of solvent was added to a 25 ml beaker containing 1 g of grounded inflorescences. The beaker was shaken every 10 min for 1 h and allowed to stand for 3 h at room temperature. The solvent uptake was obtained by the difference between the volume occupied by the plant material at the end of the test and its initial volume.

### *Extraction procedure*

The hydroalcoholic extracts of the *M. chamomilla* were obtained by dynamic maceration in a multipoint magnetic stirrer with thermostatic air bath TAB15 (Labmaq do Brasil Ltda, Ribeirão Preto, SP, Brazil) and using 250 ml Erlenmeyer and according to the extraction conditions defined by the experimental designs. At the end of the experiment, the extracts were filtered using qualitative filter paper.

A fractional factorial design $2^{5-2}$ with two levels (−1 and 1) and five factors (Barros-Neto et al., 2003) was applied to study preliminarily the influence of the extractive factors extraction temperature (TE), percent ratio of plant to solvent (P/S%), percent ethanol strength (Et%), extraction time ($t$) and stirring speed ($S_S$) on the extract solids content (SC) and total flavonoid content (TFC). Table 1 shows the five factors with their levels in the coded form (−1 and +1) and actual values in low and high levels.

After the choice of the most important factors on the dynamic maceration, a central composite design (CCD) was set to study the effect of TE, P/S% and Et% on the extract properties. The CCD is shown in Table 2. The experiments were performed according to the conditions established in Table 2 using a stirring speed of 900 rpm and extraction time of 1 h. The dependent variables evaluated in the CCD were solids content (SC), total flavonoid content (TFC) and *in vitro* antioxidant activity ($IC_{50}$) of the extracts.

**Table 1**
Fractional factorial design ($2^{5-2}$) applied to evaluate the effects of extraction temperature (TE), percentage of plant to solvent (P/S%), percentage of ethanol (Et%), extraction time ($t$) and stirring speed ($S_S$) on *Matricaria chamomilla* extract.

| Experiment | TE (°C) | P/S% (w/v) | Et% (v/v) | $t$ (h) | $S_S$ (rpm) |
|---|---|---|---|---|---|
| 1 | −1 (25) | −1 (10) | −1 (50) | −1 (1) | +1 (900) |
| 2 | −1 (25) | +1 (30) | −1 (50) | +1 (18) | −1 (300) |
| 3 | −1 (25) | −1 (10) | +1 (90) | +1 (18) | −1 (300) |
| 4 | −1 (25) | +1 (30) | +1 (90) | −1 (1) | +1 (900) |
| 5 | +1 (50) | −1 (10) | −1 (50) | +1 (18) | +1 (900) |
| 6 | +1 (50) | +1 (30) | −1 (50) | −1 (1) | −1 (300) |
| 7 | +1 (50) | −1 (10) | +1 (90) | −1 (1) | −1 (300) |
| 8 | +1 (50) | +1 (30) | +1 (90) | +1 (18) | +1 (900) |

**Table 2**
Central composite design applied to evaluate the effects of extraction temperature (TE), percent ratio of plant to solvent (P/S%) and percentage of ethanol (Et%) on *Matricaria chamomilla* extracts.

| Experiment | [a]$X_1$ | [a]$X_2$ | [a]$X_3$ | P/S% (w/v) | Et% (v/v) | TE (°C) |
|---|---|---|---|---|---|---|
| 1 | −1 | −1 | −1 | 10 | 40 | 30 |
| 2 | +1 | −1 | −1 | 30 | 40 | 30 |
| 3 | −1 | +1 | −1 | 10 | 80 | 30 |
| 4 | +1 | +1 | −1 | 30 | 80 | 30 |
| 5 | −1 | −1 | +1 | 10 | 40 | 60 |
| 6 | +1 | −1 | +1 | 30 | 40 | 60 |
| 7 | −1 | +1 | +1 | 10 | 80 | 60 |
| 8 | +1 | +1 | +1 | 30 | 80 | 60 |
| 9 | [b]$-\alpha$ | 0 | 0 | 3.2 | 60 | 45 |
| 10 | [b]$+\alpha$ | 0 | 0 | 36.8 | 60 | 45 |
| 11 | 0 | [b]$-\alpha$ | 0 | 20 | 26.4 | 45 |
| 12 | 0 | [b]$+\alpha$ | 0 | 20 | 93.6 | 45 |
| 13 | 0 | 0 | [b]$-\alpha$ | 20 | 60 | 19.8 |
| 14 | 0 | 0 | [b]$+\alpha$ | 20 | 60 | 70.2 |
| 15 | 0 | 0 | 0 | 20 | 60 | 45 |
| 16 | 0 | 0 | 0 | 20 | 60 | 45 |
| 17 | 0 | 0 | 0 | 20 | 60 | 45 |

