IMPACT OF DIC TREATMENT ON THE ANTIOXIDANT PROFILE OF *RHEUM RIBES* L. RHIZOME EXTRACTS

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Abstract

Rheum ribes L. *(rhubarb), which belongs to the family of Polygonaceae, is one of the most important medicinal Mediterranean plants. Its aqueous decoctions have become a common practice among patients suffering from diabetes, hypertension, obesity, and diarrhea. Therefore, it is worth using techniques capable of increasing the extraction of valuable compounds while providing effective microbiological decontamination of the raw material.*

Instant Controlled Pressure-Drop (DIC) is a high temperature-short time treatment (HTST) followed by an abrupt pressure-drop ($\Delta P/\Delta t$>500 kPa s^{-1}) towards a vacuum (about 5 kPa). It allows a controlled expansion to ensure better diffusivity and higher availability of the active molecules. Indeed, DIC treatment has already shown its potential in improving the conventional extraction of active molecules and essential oils from plants.

Rheum ribes L. *rhizomes were collected from Chouf, Lebanon, dried from 1.33±0.28 to 0.16±0.02 g H_2O/g dry-basis, ground, and sieved into small particles of 0.4-2 mm. DIC treatment was used with a saturated steam pressure ranged from 200 to 600 kPa for 10 to 40 s. The antiradical characteristics (DPPH· test) of aqueous extracts of DIC-treated samples were determined. At low levels of saturated steam-pressure, DIC treatment showed a significant impact on the scavenging capacity of the rhizomes. Thus, at 260 kPa, it recorded the lowest IC50 value (54.56±0.19 µg/mL) versus 71.29±0.499 µg/mL for the untreated rhizomes (RM for raw material) used as a control.*

DIC processing parameters will be optimized in our future work to obtain the highest yield of phytochemicals while assessing the antimicrobial, cytotoxic, and bioactive properties of the aqueous extracts of R. ribes L. *rhizomes.*

Keywords: *Rheum ribes L., DIC, DPPH, Antioxidant*

1. INTRODUCTION

Antioxidants are compounds capable of acting as scavenging agents of free radicals thus playing the role of defenders against many human ailments such as cardiovascular diseases, asthma, and cancer. Antioxidants can be either synthesized (butylated hydroxytoluene, butylated hydroxyanisole, etc.) or naturally derived from plants [1]. The worldwide trend is for the extraction and usage of natural antioxidants, that are found in plants as secondary metabolites, i.e., phenolic compounds with one or more hydroxyl groups [2,3].

A wide group of medicinally important species belongs to the *Rheum* genus. Phenolic compounds that are abundantly present in their rhizomes make them one of the most important medicinal Mediterranean plants [4]. One of these species is *R. ribes* L. that was the subject of many studies, performed in particular on the antioxidant activity of its rhizomes. Abdulla *et al.*, (2014) have compared the antioxidant activity of Iraqi *Rheum ribes* L. rhizomes extracted in two different solvents. The ethanol extract was a better radical scavenger than the aqueous extract [5]. The antioxidant potential of Turkish chloroform and methanol extracts of *Rheum ribes* L. rhizomes were also assessed through different antioxidant tests, such as DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, total antioxidant (lipid peroxidation

inhibition activity), ferric reducing power, cupric reducing power (CUPRAC), superoxide anion radical scavenging, and metal chelating activities. Results have shown that both extracts demonstrated stronger activity than standards compounds, namely α-tocopherol and butylated hydroxytoluene (BHT) [4]. Since people believed for ages in the curative effect of the aqueous decoctions of *Rheum ribes* L. rhizomes, it would be interesting to enhance the extraction of antioxidant molecules. However, plants naturally tend to resist solvent diffusivity during extraction due to their compact structure [6]. To improve the extraction process, changes in the stiff cellular structure are then necessary.

