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ORIGINAL ARTICLE

Green extraction process of tannins obtained from Moroccan *Acacia mollissima* barks by microwave: Modeling and optimization of the process using the response surface methodology RSM

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Abstract The effect of extraction conditions on polyphenols contents and condensed tannins by microwave-assisted extraction (MAE) was studied for the first time to our knowledge. Moroccan barks of *Acacia mollissima* was used to extract phenolic compounds. The variables studied are the following: power extraction, time extraction and solvent nature. Five powers extraction were tested: 150 W, 250 W, 300 W, 450 W and 600 W. A significant effect of power extraction on the extractable nature was proved by ANOVA and Student test. The yields were also affected by time extraction. Different solvent (water, ethanol, methanol and ethyl acetate) were tested to evaluate the best extraction solvent according to the extractable nature. Highest polyphenols contents were obtained with methanol. The proportion of this solvent, time extraction and power extraction were optimized using the response surface methodology (RSM). A face-centered composite design (FCCD) was applied to evaluate the effects of these variables on the polyphenols and condensed tannins contents. For each experiment, the extraction yield, the total polyphenolic contents and

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the condensed tannins contents were quantified using colorimetric essays. The extracts were characterized by their reactivity to formaldehyde and reverse phase high pressure liquid chromatography (RP-HPLC). The highest polyphenols content was obtained at 156 W using 80% of methanol during 5 min. For condensed tannins, the highest content of cyanidin was obtained at 182 W using 20% of methanol during 3.66 min. RSM applied in MAE, permitted to develop green extraction process of polyphenols and tannins extracted, using lower microwave power and methanol proportion with a shortest time extraction and in the same time improve the quantity of extractables obtained from renewable natural resource.

1. Introduction

The barks of *Acacia mollissima* contain 46% of the extractables (Gandini and Belgacem, 2002). This category includes a whole range of compounds with majority being secondary metabolites, such as polyphenols, mostly condensed tannins and hydrolysable tannins (Stevanovic and Perrin, 2009). The high level of extractables in the barks of *Acacia* rich in natural tannins constitutes a potential substitute to synthetic phenols. Recently, the tannins of the *A. mollissima* were used in lot of applications: wood protection (Tascioglu et al., 2013; Hayoz et al., 2003), inhibition of corrosion (Gerengi and Halil, 2012; Silveira et al., 2012), elaboration of carbon foam (Basso et al., 2013; Tondi et al., 2010; Zhao et al., 2010), elaboration of natural adhesive with low emission of formaldehyde (Bertaud et al., 2012; Sumin, 2009; Stefani et al., 2008), elaboration of thermoplastic (Nicollin et al., 2013), fertilization of sea urchins and development of the growth algal of sea (De Nicola et al., 2007). After tannin extraction, the residual bark of *A. mollissima* may also be used in the composting to produce the organic fertilizer, or as biomass fuel (Foelkel, 2008).

The *A. mollissima* was introduced in 1980 in Morocco to satisfy the demand of tanning products. The tannins of the *Acacia* are widely used in the tanning industry (Khatouri and Berbich, 1994). Tannins do not pollute the environment and are not toxic for the user (Lemmens and Wulijarni-Soetjipto, 1991). This species regenerates naturally and is adapted very well to a wide range of ecological conditions including the degraded sites and also is adapted very well to the climate of Northwestern Morocco (Khatouri and Berbich, 1994). The importance of Moroccan *Acacia* resides in the high quality of tannins extracted from the bark (Hillis, 1997) and also in the high content of condensed tannins, which can reach 70%. This content can vary depending on the thickness of the bark, the age of the tree and the average annual precipitation (Stevanovic and Perrin, 2009).

