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Intensification of the Solvent Extraction of *Rhus tripartitum* Bioactive Molecules Using Instant Controlled Pressure Drop (DIC)

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Authors' contributions

This work was carried out by author KE, PhD student in the frame of collaboration work between university of La Rochelle, Soran University and University of Gabes. The fundamentals and kinetics modeling were designed by Prof. Author KA while a main part of chemical analysis and identification of AOA was performed by Prof. Author IK wrote the first draft of the manuscript and managed literature searches. Other authors contributed to the statistical analysis, wrote the protocol and managed the analyses of literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This article discusses the use of Instant Controlled Pressure Drop (DIC) as a pre-treatment stage to intensify the solvent extraction of total phenols and more especially tannins from the bark of African sumac (Tunisian *Rhus tripartitum*). Total phenol land tannin contents were determined using the spectrophotometric Folin–Ciocalteau method and external calibration with Gallic acid. We used DIC with two processing parameters in a 5-level central composite Design of Experiment to study the yields of total phenol and tannin as the dependent variables. The results obtained confirmed that the DIC operating parameters, which were saturated steam pressure and total heating time, were significant for both dependent variables. The optimum predictive values for DIC treated bark were 280.66 Gallic Acid Equivalent/g dry basis (GAE/g db) for total phenol and 51.79mgGAE/g

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db for tannins, compared with 207.5 and 33.4mg GAE/g db with untreated raw material. In terms of total phenol extraction kinetics, the starting accessibility δX_s and effective diffusivity D_{eff} were calculated to be 0.17gGAE/g db and $1.26\times10^{-10}m^2s^{-1}$, respectively, for DIC treated material at a steam pressure of 0.37 MP and a processing time of 37s, compared with 0.15 g GAE/g db and $1.18\times10^{-10}m^2s^{-1}$ for raw material. Moreover, all DIC treated samples exhibited higher antioxidant activity compared to the raw material. A positive correlation was established between the total phenol content and the % DPPH free radical scavenging activity. The overall findings demonstrate that DIC increases the extraction efficiency for both total phenols and tannins, thus rendering *Rhus tripartitum* bark a promising source of natural antioxidants.

Keywords: Rhus tripartitum; Instant controlled pressure drop DIC; polyphenols; tannins; extraction kinetics; antioxidant activity.

1. INTRODUCTION

Sumac is the common name for a genus (Rhus) that contains over 250 individual species of flowering plants in the *Anacardiaceae* family [1]. This genus is found in temperate and tropical regions worldwide, with representative members in different geographic locations. In general, sumac can grow in arid or semi-arid agricultural regions, and various species have been used by indigenous cultures for medicinal and other purposes [2,3]. *Rhus tripartitum* is found primarily in North Africa [4]. It is a wide-spreading tree with thorny branches that has many traditional uses. It is commonly for making charcoal. Rhus fruits are consumed fresh, and can be also be stored (sweetened) or soaked in sour milk. They are added to drinking water to give it an acceptable taste [5]. *Rhus tripartitum* buds can be chewed to quench thirst as they give the impression of water in the mouth. The bark can be ground to make a powder that is used to treat ulcers.

Rhus is rich in antioxidants including tannins [6], which are a complex and heterogeneous group of polyphenols with molecular weights of between 500 and 20,000 Da. They share the ability to bind to and precipitate proteins, alkaloids, polysaccharides, nucleic acids and minerals, etc. [7,8]. This particular reactivity with proteins is named astringency [9,7]. The bark of *Rhus tripartitum* roots are used for tanning hides and directly staining them red.

Tannins are defensive compounds that counteract bacteria and fungi by interfering with their surface proteins. They deter herbivores by virtue of their astringent effect on the mouth and their interference with digestion [10,11]. Experimental studies performed on tannin plants have also shown that tannins have detrimental effects on insects: they deter feeding, reduce growth and survival, correlate negatively with pupal mass, cause lethal deformities and increase parasitism [10,8].

The composition and concentration of tannins vary considerably with the plant species, age and organ [7]. In general, sumac is a rich source of hydrolysable tannins [6]. The sumac leaves (*Rhus typhina*) contain 12%db (dry basis) tannin based on Gallic acid, most commonly referred to as tannic acid. The hard carapace of Chinese galls (leaves, *Rhus semialata*) contains up to 70% of the same tannin [7].

The study of tannins is difficult because of their structural complexity [9,12]. Many analytical methods have been used to quantify tannins in plant materials. Commonly used methods include oxidative proanthocyanidinde-polymerization and oxidation–reduction reactions [13].

Other methods involve acid cleavage reactions, precipitation, enzyme and microbial inhibition, gravimetric procedures [14,6,15] and titrimetric methods [16]. Mahjoub et al. [17] studied Tunisian *Rhus tripartitum* (Ucria) and identified new biflavonoids and isobiflavonoids from the crude extract of leaves, stems and fruits. They also investigated their anti-inflammatory and antioxidant activities [2].

Extraction methods can be used to quantify plant polyphenol contents and antioxidant capacity [1,6,18,19]. Several methods have been developed to increase the yields and kinetics of polyphenol extraction [18,19,24]: microwave assisted extraction (MAE) [20], pulsed electric field (PEF)-assisted diffusion and high-voltage electrical discharge (HVED)-assisted diffusion [21], ultrasound-assisted extraction [22] and using a high-temperature, high-pressure (PARR) reactor [23,24].