[a] $X_1$, P/S% coded; $X_2$, Et% coded; $X_3$, $T_E$ coded.
[b] $\alpha = 1.68$.

*Extraction optimization and extracts characterization*

Extraction optimization was carried out by the desirability functions method using the module *Response Optimizer* from the software Minitab 14.0 (Lead Technologies Inc.) and the SC, TFC and $IC_{50}$ data obtained in the CCD. Hydroalcoholic extracts were characterized by their solids content (SC), total flavonoid content (TFC), *in vitro* antioxidant activity ($IC_{50}$), density ($\rho$) and apigenin ($Q_A$) and apigenin-7-glycoside ($Q_{A7}$) contents.

The SC was determined gravimetrically by sampling and weighting 2 g aliquots of the extracts in Petri dish and taking to an oven at 105 °C until constant weight. The analyses were performed in triplicate and result was calculated as percent weight (w/w) (Brazilian Pharmacopoeia, 2010; Tacon, 2012; Martins et al., 2013).

The TFC was measured by the method of complexation with 2% aluminum chloride in methanol and using quercetin (Sigma–Aldrich) as reference. The samples were analyzed in a UV–vis spectrophotometer model M 330 (Camspec Ltd., Garforth, UK) at 425 nm after 1 h of reaction under light (Costa-Machado et al., 2013). The analyses were performed in triplicate and results were expressed as weight percentage (w/w). The extracts density was determined by pycnometry according to Brazilian Pharmacopoeia (2010).

The *in vitro* antioxidant activity was determined by the 2,2-diphenylpicrilhydrazyl radical (DPPH) method. Each extract sample was diluted in methanol solutions in five concentrations. Then, 50 μl of each extract dilution was mixed with 2 ml of 60 μM of DPPH in methanol. The samples were then held sheltered from the light for 1 h and analyzed by UV–vis spectrophotometer model M 330 (Camspec Ltd., Garforth, UK). The results were expressed as $IC_{50}$, which is the concentration corresponding to the 50% inhibition of DPPH. Antioxidant activities of quercetin and α-tocopherol were also determined following this same methodology. The analyses were performed in triplicate and the IC for each sample was calculated based in Eq. (1) in seven concentrations (Abdoul-Latif et al., 2011; Kulisic et al., 2004) and the $IC_{50}$ was obtained the extract concentration (μg/ml) corresponding to an inhibition of 50%.

$$\text{IC}\ (\%) = \left(\frac{\text{Abs}_{517\,\text{nm}}\text{Control} - \text{Abs}_{517\,\text{nm}}\text{Sample}}{\text{Abs}_{517\,\text{nm}}\text{Control}}\right) \quad (1)$$

*High performance liquid chromatography*

The quantification of apigenin and apigenin-7-glucoside in the hydroalcoholic extract was done by high performance liquid chromatography (HPLC) on an Ultimate 3000 chromatograph (Thermo Scientific Inc, Waltham, USA) with a diode array detector operating at wavelengths of 335 nm. The analyses were run with a Phenomenex Luna C-18 column (4.6 mm × 250 mm, particle size of 5 μm and aperture of 100 Å) and a safety guard column, Phenomenex Luna. The sample volume injected was 20 μl and the mobile phase was acetonitrile/water acidified with phosphoric acid (pH 3). The method was validated by determination of the selectivity, linearity, accuracy, detection and quantification limits (Cabral et al., 2017).

*Statistical analysis*

The experimental designs were analyzed by ANOVA using the response surface methodology and the software's Statistica 10 (Statsoft Inc, USA) and Minitab 14 (Minitab Inc., USA). The effects of factors were considered significant only when $p < 0.05$.