Instant Controlled Pressure-Drop (DIC that stands for *Détente Instantanée Contrôlée* in french) is a high temperature-short time treatment (HTST) followed by an abrupt pressure-drop ($\Delta P/\Delta t>500$ kPa.s^{-1}) towards a vacuum (about 5 kPa). It has been shown that DIC technology is capable of intensifying the extraction process by puffing the plant tissues and consequently reducing the mass transfer resistance [7]. When applied to plants, it was remarkable that DIC treatment increases the antioxidant potential by rising the availability of phenolic compounds in strawberries [8], pomegranate peels [6], and many other plant matrices. Being already a high antioxidant agent, it will still be interesting to increase the *Rheum ribes* L. scavenging activity, which will consequently add a therapeutic value to treated rhizomes. Therefore, the objective of this study was to investigate the effect of DIC treatment on the antioxidant activity of a Lebanese species of *Rheum ribes* L. rhizomes using DPPH free radicals.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant materials

Rheum ribes L. rhizomes were collected in July 2020 from Chouf, Lebanon at an altitude of 1140 m. Firstly, rhizomes were dry cleaned from dirt and soil to avoid risks of contamination, then they were kept in a well-aerated dark room. After fifteen days, rhizomes became dry (0.16±0.02 g H_2O/g dry-basis*), they* were crushed into small particles (0.4-2 mm) and treated by DIC technology.

2.1.2. Chemicals

L-ascorbic acid was obtained from Sigma-Aldrich Co. (Schnelldorf, Germany). Absolute ethanol was obtained from Carlo Erba Reagents (Val-de-Reuil, France). DPPH free radicals were obtained from Merck Group (Fontenay sous Bois, France).

2.2. Methods

2.2.1. DIC treatment

Allaf *et al*., (2014) have described the experimental setup of a DIC machine (Fig1) which is mainly made of a steam generator, a processing vessel, and a large vacuum tank [9]. Thirty-five grams of dried rhizomes were placed in the processing vessel, to be subsequently injected with high pressure/high temperature saturated steam fed by a steam generator. At this stage, condensation on the cold surface of rhizomes will occur. The injection period is then maintained for some time to ensure that the condensed water and heat evenly reach the totality of the treated particles. This phase is then followed by an abrupt pressure drop toward a vacuum that was initially created in a vacuum tank, which initiates the auto-vaporization of water contained in the treated rhizomes. Due to this pressure drop, particles gain a new puffed and porous structure which consequently increases the specific surface area of rhizomes with the extracting solvent, as well as significant cooling that limits thermal degradation of the product.

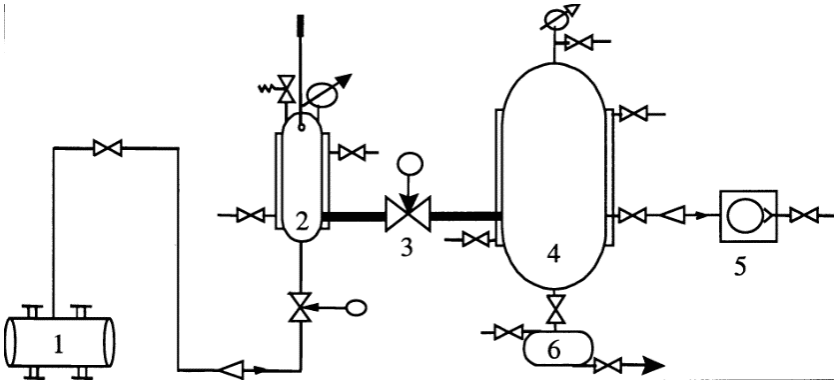


Figure 1. Diagrammatic DIC setup [10]: (1) Saturated steam generator, (2) Processing vessel, (3) pneumatic valve, (4) vacuum tank, (5) vacuum pump, (6) condensate collection trap

2.2.2. Experimental design

We used a 2-variable central composite rotatable design of experiments with the aim of assessing the effect of the DIC independent variables by minimizing the number of experimental trials. This methodology was established and developed in 1992 by Box and Wilson for being an assemblage of mathematical technics leading to empirical models [11]. In this work, DIC experiments were carried out considering two operating parameters: absolute saturated steam pressure ranging from 200 to 600 kPa and processing time varying from 10 to 40 seconds. The goal of our study was to maximize the antioxidant activity of the *Rheum ribes* L. extracts. Since two factors are studied, the design of experiments (DoE) implies 13 experimental points as listed in table 1: five central-points (points number 1, 4, 7, 10, and 13) treated at the same condition of saturated steam pressure (400 kPa) and time (25 s), four factorial-points (points 5, 6, 8, and 9), and four star-points (points number 2, 3, 11, and 12).