There is abundant literature supporting the observation that the total phenols extracted can vary widely as a function of the material and the extraction conditions such as the polarity of the solvent, temperature or particle size [25]. The solvents most commonly used for extractingpolyphenols from plants are polar organic solvents such as ethanol and acetone. Furthermore, mixtures of polar organic solvents and water were found to be more efficient in extracting phenolic constituents than the corresponding mono-component solvent system [26,27]. The principal objective of the present work was to study the impact of a thermo-mechanical treatment, the Instant controlled Pressure Drop process (DIC), on the extraction of total phenols, including tannins, from the bark of the Tunisian *Rhus tripartitum*. The work included adopting a novel protocol for tannin determination using a MCP (Methyl Cellulose Precipitable) precipitation assay starting from crude extracts. Response Surface Methodology was employed to optimize the yields as well as the antioxidant activity, which was determined using a DPPH Assay. The effect of time and temperature on total phenol extraction kinetics was also investigated.

2. MATERIALS AND METHODS

2.1 Raw Material

Brown *Rhus tripartitum* bark was purchased from a local Tunisian market. The raw material was ground to small particles (1-4 mm) before DIC treatment. The green *Rhus tripartitum* barks were harvested from Mareth (Gabes; Tunisia) in March 2012. The green barks were manually separated, cleaned and hot-air dried in the dark at room temperature (28°C for 20 days). The plant was identified by a plant biology specialist from Gabes University, and also compared with the description given in “Arid and Saharan zones of Tunisia” [28]. Dried barks were ground using an electric grinder before DIC treatment. A particle size of 1 to 4 mm of ground bark was measured using a sieve machine (FRITSCH) with a 1.5 mm amplitude and 10 min sieving time. The samples were stored at 4ºC until needed for analyses.

2.2 Chemicals and Reagents

Analytical grade solvents and chemicals were used. Deionized water was produced using the Milipore system (Millipore Corporation, USA). Acetone was acquired from 27106 Val-de-Reuil, France; Ammonium sulfate from Oulchy-le-Chateau/France; and Methyl Cellulose (viscosity 3755m Pas) and DPPH(2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) from Sigma-Aldrich, USA.

2.3 Instant Controlled Pressure Drop (DIC)

The well-known Instant Controlled Pressure Drop (DIC) process is a thermo-mechanical treatment developed and patented some years ago [29,30]. It has shown promising results in numerous industrial unit operations for the drying, extraction and decontamination of both foods and non-edible materials [31,32,33]. In various cases, DIC improves both the process performance (kinetics, energy consumption, environmental impact, etc.) and the end product quality (functional, hygienic, organoleptic and sensorial features). Recently, DIC technology was introduced to extract polyphenols from Lebanese sumac [34].

DIC treatment consists in placing a moistened product in a processing chamber and exposing it to saturated steam pressure (up to 0.8 MPa) at high temperature (up to 170°C) for a short time (a few seconds to 1 min). The thermal treatment is ended by an instant pressure drop (5 kPa), which is achieved by opening a valve between the treatment vessel and a vacuum tank with a 50-times greater volume. There is a sharp fall in pressure within the chamber, leading to a partial auto vaporization of the water in the product Fig. 1.

Depending on the thermo-hydro-rheological behavior of the treated polymers, this results in a well-controlled expansion process that strictly depends on the operating conditions (e.g. temperature and pressure drop rate, processing time). The short duration of the thermal treatment and the immediate temperature drop prevent further thermal deterioration and provide a high quality end product. Fig. 2 shows the different stages of DIC treatment.

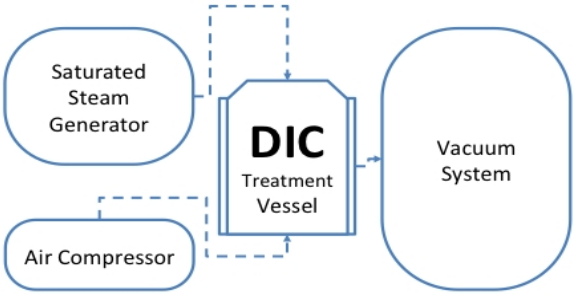


Fig. 1. Diagrammaticlay out of DIC reactor (ABCAR-DIC Process; La Rochelle; France)

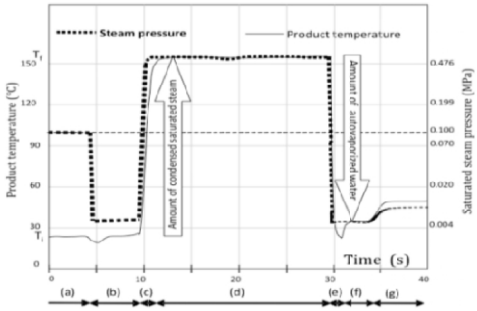


Fig. 2. DIC process: Pressure/temperature vs time

2.4 Experimental Design

After preliminary experimental trials, DIC operating parameters and their ranges were selected for saturated steam pressure (P) of between 0.2 and 0.4 MPa and thermal treatment duration (t) of between 20 s and 40 s. Conventional extraction of anthocyanins is typically conducted at temperatures ranging from 20 to 50ºC [35]. The initial water content of the raw material was determined to be 9.34 g H_2O/100 g dry basis.A 2-parameter 5-level central composite Design of Experiment (DoE) was defined with 2^2=4 factorial points, 2*2=4 star points and 3 repetitions of the central point. The experimental design for the extraction of *Rhus tripartitum* bark is presented in Table 1.