## Results and discussion

The powder size distribution of the dried and grounded inflorescences of *M. chamomilla* showed a Gaussian shape, as seen in Fig. 1. Fig. 1 also shows the accumulative size distribution of the

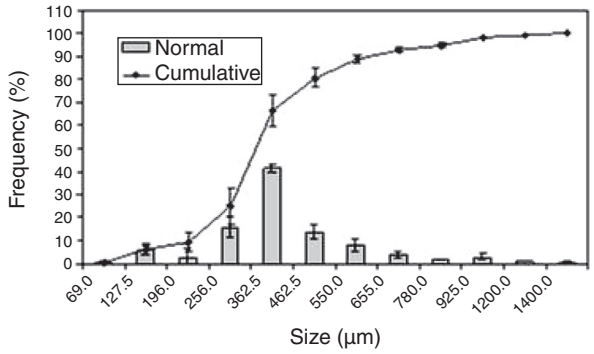


**Fig. 1.** Size distribution of the dried and grounded inflorescences of *Matricaria chamomilla*.

*M. chamomilla* powder and particle sizes from 127 to 1400 μm. From the accumulative size distribution the D50 and Spam could be obtained and resulted in 410 μm and 1.25, respectively. The analysis of the vegetal material granulometry is important for the extractive process, since the mean size and spam of the drug powder influences the extraction of the chemical constituents. It is already known that smaller particle sizes result in faster and sometimes more efficient extraction process. In the literature, extraction studies have used plant materials with particle size ranging from 150 to 825 μm (Jacques et al., 2007; Luthria et al., 2007; Fan et al., 2008; Diouf et al., 2009; Costa-Machado, 2011). The mean particle size and spam found for *M. chamomilla* herein are adequate for a dynamic maceration extraction.

The determination of drug solvent uptake is important to extraction studies since it is related to the amount of solvent which is retained by the solid vegetable matrix and cannot be recovered (Tacon, 2012). The solvent uptake of the dried and ground *M. chamomilla* inflorescence was determined for all the solvents used as extractive liquid in this study. The solvents used were ethanol 26.4%, 40%, 50%, 60%, 80%, 84.8%, 90% and 93.6%, and the respective swelling indexes obtained were 7.0, 6.0, 4.2, 2.4, 1.9, 1.8, 1.6, 1.4 and 1.3 ml/g.

The HPLC method was validated for apigenin and apigenin-7-glycoside and showed to be selective to the analytical standards compared to the chamomile extracts obtained under the several conditions studied. The method showed to be linear within the ranges of 0.1–6 μg/ml ($r = 0.9996$) and 0.1–40 μg/ml ($r = 0.9998$) for apigenin and apigenin-7-glycoside, respectively. The precision and accuracy were evaluated by the relative standard deviation of five injections and resulted in maximum deviations of 2.08 and 2.17% for apigenin and 2.19 and 3.62% for apigenin-7-glycoside, respectively. Quantification limits were 0.05 μg/ml for both chemical markers (Cabral et al., 2017).

*Fractional factorial design*

The response surface analysis carried out on the influence of the five factors on SC and TFC is shown in Fig. 2. Although only the P/S% effect on SC was significant at 5% level, the estimated effect can still be used to identify the strongest influences among the factors studied. As can be seen in Fig. 2A, the three factors that influenced most the SC responses were P/S%, Et% and TE with estimated effects of 6.7, 4.2 and 2.0, respectively. The influence of P/S% on SC and TFC is theoretically expected since increasing the proportion of plant a larger amount of soluble solids and phenolic compounds are available to be extracted for as much as the solvent does not become saturated. Another important estimated effect is for Et% on SC and TFC due to the increasing polarity for the increasing strength of ethanol in hydroalcoholic solutions, favoring the extraction of