In recent years, the use of microwave-assisted extraction (MAE) to extract polyphenols and tannins from vegetables has shown tremendous research interest and potential. Compared with the traditional methods, MAE has many advantages, such as shorter time extraction, less solvent used, higher extraction yield and better products with lower cost because microwave treatment has mechanical effects that heats the solvent mixture directly, and interacts directly with the free water molecules present in the glands and vascular systems, which results in rupture of the plant tissue and release of the active constituents into the solvent (Al-Harahsheh and Kingman, 2004). Currently, the extraction by microwave is widely used for the extraction of polyphenols from several vegetal materials, including black tea (Spigno and De Faveri, 2009), tomatoes (Hongyan et al., 2012), and grape peel (Yuan et al., 2012).

Response surface methodology (RSM) was an effective and powerful statistical method to optimize the extraction process while giving a maximum of information, reducing the number of experimental trials required and giving the best precision of the results calculated with the established model (Goupy, 2000). The first step for the development of the experimental design was not only to determine the factors which had a dominating influence on the required properties but also to choose the favorable levels of certain factors. The second step was modeling the response. It consists of choosing the suitable plan according to the searched criteria, and validating the adopted model. The last step was the optimization of the RSM, which allowed to optimize the multi-criteria of the process and determine the optimal conditions for the operating of the process (Goupy, 1997). To elaborate an experimental design, it was necessary to determine all the controlled parameters (or the factors), to which a set of distinct states (or levels) can be imposed. The experimental factors can be quantitative or qualitative and can adopt various levels according to the searched interest (Goupy, 1997). Each line of an experimental design represents the experimental conditions of an experiment, and it is represented by a coded variable, of which the values correspond to the levels of the factor which it is associated (Mathieu et al., 2000). The experiments were carried out in a random order. The estimate of the coefficients of the effects was calculated using the method of least squares (Delacroix and Porte, 1987). For the decision of the choice of the significant factors, we based on the degree of freedom of the factors to evaluate the signification of the coefficients, or on the visual methods, such as the Lenth (1989) or the Box–Meyer method (Box and Meyer, 1986).

To optimize the response obtained of the validated model, it was necessary to complete the model with experiments of the star points. For this objective, we used a faced central composite design, to determine the optimum conditions to extract polyphenols and tannins. This experimental design has the advantage that it lends itself to the sequential course of a study and requires a relatively low number of experiments (Goupy, 2000). For k factors, the experiment matrix of a faced central composite design contains the following experiments:

- $N_F = 2^k$: corresponds to the experiments of the complete factorial with two levels design (k is a number of factors).
- $N_a = 2k$: corresponds to the experiments located on the axes representing the factors.

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- N_0 = corresponds to the experiments located in the center of the field. These points permitted to evaluate the variation due to the experimental error and analyze the lack of adjustment of the model (Sarabia and Ortiz, 2009).

In this study, we used this methodology to optimize extraction process of polyphenols and tannins extracted by microwave from Moroccan barks of *A. mollissima*. The variables studied are the following: *time extraction* (X_1), *methanol proportion* (X_2) and *microwave power* (X_3). The responses measured were the following: the yields of polyphenols (Y_1) and the yields of condensed tannins: cyanidin content (Y_2). The results of this study were evaluated with colorimetric assays. The first aspect of this research reside at exploring experimental design (RSM) to optimize the extraction conditions using a lower number of experimental trials. For example, to optimize the conditions for seven factors having each one four levels, 16,384 experiments must be carried out (4^7 = 16,384). This experimental number represents a huge work most often it is unrealizable. The use of experimental design in this case reduces this number to 8 experiments. The main difference with the intuitive method was that RSM permits to vary all levels of both experiments in the same time. The second aspect was to model extraction conditions of phenolic extracts obtained by microwave. The modeling extraction process create a mathematical model between extraction conditions (factors studied) and the response measured (propriety searched). It permits to define range of the variables studied and their levels. The main objective to model the response was to determine the value of the yield extracts or the propriety searched, without carrying out the experiments. After validation of the established model, the equation established permits to predict the response measured. The last aspect of this research was to define green extraction process to extract polyphenols and condensed tannins using low methanol proportion, microwave power and shorter time extraction.