Each response parameter was studied statistically using Statgraphics for Windows (5.1 version) to calculate the impact of the operating parameters. The analysis for each sample was triplicated [36,37]. The non DIC treated raw material was taken as a control point.

Table 1. Level of independent variables used in developing experimental data

Coded level	-α	-1	0	+1	+α
Saturated Steam pressure (MPa)	0.2	0.23	0.3	0.37	0.4
Processing time (s)	20	23	30	37	40

2.5 Process Analysis

2.5.1 Water content

The water content was determined by drying three samples (3g of raw material) in a drying oven at 105ºC until a constant weight was obtained (24h).

2.5.2 Extraction method

The raw material and DIC treated samples of *Rhus tripartitum* bark were dried and ground to a fine powder. Approximately 1 g of sample was weighed using an analytical balance (Kern 770, readability 0.0001 g). The powder (1 g) was extracted by maceration into 20 ml of aqueous acetone (acetone/water, 70:30, v/v) with reflux and continuous shaking (400 rpm) at 40ºC for 30 min. After that, the extract was filtered through membrane filters (45 micron). The crude extracts were analyzed to determine Total Phenol Content (TPC), tannin content and Antioxidant Activity (AOA). The same TPC extraction procedure was used to determine the extraction kinetics, carried out under different time intervals.

2.6 Determination of Total Phenol Content (TPC)

The Gallic Acid Equivalence method (GAE), also referred to as the Folin–Ciocalteau method, was used to determine the amount of total phenols in the crude extracts. A mixture of phosphotungstate and phosphomolybdate was prepared and used to oxidize the phenols in alkaline solution to convert them first to phenolate ions and, just after, to phosphotungstic blue [38]. The absorbance of this phosphotungstic blue is proportional to the number of aromatic phenolic groups, which is expressed in Gallic Acid Equivalents [39]. 2.5 ml of 10% (v/v) of Folin-Ciocalteu reagent was mixed in a test tube with 0.5 ml of a diluted sample and left for 30 s to 5 min. 2 ml of 20% of sodium carbonate solution was then added and the test tube was vigorously shaken for 10 s before being incubated at 45ºC for 5 minutes in a water bath. The absorbance of the sample was measured at 765 nm using a Helios Omega UV/VIS Spectrophotometer (Thermo Scientific Merk and Co.) with reference to a blank sample, i.e., distilled water. Total phenolic compounds were quantified on the basis of a standard calibration curve of Gallic acid. Eq. 1 for the standard calibration curve of extinction against Gallic acid concentration is shown below (R^2=0.9955):

$$y = 0.0092x + 0.0144 \quad (1)$$

The results were expressed as milligrams of Gallic Acid Equivalents (GAE) per g of dry base (mg GAE/g db). The analyses were performed in triplicate and the standard deviation was calculated. Total phenol content of the crude extracts of raw and DIC treated samples are shown in Table 2.

Table 2. Two DIC variable central composite design used to optimize the effect of DIC on Total Phenol Content (TPC), tannin content and % Inhibition yields (Anti-Oxidant Activity AOA) from *Rhus tripartitum* bark. Response values are also included

Trial no.	Pressure (MPa)	Time (s)	TPC (mgGAE/g db)	Tannins (mgGAE/g db)	AOA % Inhibition
1	0.4	30	271.5±3.1	48.3±1.3	96%
2	0.3	40	275.6±3.6	53.3±2.1	97.5%
3*3	0.3	30	262.5±1.2	47.5±1.6	93±1%
4	0.37	37	271.5±4.1	48.7±3.2	95.5%
5	0.37	23	271.9±2.2	48.3±2.4	95.5%
7	0.23	23	238.3±2.2	43.7±1.7	91%
8	0.23	37	271.5±1.5	48.3±1.3	95.5%
9	0.2	30	250.8±3.6	44.1±2.2	91%
10	0.3	20	248.3±3.7	44.1±2.1	91%
Control	-	-	207.5±3.1	33.4±1.3	81%

2.7 Determination of Tannins using a Precipitation Assay

2.7.1 Preparation of the reagents

2.7.1.1 Saturated ammonium sulfate solution

Super saturated ammonium sulfate solution was prepared by adding an excess amount of ammonium sulfate crystals to deionized water with stirring till the crystals no longer dissolved (approximately 1.5 cm of ammonium sulfate crystals remain on the bottom of the flask).

2.7.1.2 Methyl cellulose solution of 0.04%

0.04 g of methylcellulose was dissolved in a small amount of deionized water then homogenized with ultrasound (Fisherb and FB 11010) and made up to 100 ml with deionized water. The solution is stable at room temperature for 2 weeks.

2.8 The Optimized Protocol

A novel protocol for the precipitation of tannins from *Rhus tripartitum* was studied, optimized and adopted in this study. Multiple experiments carried out allowed us to identify the various volumes of regents needed to precipitate all the tannins in our material. The precipitation procedure was carried out following AWRI standard methods [40].