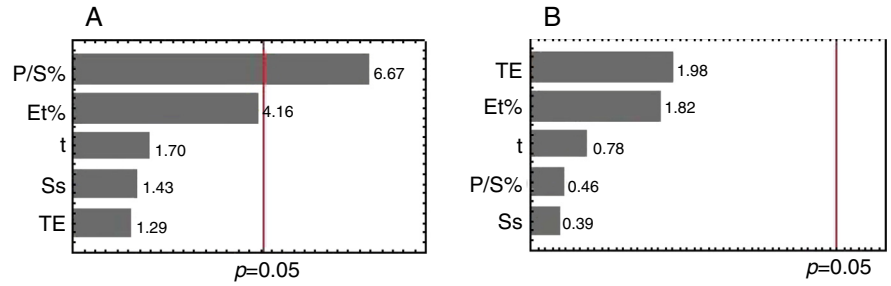


**Fig. 2.** Standardized effects of the factors on the: (A) SC and (B) TFC of extracts.

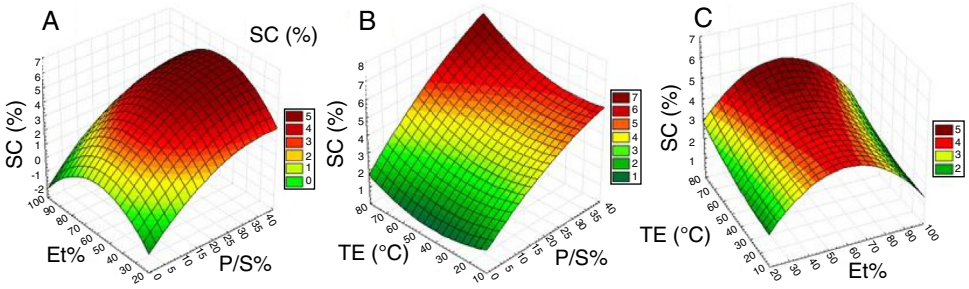


**Fig. 3.** Surface response plots of extracts solid contend (SC) obtained in central composite design as function of: (A) Et% and P/S%, (B) TE and P/S% and (C) TE and Et%.

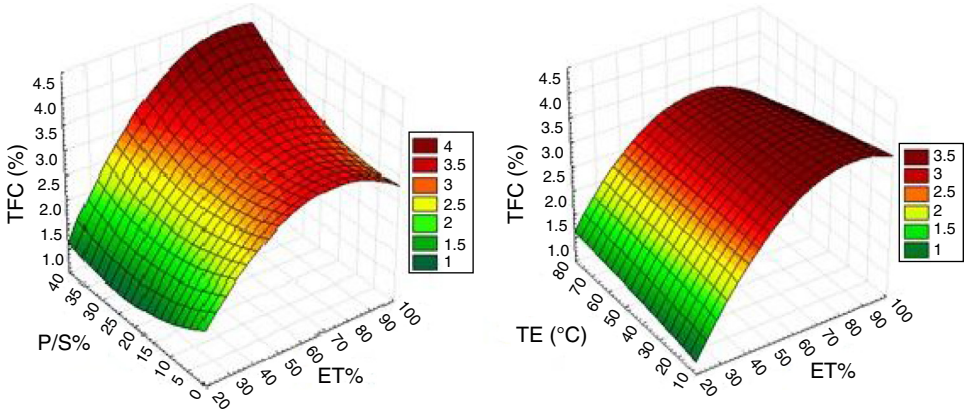


**Fig. 4.** Surface response plots of total flavonoids content (TFC) of extracts obtained in central composite design as function of: (A) P/S% and Et% and (B) TE Et%.

flavonoids and soluble solids (Pinelo et al., 2006; Huang et al., 2009; Costa-Machado et al., 2013). Finally, TE can influence SC and TFC, because the temperature interferes with solute solubility, diffusion coefficient and dielectric constant of water (Costa-Machado, 2011; Costa-Machado et al., 2013; Pinelo et al., 2006).

In equilibrium extractive methods (Martins et al., 2017), such as dynamic maceration, the extraction time t is a factor that must be considered because there is a minimum time required for the solvent to swell and moisturize the plant, solubilize the compounds within the vegetable cells and then diffuse them into the solvent (Prista and Morgado, 1995; Costa-Machado et al., 2013; Martins et al., 2017). However, in the present study t (Fig. 2A and B) was not among the factors that influenced mostly SC and TFC. The agitation speed probably promoted a rapid homogenization of the solute in the solvent in this case, causing the equilibrium to be reached within 1 h. According to the literature, other factors, such as temperature and agitation speed, may influence extraction time (Costa-Machado et al., 2013; Martins et al., 2017).