2. Materials and methods

2.1. Material

In this study we used the bark of *A. mollissima,* collected in September 2012, dried in the shade at room temperature. The barks of fives trees of ten years were taken from the Moroccan Mamoura forest, from the Kenitra area, and they contain 17% moisture.

2.2. Preparation of sample

The bark of *A. mollissima* was dried to a stable moisture content, and finely grounded (approximately 0.5 mm in diameter) using a grinder (Retsch SK1 rotary knife) to prepare the extracts of polyphenols and tannins.

2.3. Screening factors

Microwave extraction was carried out using closed system, type (Mars V, CEM). Closed vessel reactions were carried out in multimode system, using old bark with ten years of age and automatically moderate stirring. The variables studied are the following: time extraction, power extraction and solvent nature.

- To evaluate effect of time extraction, different extractions using water at 250 W were carried out during 1–3 min.
- To determine the adequate power extraction, different extractions using water during 1 min were carried out at: 250 W, 300 W, 450 W and 600 W.
- To choose the best extraction solvent, different extractions during 5 min at 150 W were carried out with: water, ethanol (80%), methanol (80%) and ethyl acetate. We used a low power extraction (150 W), for best managing of solvents reaction in a closed system.

The effect of these factors on yield extract was determined using colorimetric assays.

2.4. Extractions

The extraction method described by Scalbert et al. (1989) was used according the conditions of the study carried out: evaluation of the effect of time, solvent or power extraction. The ratio used in this study is 1/20 (m/v) (1 g sample with 20 mL of solvent). The solvent used to extract polyphenols and tannins is a mixture of water and methanol with a ratio of 20/80. The supernatant was collected after each extraction by filtering through Whatman paper n°1. The methanol was evaporated under reduced pressure at 40 °C. To separate the phenolic phase and non-phenolic phase, an acid hydrolysis using two drops of HCl (6N) is performed on the water volume remained after evaporation of methanol and an extraction with diethyl ether (3×5 mL) is performed. Non-phenolic phase (organic phase) containing some oligomers of phenolic compounds and sugars, was dried with anhydrous sodium sulfate and after evaporation of the diethyl ether, the residue was dissolved in 5 mL of methanol. Phenolic phase (aqueous phase) containing the condensed and hydrolysable tannins, was regrouped and adjusted to 10 mL with distilled water. The procedure for extraction of polyphenol and tannins is illustrated in Fig. 1.

2.5. Determination of content

2.5.1. Determination of total polyphenolic content

Total polyphenolic content was determined with the Folin–Ciocalteu method (Singleton and Rossi, 1965): 2.5 mL of Folin reagent (diluted 10 times) was added to 0.5 mL of aqueous extract (diluted 200 times). 2 mL of sodium carbonate (75 g L^{-1}) was then added. The mixture was then put in a water bath at 50 °C for 5 min before the absorbance was read at 760 nm. A calibration curve was done with a solution of gallic acid (80 mg L^{-1}, Jenway 6300 Spectrophotometer). The results were obtained as milligram of gallic acid equivalent (GAE) per gram of dry bark (mg GAE/g bark) and as milligram of GAE per gram of dry extract (mg GAE/g ext).

2.5.2. Determination of condensed tannins: Cyanidin equivalent content

Proanthocyanidin content was determined with a BuOH/HCl test as described by Scalbert et al. (1989): 0.5 mL of aqueous extract (diluted 100 times) was added to 5 mL of an acidic