The adopted protocol optimized from the experimental results for tannin content shown in Table 3 was used to precipitate total tannins from raw material and DIC treated *Rhus tripartitum* samples.

Table 3. Optimized volumes of samples and reagents for the MCP tannin assay for *Rhus tripartitum* extracts

Sample	Assay format	Sample volume	Polymer	Salt	Water
Rhus extract	10 ml	0.1 ml	3 ml	2.5 ml	4.4 ml
Control	10 ml	0.1 ml	0 ml	2.5 ml	7.4 ml

A 3ml methyl cellulose solution was added to 0.1 ml of the crude extract sample. The mixture was shaken lightly several times and left to stand for approximately 2-3 min. 2.5 ml of saturated ammonium sulfate solution was then added and the volume was made up to 10 ml with deionized water. The solution was left for 10 min at room temperature, and then filtered through a 45 µm micro filter. Supernatant absorbance was recorded at 765 nm. Control samples with a total volume of 10 ml were prepared with a similar procedure using deionized water instead of the methylcellulose solution. Tannin content was expressed as mg GAE/g db based on the recorded tannin absorbance from Eq.2:

$$\mathrm{A765\ (tannin)} = \mathrm{A765_{control}} - \mathrm{A765_{supernatant}} \quad (2)$$

2.9 Antioxidant Activity (AOA)

In terms of antioxidant activity, the extracts of *Rhus tripartitum* bark were analyzed by DPPH (2.2-diphenyl-1-picryl-hydrazyl), which was used to evaluate the Antioxidant Capacity (AOC) of the scavenging free radicals of DPPH that were formed.

2.10 DPPH Method

A solution of 0.04 g of the DPPH radical dissolved in 100 ml methanol was prepared. Then, 3 ml of this solution was mixed with 1 ml of the crude bark extract (dissolved in methanol) and the mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 517 nm. The blank was amixture of 1 ml of extract with 3 ml of methanol. The amount of DPPH remaining in the reaction medium was calculated using a linear regression equation from a calibration curve. It should be taken into account that a very low value for the absorbance of the reactant mixture indicates a high sequestrant capacity of the free radicals (% Inhibition), expressed numerically by Eq. 3:

$$\mathrm{Inhibition\ \%} = \frac{\mathrm{Abs_o} - \mathrm{Abs_{30min}}}{\mathrm{Abs_o}} \quad (3)$$

$Abs_o$is the absorbance of DPPH at t=0, and$Abs_{30min}$is its absorbance after a 30 min incubation. The percentage of the remaining DPPH was plotted against the sample or standard concentrations to obtain the amount of antioxidant necessary to reduce the initial concentration of DPPH.

3. RESULTS AND DISCUSSION

3.1 Total Phenol Content: RSM Analysis of the Extraction Yield

The extraction of total phenols and tannins from *Rhus tripartitum* bark was optimized through a RSM approach. A fixed liquid-to-solid ratio (20:1, ml:g) and a fixed particle size (0.60–0.84 mm) were chosen. The results for Total Phenol Content (TPC) and tannin quantity for all runs are reported as multiple linear regressions (obtained using the second-order polynomial model) that were performed using the results of Table 2. The significance of DIC parameters on TPC extraction yield was estimated from the Response Surface Analysis. The vertical line on the Pareto chart determines the effects that are statistically significant. The standardized effect is the estimated effect divided by its standard error. Hence a low standardized effect can mean either a slight effect or a large experimental error. The response surface analysis

was statistically significant and suggested that the two independent parameters explain the experimental variation for total phenol and tannin content in relation to the average response. Fig. 3 shows the effects of the DIC operating parameters steam pressure P and heating time t in terms of TPC yields. It is clear that the two independent variables had a significant effect since TPC increased when P and t were increased. This finding can easily be explained given that both DIC processing pressure and heating time positively affect plant morphology, resulting in a higher availability of TPC. The negative interaction (cross-effect) between P and t may be imputed to the maximum yields of these molecules. The polynomial model Eq. 4 estimated from the RSA reflects the regression empirical model of TPC versus P and t, with a regression factor =98.90:

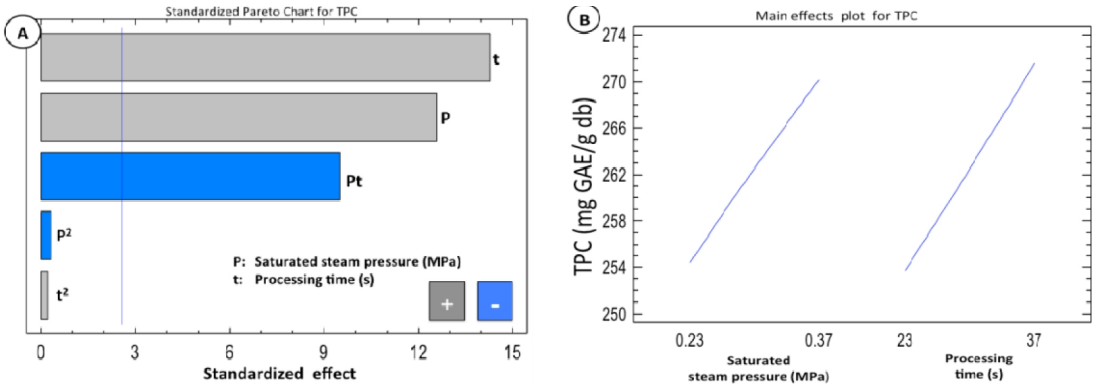
$$TPC = 34.90 + 655.64P + 6.22t - 48.4695P^2 - 17.1429Pt + 0.0033t^2 \quad (4)$$

The high value of R^2indicates that the model is capable of giving a good description of the results.