*Central composite design*

The results of total solid contents and flavonoid contents in *M. chamomilla* extracts originated from the CCD are shown in Figs. 3 and 4, respectively. The SC ranged from 1.02 to 6.24%, and was affected significantly by the factors P/S%, Et% and TE at the significance level of 5%. The results obtained here agree with literature on dynamic maceration (Pinelo et al., 2006; Huang et al., 2009; Costa-Machado et al., 2013; Tacon and Freitas, 2013). Fig. 3A shows SC as function of Et% and P/S% and as seen also in Table 3, the both the linear and quadratic effects of these two factors on SC are significant at 5%. The response surface in Fig. 3A is curved due to the quadratic effect and shows that the highest values of SC were observed for intermediate values of Et%. One possible explanation for this result is that hydroalcoholic solutions of intermediate polarities are capable of extracting both polar and nonpolar compounds, which results in higher amounts of soluble solids extracted. Also in Fig. 3A it is possible to see that maximum SC is observed for the highest P/S%. This effect is also well notified in literature

**Table 3**
Summary of ANOVA on extracts solid content (SC), total flavonoid content (TFC) and *in vitro* antioxidant activity ($IC_{50}$) in central composite design.

| Studied factors | *p* value | | |
|---|---|---|---|
| | SC | TFC | $IC_{50}$ |
| P/S% | 0.000005[a] | 0.054763 | 0.656327 |
| P/S%$^2$ | 0.016715[a] | 0.073836 | 0.159313 |
| Et% | 0.528548 | 0.000011[a] | 0.069868 |
| Et%$^2$ | 0.000419[a] | 0.002062[a] | 0.000145[a] |
| TE | 0.032055[a] | 0.501449 | 0.247743 |
| TE$^2$ | 0.674875 | 0.626947 | 0.426903 |
| P/S% × Et% | 0.687189 | 0.119734 | 0.249776 |
| P/S% × TE | 0.506638 | 0.298957 | 0.793896 |
| Et% × TE | 1.000000 | 0.362543 | 0.742624 |

[a] $p < 0.05$.

(Martins et al., 2017) since higher ratio of plant in relation to solvent gives more soluble solids available to be extracted, and in this case revealing the solvent is not saturated. The influence of TE on SC can be seen in Fig. 3B, where it is possible to observe that the SC increases with increasing the temperature in the extraction. Although there is some divergence in the literature regarding the effect of temperature in extraction (Moure et al., 2001), the most accepted idea is that the increase in temperature promotes an increase in solute solubility, diffusion coefficient and dielectric constant of water which may improve compounds extraction (Costa-Machado, 2011; Pinelo et al., 2006). However, the data in Table 3 indicates that the effect of TE on SC is linear, which is shown in Fig. 3C. In the response surface in Fig. 3C the effect of TE seems to be not so pronounced such as in Fig. 3B, showing that Et% effect is more important than TE. Also, Fig. 3C confirms that Et% effect is quadratic on SC and there is a maximum SC at intermediary values of Et%.

The data from central composite design shows that TFC results were between 1.49 and 3.95% (w/w). These values are consistent with the literature (Franke and Schilcher, 2005; Reis et al., 2011). However, the minimum content of total flavonoids in extracts of *M. chamomilla* is usually reported to be 2.5% (Dowd, 1959; Reis et al., 2011). The effect of Et% on the TFC is presented in Fig. 4A and B, showing that extract TFC increases with Et% in a asymptotic behavior. This result is consistent with the literature, since higher proportions of ethanol in solution favor the extraction of phenolic compounds (Costa-Machado, 2011; Huang et al., 2009; Pinelo et al., 2006). Fig. 4A and B also show that effects of P/S% and TE are not so expressive. All the effects observed in the response surfaces Fig. 4A and B are confirmed by the analysis of variance, as shown in Table 3.