Table 1. DIC processing parameters

Sample	Pressure (kPa)	Time (sec)
Central points	400	25
2	600	25
3	400	40
5	540	36
6	540	14
8	260	14
9	260	36
11	200	25
12	400	10

2.2.3. Extract preparation

Ten grams of treated and control rhizomes were macerated in 100 ml of distilled water under continuous magnetic stirring for 24 hours. The extracts were filtered using Whatman No.1 filter papers and then frozen for 48 hours. Finally, extracts were frozen-dried at -55°C under 7.9 Pa using a Martin Christ-Alpha 1-2 LD plus freeze drier. The resulting powders were stored at room temperature in small amber glass containers until usage.

2.2.4. Free radical scavenging activity

The free radical scavenging capacity of treated and control *Rheum ribes* L. rhizomes was evaluated by using stable DPPH free radicals as described by Rammal *et al*., [12]. Equal volumes of DPPH priorly dissolved in absolute ethanol (0.15 mM) and diluted extract of concentrations ranging between 100 and 1000 µg/ml were mixed. The mixture was then shaken and left to stand in the dark at room temperature. Thirty minutes later, the absorbance of the solutions was assessed using a Thermo Scientific-Helios Omega UV-VIS spectrophotometer at 517 nm. The antioxidant activity of the various extracts was calculated using the following formula:

$$\%\ Scavenging\ capacity = \left(\frac{Abs_{\text{control}} - Abs_{extract}}{Abs_{\text{control}}}\right) * 100$$

The blank solution was made of absolute ethanol and distilled water, while the negative control consisted of DPPH and distilled water (1:1 v/v). The IC50 value of each sample was determined to better compare the antioxidant activity of the extracts. The IC50 represents the concentration causing 50% inhibition of free radicals. The lower the IC50 value the more potent the inhibitor.

2.2.5. Statistical analysis

The response variable studied is the antioxidant activity of *Rheum ribes* L. rhizomes. IC50 values were statistically analyzed using Statgraphics Centurion XV (15.2.06).

3. RESULTS AND DISCUSSIONS

The values of antioxidant activity against DPPH free radicals of DIC treated rhizomes and control samples or RM (raw material) are shown in Fig 2. The highest IC50 value was recorded for sample 2 (89.97±0.871 µg/ml) treated at 600 kPa for 25 seconds, followed by sample 5 (89.39± 0.358 µg/ml) and 6 (86.34±0.154 µg/ml) that were both treated at 540 kPa for correspondingly 36 and 14 seconds. Whereas the lowest IC50 values were attributed for sample 9 (54.56± 0.186 µg/ml), 11 (57.34± 0.093 µg/ml), and 8 (64.61± 0.435 µg/ml) that were respectively treated at 260, 200, and 260 kPa at 36, 25, and 14 seconds. It is also notable that samples 2, 3, 5, and 6 antioxidant activities were significantly lower than that of control sample ($p < 0.05$). This can be due to the thermal degradation of antioxidant plant molecules, i.e., phenolic compounds, that were contrarily conserved and even more available in aqueous samples treated at low saturated steam pressures. Similar findings have been reported in DIC treated plant matrices where high pressures caused phenolic degradation. Kamal *et al*., (2015), have stated losses of polyphenolic compounds in henna leaves when treated at high saturated steam pressures [13]. Moreover, a decrease in total phenolic compounds was observed in treated okra pods for severe conditions of DIC such as 600 kPa for 50 seconds, which can be attributed to thermal degradation of phenolic compounds [14].