It was found experimentally that the DIC treated materials produced higher polyphenol contents: 238-276 mg GAE/g db compared with 207 mg GAE/g db for the raw material. The highest value of TPC (281 mg GAE/g db) was achieved with a DIC treatment at P=0.2 MPa, t=40 s. These results indicate that DIC used as a pre-treatment of *Rhus tripartitum* bark resulted in an increase of about 33% in the yield of extracted TPC.

3.2 Total Phenol Content: Extraction Kinetics

Papers on the conventional solvent extraction of phenolsindicate that water is one of the relevant solvents in a temperature range from 40 to 90ºC [41,42]. However, mixtures of polar organic solvents and water have been found to be more efficient in extracting phenolic constituents than the corresponding mono-component solvent system [43,27]. In the present work, the polyphenol extraction process was conducted with aqueous acetone (acetone/water, 70:30, v/v) by reflux, varying in temperature (20, 30, 40 and 50ºC). An increase in extraction temperature from 20 to 50ºC led to a slight increase in total phenol content. The kinetics results were obtained for times of between 2 and 60 min for both the raw material and DIC treated samples of *Rhus tripartitum* bark. Fig. 4 shows conventional kinetic behavior similar to that obtained with other plant materials under different extraction conditions [44,27].



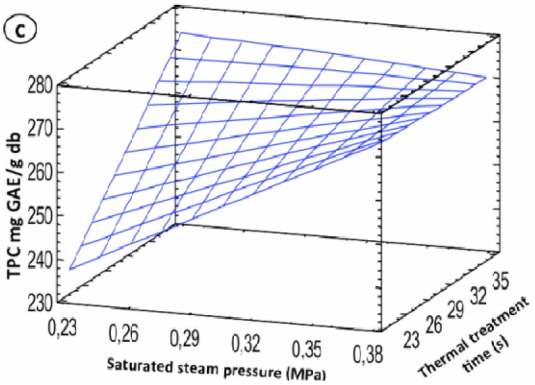


Fig. 3. Pareto chart of DIC operating parameters (A), general trends (B) and the response surface (C) for DIC treatment prior to TPC extraction from *Rhus tripartitum* bark.

The results of solvent extraction kinetics with solute extracted (TPC) from a solid matrix (*Rhus tripartitum* bark) can be interpreted following the method given by Ben Amor and Allaf [45]. Allaf et al. [46] stipulated that, after having selected the appropriate solvent, the extraction operation begins by washing the surface with solvent and immediately dissolving the solute. The density of the extraction rate of solute from the surface per unit of dry matter is done by Eq. 5:

$$\frac{dX_s}{dt} = k_e \, SESA \, (\varpi_e - \varpi_{solvent}) \quad (5)$$

Where:

k_e: coefficient of dissolution of solute by the solvent (kg of solvent m^{-2} s^{-1})
SESA: specific exchange surface area between the solvent and the product per unit of dry basis (m^2kg^{-1} dry basis db)
X_s: density of solute per unit of dry matter extracted by interaction between the solvent and the surface of the product (kg of solute per kg of dry matter or % db).
ϖ_e: dissolving coefficient at equilibrium (kg of solute kg^{-1} of solvent)
$\varpi_{solvent}$: solute dissolved in solvent (kg of solute kg^{-1} of solvent).

Increasing the agitation of the solvent increases the dissolution coefficient of the solute by the solvent (k_e), as well as the kinetics of the first “washing” process.

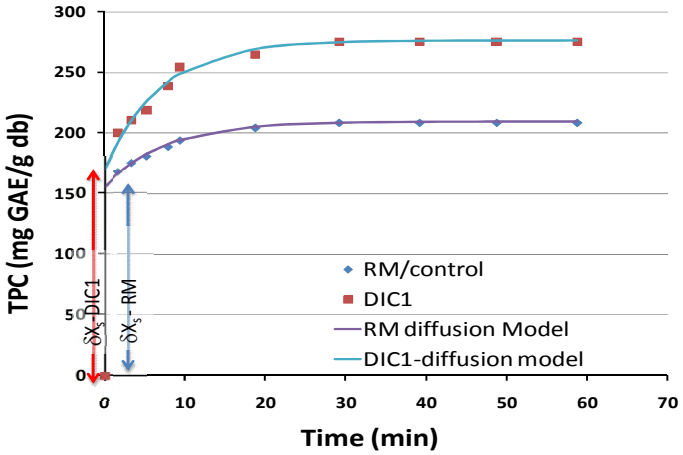


Fig. 4. Typical TPC extraction kinetics of raw material and a DIC treated sample

"External" intensification processes are generally easy to carry out. Once the external transfer phenomenon is perfectly intensified, it is no longer the limiting process. Solute extraction kinetics from the surface can then reach their highest level, and the total effect can be shown through the "starting accessibility" δX_s parameter done in Eq. 6:

$$\delta X_s = k_e SESA_{eff}(\varpi_e - \varpi_{solvent})\delta t \quad (6)$$

Where$\delta X_s$is expressed in g of extract per g of dry material. Subsequently,the process involvesa solvent within the structure and the solute-in-solventdiffusion phenomena. Both the granulometry and the agitation rateonly modify the starting accessibility δX_s [46].