Fig. 5A and B shows the influence of the P/S%, TE and Et% on the $IC_{50}$. The *in vitro* antioxidant activity by DPPH indicates that the extracts obtained in the CCD presented $IC_{50}$ between 13.38 and 36.23 μg/ml. These $IC_{50}$ indicate that the extracts presented good antioxidant activity, since a small extract concentration is required to inhibit the DP/S%H radical, which is in accordance with the literature (Abdoul-Latif et al., 2011) and it is similar to the antioxidant activity of other medicinal plants (Lima et al., 2006; Sousa et al., 2007; Costa-Machado, 2011; Tacon and Freitas, 2013). However, extracts $IC_{50}$ were higher than reference compounds studied herein, since their $IC_{50}$ were 1.14 ± 0.28 μg/ml and 8.23 ± 0.56 μg/ml for quercetin and α-tocopherol, respectively. These quercetin and α-tocopherol $IC_{50}$ are in good agreement with literature (Kulisic et al., 2004; Costa-Machado, 2011).

The effects of extraction conditions on $IC_{50}$ shown in Fig. 5A and B. The surface responses in these figures indicate that $IC_{50}$ was influenced by Et% but not by P/S% and TE. The effect of Et% is somewhat unusual since there a minimum $IC_{50}$ for intermediate values of Et%, meaning that the antioxidant activity of the hydroalcoholic extracts are higher for intermediate Et%. This is an indicative that $IC_{50}$ is probably related not only to the total flavonoids, but also to other compounds of *M. chamomilla*. Similar effect was observed by Kazan et al. (2014) and Kukula-Koch et al. (2013).

The summary of statistical analysis on SC, TFC and $IC_{50}$ are presented in Table 3. The linear and quadratic term of percentage of plant (P/S% and P/S%$^2$), the quadratic term of the ethanol percentage (Et%$^2$) and the extraction temperature (TE) have a significant influence on SC. The extracts TFC and $IC_{50}$ were influenced only by the Et%.

### *Optimization of extraction process*

The optimization of the *M. chamomilla* dynamic maceration extraction process was made to obtain maximum extraction of total solids and flavonoids, as well as maximum antioxidant activity (minimum $IC_{50}$) of the extracts. The optimization was done by the method of desirability functions using the set of equations fitted to the response surfaces of SC, TFC and $IC_{50}$. The desirability is widely used because it is very useful when there are a large number of responses to be considered (Freitas et al., 2017).

The optimal condition obtained for the *M. chamomilla* dynamic maceration were percent plant to solvent ratio of 36.8% (w/w), 74.7% (w/w) ethanol solution and temperature of 69 °C. In order to verify the accuracy of extraction performance, experiments were run in triplicate at this combination of P/S%, Et% and TE, and also at a stirring speed of 900 rpm for 1 h. Table 4 shows the comparison between the predicted and experimental values for each response studied. Under this set of conditions the extract presented solids content of 5.38 ± 0.03% (w/w), total flavonoid content of 4.11 ± 0.07% (w/w) and antioxidant activity ($IC_{50}$) of

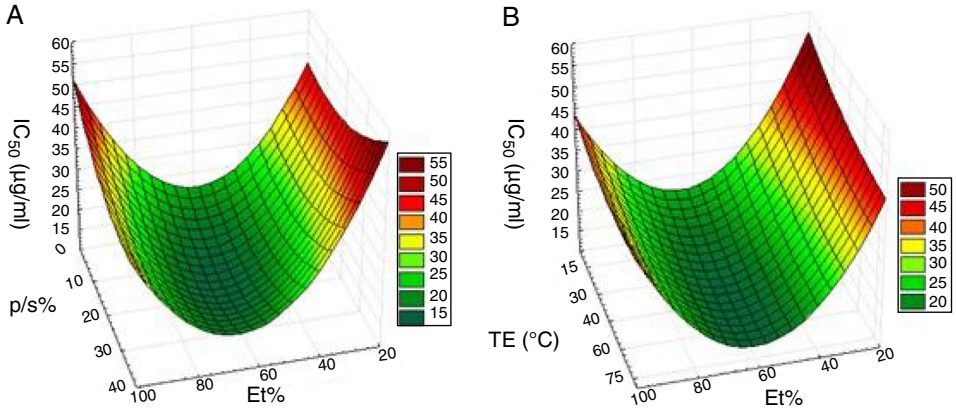


**Fig. 5.** Surface response plots of antioxidant activity *in vitro* ($IC_{50}$) of extracts obtained in central composite design as function of: (A) P/S% and Et% and (B) TE Et%.