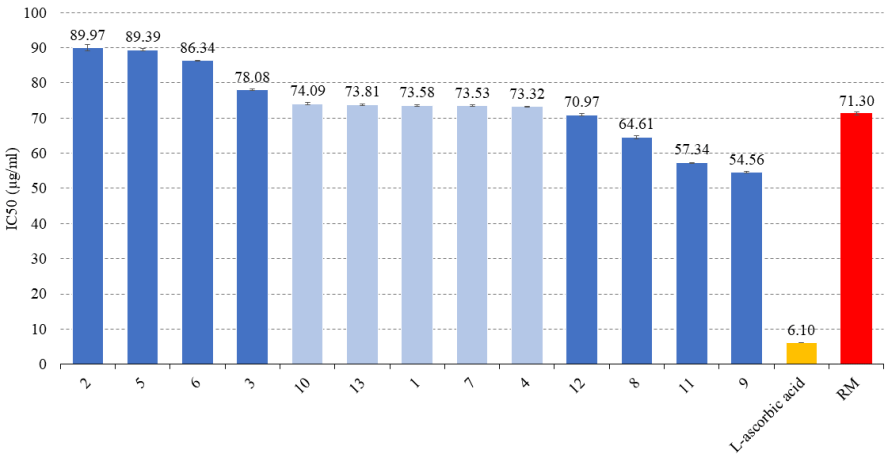


Figure 2. IC50 values of DIC treated, control samples (RM), and L-ascorbic acid

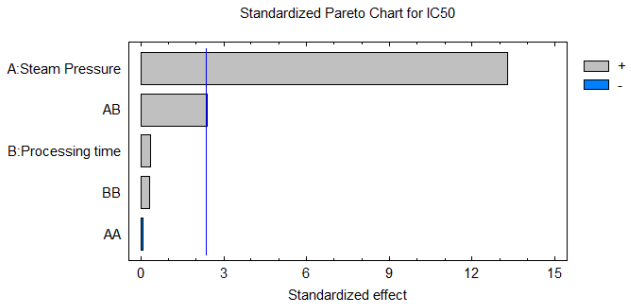
When comparing our results to those obtained by Abdulla *et al*., (2014) it must be pointed out that the IC50 of Iraqi aqueous *Rheum ribes* L. rhizomes is 25.62 µg/ml which means that the Iraqi species has a higher antioxidant activity than the Lebanese one. This can be explained by the climatic difference between the two countries, as it was found that when plants are under hydric and thermal stresses, they increasingly produce secondary metabolites (phenols) [15]. This could explain the higher antioxidant capacity of Iraqi *Rheum ribes* L. rhizomes.

The effect of the DIC parameters on IC50 values of aqueous extracts is presented in Fig 3A that is a presentation of ANOVA for DIC processing parameters and the mutual interaction between them. The operating parameters are considered statistically significant when they exceed the vertical line ($p < 0.05$). The Pareto chart below showed that the variation in saturated steam pressure is the only parameter to have a significant effect on IC50. A positive correlation exists between IC50 of treated rhizomes and the saturated steam pressure. Optimum conditions of DIC parameters with the aim of minimizing the IC50 of *Rheum ribes* L. rhizomes are a saturated steam pressure of P=200 kPa, and a thermal treatment time of t=40 s. Consequently, the optimized value of IC50 is 49.97 µg/ml, which is about 29.9% lower than the IC50 of the raw material. The response surface methodology of Fig 3B allowed us to estimate the below polynomial equation of the fitted model that explains 96.3% of the variability of IC50 (R^2= 96.3%).

$$IC50 = 58.64 + 0.04215P - 0.96328t - 0.0000051P^2 + 0.00213Pt + 0.002895t^2 \quad (1)$$

Where: P = absolute pressure of saturated steam (kPa) and t = processing time (s)

(A)



(B)

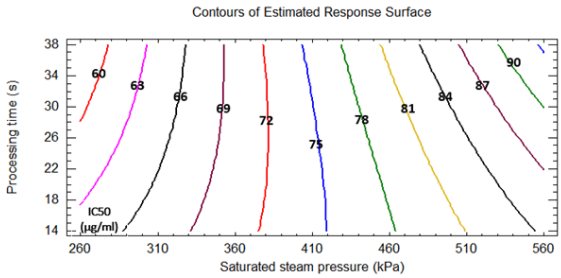


Figure 3. Pareto chart (A) and contours of estimated response surface (B) for IC50

4. CONCLUSION

In conclusion, the findings of the present work have shown that DIC process has an impact on the antioxidant activity of *Rheum ribes* L. rhizomes. The design of experiments revealed the optimized DIC parameters which were P= 200 kPa and t= 40 s. Indeed, the highest antioxidant activities were attributed to samples treated at low pressure/temperature values. Sensitive bioactive molecules towards high pressures/temperatures may be affected. A mild DIC treatment in terms of saturated steam pressure might be more suitable for *Rheum ribes* L. rhizomes.

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