Subsequently, most of the operation is controlled through various processes involving penetration of the solvent within the material (capillarity, molecular diffusivity, etc.) and then the solute inside the matrix according to a Fick's type Law [47], where the gradient of the apparent density ratio of solute to solvent is the driving force. In such conditions, the solute transfer driving force is the gradient of apparent densities ratio of solute to solid, with $D_{eff}(m^2s^{-1})$ done in Eq. 7 as the effective diffusivity [46,47].

$$\frac{\rho_{solute}}{\rho_d}(\vec{v}_{solute} - \vec{v}_d) = -D_{eff}\vec{\nabla}\left(\frac{\rho_{solute}}{\rho_d}\right) \quad (7)$$

Due to the absence of expansion, Eq. -8 reveals the special case of $\vec{v}_d = 0$ and $\rho_d = constant$:

$$\rho_{solute}\vec{v}_{solute} = -D_{eff}\vec{\nabla}\rho_{solute} \quad (8)$$

Although the effective diffusivity D_{eff} varies considerably with the temperature in the system, it is assumed to be constant based on the thermal homogeneity hypothesis, in which case Eq. 9 becomes [48]:

$$\frac{\partial \rho_{solute}}{\partial t} = -\vec{\nabla}.\left(D_{eff}\vec{\nabla}\rho_{solute}\right) \quad (9)$$

Using the mass balance, we obtain Eq. -10:

$$\frac{\partial \rho_{solute}}{\partial t} = -D_{eff}\nabla^2\rho_{solute} \quad (10)$$

And, the one-direction radial flow is revealed through Eq. -11:

$$\frac{\partial \rho_{solute}}{\partial t} = -D_{eff}\frac{\partial^2 \rho_{solute}}{\partial r^2} \quad (11)$$

with:

ρ_{solute}: apparent density of the solute within the solid matrix (kgm^{-3}),
ρ_d: apparent density of the solid dry material(kgm^{-3}),
v_{solute}: velocity of the solute(ms^{-1}),
v_d: v elocity of the solid dry material(ms^{-1}),

The solutions required for this diffusion equation, such as the standard Crank's solutions, are highly dependent on the initial and boundary conditions and the geometry of the products [49]. As an example, Crank's solution for a sphere with a radius d_p is done by Eq. 12:

$$\frac{X_\infty - X}{X_\infty - X_{t_o}} = \sum_{i=1}^{\infty}\frac{6}{i^2\pi^2}\exp\left(-\frac{i^2\pi^2 D_{eff}}{{d_p}^2}(t - t_o)\right) \quad (12)$$

Where:

d_p: average radius
X: amount of solute extracted at time t $(g.g^{-1}drymaterial)$
X_{to}: amount of solute extracted at the starting diffusion time $t_o(g.g^{-1}drymaterial)$
X_∞: amount of solute within the matrix $(g.g^{-1}dry\ material)$
δX_s: starting accessibility $(g.g^{-1}dry\ material)$.

Limited to its 1st term, this solution becomes (Eq. 13 and Eq. 14):

$$\frac{X_\infty - X}{X_\infty - X_{t_o}} = A\exp\left(-k(t - t_o)\right) \quad (13)$$

$$Ln\left(\frac{X_\infty - X}{X_\infty - X_{t_o}}\right) = -k(t - t_o) \quad (14)$$

The effective diffusivity can be estimated as (Eq. -15):

$$D_{eff} = k\frac{{d_p}^2}{\pi^2} \quad (15)$$

where:

d_p: the characteristic length (mm), which was, in our case, the average radius of *Rhus tripartitum* bark powder

Experimental data are utilized to identify δX_s and D_{eff}. Never the less, the choice of t_o is dictated by the necessity of not including the "washing" part in the diffusion study. The experimental data to be used to identify D_{eff} must exclude those close to the initial time and so standard Crank's solutions should only concern the time $t>t_o$[50].The starting accessibility δX_s(Eq. 16) is then calculated by extrapolating the diffusion model to t=0; $X_o \neq (X_i = 0)$:

$$\delta X_s = X_o - X_i = X_o \quad (16)$$

The results of TPC extraction kinetics based on the exchange surface and diffusion model gave starting accessibility and effective diffusivity values of 0.232 g GAE/g db and $1.26\times10^{-10}m^2s^{-1}$, respectively, for DIC treated material at a steam pressure of 0.37 MPa and a processing time of 37 s, compared with 0.184 gGAE/g db and $1.18\times10^{-10}m^2s^{-1}$ for the raw material. These results indicated that pretreatment of raw material by DIC accelerated the kinetics of TPC extraction compared to the raw material. This is due to the fact that DIC treatment may cause the rupture of cell walls and structural modifications in the plant; rupturing cell walls increases the availability and initial accessibility of polyphenol compounds. Expansion of the solid matrix leads to an increase in porosity, which improves the diffusivity of the solvent within the plant [36].