**Table 4**
Comparison between predicted and experimental values in optimal conditions of extractive process, considering solid content (SC), total flavonoids content (TFC) and *in vitro* antioxidant activity ($IC_{50}$).

| Results | Predicted | Experimental[a] |
|---|---|---|
| Solid content (SC) | 5.94% (w/w) | 5.38 ± 0.03% (w/w) |
| Total flavonoids content (TFC) | 3.66% (w/w) | 4.11 ± 0.07% (w/w) |
| *In vitro* antioxidant activity ($IC_{50}$) | 18.45 μg/ml | 18.19 ± 0.96 μg/ml |

[a] Mean ± standard deviation ($n = 3$).

18.19 ± 0.96 μg/ml. In previous works the solid liquid extraction of *M. chamomilla* resulted in TFC of 0.11–0.18% (Osman et al., 2016) while subcritical aqueous extraction resulted in 5.16–6.43% (Cvetanovic et al., 2014). The TFC yield of (Cvetanovic et al., 2014) is similar to the one obtained herein but these authors used a drug/solvent ratio of 1:50 while the one used here was only around 1:3, showing that our optimized process is more eco-friendly. The predicted and experimental values of SC, TFC and IC50 were close to each other, with deviations of −10.4%, 10.9% and −1.43%, respectively. The agreement between these values shows that the desirability method is a reliable and adequate tool for the optimization of this process and validates the optimum condition obtained for *M. chamomilla* dynamic maceration.

The hydroalcoholic extract obtained under the optimized condition was also characterized relatively to the density and apigenin and apigenin-7-glycoside contents. The markers of selected hydroalcoholic extract selected in the present study were apigenin and apigenin-7-glycoside. These two markers were selected to better characterize *M. chamomilla* extract because these flavonoids are among the most promising compounds and mostly indicated as responsible for this plant therapeutical activities (McKay and Blumberg, 2006; Weber et al., 2008; Srivastava and Gupta, 2009b; Gupta et al., 2010; Srivastava et al., 2010). Additionally, the Brazilian Normative Instruction Nr. 02 from Anvisa (Anvisa, 2014) establishes the apigenin-7-glucoside as the chemical marker of *M. chamomilla*.

The values obtained were the density of 0.89 ± 0.02 g/ml, and apigenin and apigenin-7-glycoside contents of 2.0 ± 0.1 mg/g and 20.1 ± 0.9 mg/g, respectively. In the extracts of *M. chamomilla*, apigenin is present in very small amounts in the free form, existing predominantly in the glycosylated form (Mulinacci et al., 2000; Srivastava and Gupta, 2007, 2009a,b).

## Conclusions

The multivariate approach applied herein to find optimized conditions to *M. chamomilla* extraction any dynamic maceration allowed to identify the most influential factors among the temperature, ethanol strength, stirring speed, plant to solvent ratio and time. The effects of plant to solvent ratio, ethanol strength and temperature were studied in detail by response surface modeling and the optimal condition determined by desirability functions. The best extraction condition correspond to 1 h extraction at 900 rpm, percent plant to solvent ratio of 36.8% (w/w), 74.7% (w/w) ethanol solution and temperature of 69 °C. At this condition the total flavonoid content and antioxidant activity were maximized and resulted in an extract with apigenin and apigenin-7-glycoside contents of 2.0 ± 0.1 mg/g and 20.1 ± 0.9 mg/g, respectively.

## Authors' contributions

SVP was responsible for most of experimental work, contributed in running the pharmacognostic characterization and extractions. RMSR contributed to the experimental work running chromatographic analysis and quantification of the glycosides. DCG contributed to manuscript preparation and critical reading. LAPF contributed to experimental design, data analysis and manuscript preparation. All the authors have read the final manuscript and approved the submission.

## Conflicts of interest

The authors declare no conflicts of interest.

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