3.3 RSA Analysis of Tannins

It is worth noting from the Response Surface Analysis of tannin measurements Fig.5 that the impact of the two independent variables P and t was significant; a stronger impact was seen with t. The positive result with both P and t shows that TPC increased when P and t were increased, confirming that DIC treatment does not involve thermal degradation; only an improvement of functional features occurred.

The polynomial equation Eq. 17 estimated from RSA reflected the empirical model obtained with a relatively satisfying R^2 value (R^2 = 88.97%):

$$Tannincontent = 9.95 + 169.54P + 0.29t - 142.86P^2 - 2.14Pt + 0.01122t^2 \quad (17)$$

The optimum predictive values for tannins extracted from the DIC treated bark of *Rhus tripartitum* was 52±1.6 mg GAE/g db compared with 33±1.6 mg GAE/g db for the untreated raw material, as shown in Table 2. It is noteworthy that the addition of water to acetone tremendously increased the yields of tannins from plants compared to the use of pure acetone or water since both the polar and less polar compounds were co-extracted [51].

One of the original aspects of the present study is the creation of a novel protocol to assay tannins. This new protocol may be used to determine the tannin content of other species of Rhus.

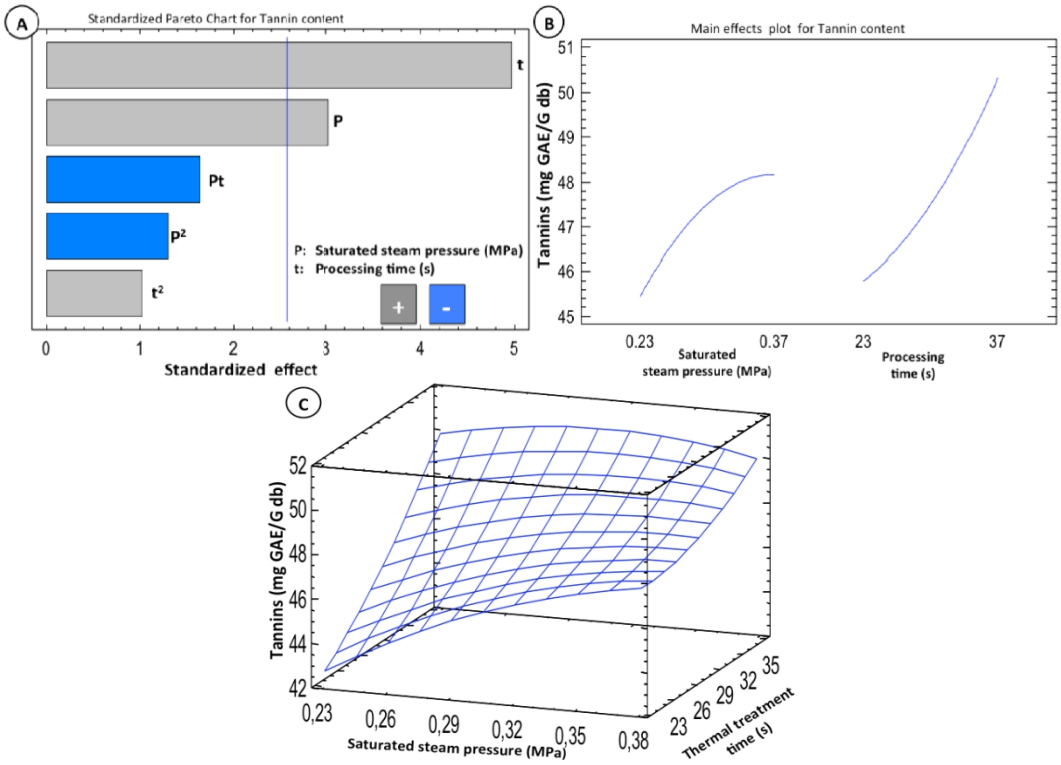


Fig. 5. Pareto chart of DIC operating parameters (A), general trends (B) and the response surface (C) for tannin content extracted from *Rhus tripartitum* bark after DIC treatment.

3.4 Free Radical-scavenging DPPH Assay

The results for the free radical scavenging activity of plant extracts are presented in Table 2. The results showed that the DIC sample extracts contained a % DPPH radical scavenging activity that was between 91% - 97.5% higher than the extracts of the untreated raw material (81%).

The Pareto chart Fig. 6 shows clearly that the two DIC operating parameters positively affected the antioxidant activity of *Rhus tripartitum* bark. However processing time seemed to be the most significant. This finding could be explained by the low thermal degradation of this activity during DIC treatment. The mechanical impact on the instantaneous drop to a vacuum results in some disruption of the cell walls, thus facilitating the extraction of phenols and other active compounds. The regression empirical model of anti-oxidant activity was established with R^2 = 88.87%:

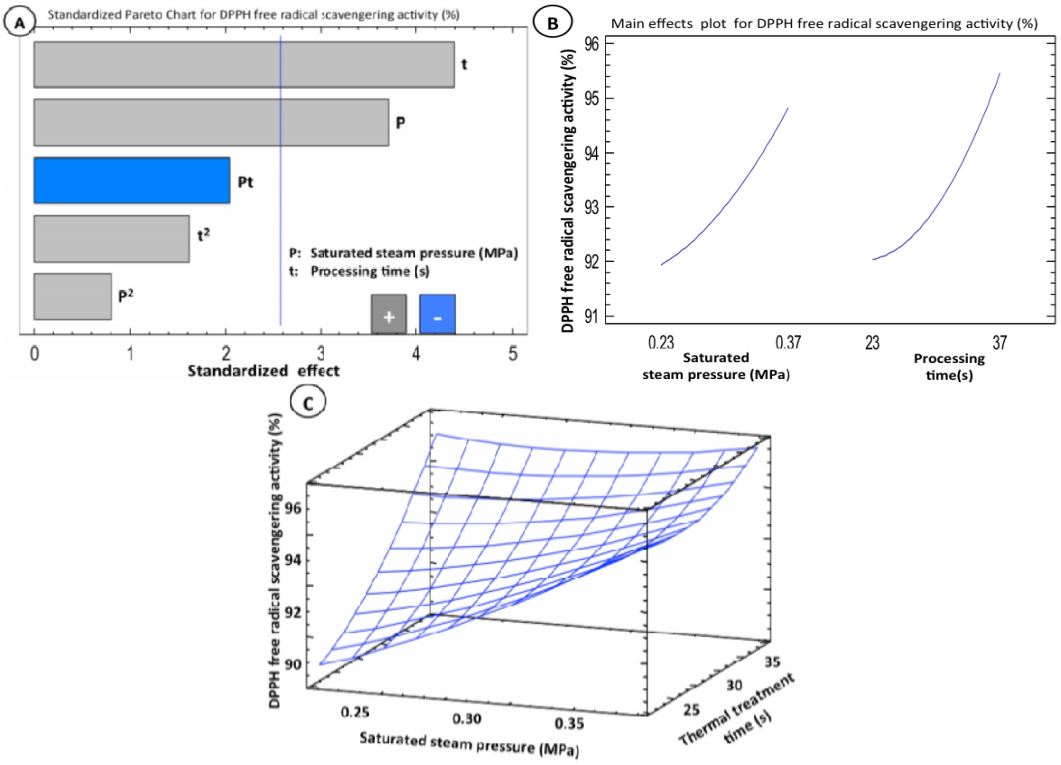


Fig. 6. Pareto chart of DIC operating parameters (A), general trends (B) and the response surface (C) for the Antioxidant Activity (AOA) of *Rhus tripartitum* bark after DIC treatment.

The optimum predictive value for DPPH free radical scavengering activity (%) from the DIC treated bark of *Rhus tripartitum* was 97.9% at (P= 0.2 MPa, t= 40 s) compared with 81% for the untreated raw material. Several studies reported that phenolic compounds in plants significantly contribute to their antioxidant properties [52,53].

Fig. 7 shows the correlation between total phenol content and a DPPH assay of extracts of DIC treated materials. The results show a highly significant positive correlation coefficient between the total phenolic content and the DPPH assay of the extracts (R^2=0.97), thus suggesting that phenols are major contributors to the antioxidant activity. However, the possible contribution of other compounds to AOA should be studied in future work using HPLC analysis.

The overall results clearly indicate that DIC-*Rhus tripartitum* bark has a strong antioxidant activity (Eq. 18):

$$AOA(\%) = 79.466 + 43.62P + 0.0149t + 76.53P^2 - 2.296Pt + 0.015t^2 \quad (18)$$

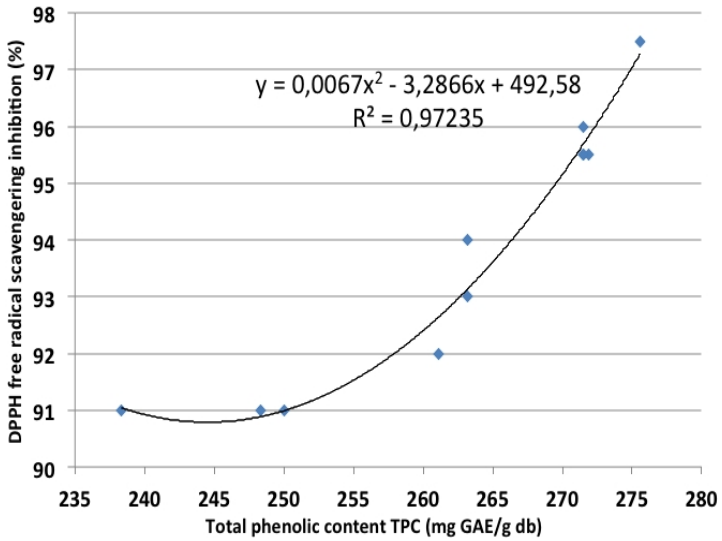


Fig. 7. Correlation between Total Phenolic Content and antioxidant activity

4. CONCLUSIONS

This work provides a method for recovering a natural waste source (the bark of *Rhus tripartitum*) using a high temperature-short time thermo-mechanical process: Instant Controlled Pressure Drop (DIC). DIC can be used to control the modifications of the texture of the material. This pretreatment enhances the extraction yield of total polyphenols, including tannins and also improves the extraction kinetics. The high antioxidant activity of the DIC samples suggests promising applications of *Rhus tripartitum* waste as an economical source of healthy ingredients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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