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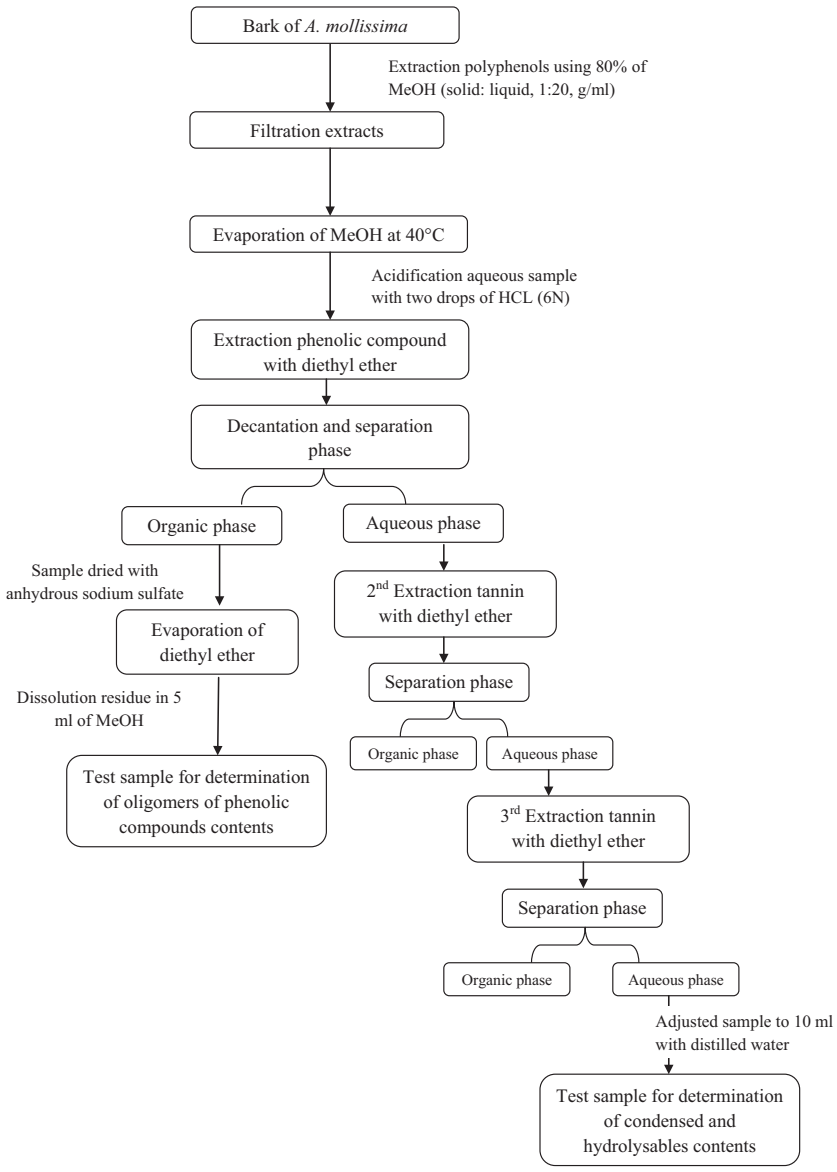

Figure 1 Scheme for extractions polyphenols and tannins from Moroccan bark of *Acacia mollissima*.

ferrous solution (77 mg of $FeSO_4{\cdot}7H_2O$ in 500 mL of Hcl/BuOH (2/3)). The tubes were covered and placed in a water bath at 95 °C for 15 min. The absorbance was read at 530 nm (Jenway 6300 Spectrophotometer) and results were expressed as follows: milligram of cyanidin equivalent (Cya) per gram of dry bark (mg Cya/g bark). The condensed tannins content was calculated using the formula given below (Eq. (1)):

$$\text{mg CyaE/g bark} = \frac{A * V * D * M * V2}{l * \varepsilon * v * m}, \quad (1)$$

where A is the absorbance of the sample at 530 nm; V is the total volume of the reaction (mL); D is the dilution factor; M is the cyanidin molar mass (g mol^{-1}); V_2 is the volume of the aqueous extract recovered after extraction with diethyl ether (mL); l is the path length (cm^{-1}); ε is the molar extinction coefficient (34.700 L mol^{-1} cm^{-1}); v is 0.5 mL; and m is the mass of dry weight of bark (g).

2.5.3. Determination of hydrolysable tannins contents

Hydrolysable tannins content was determined with potassium iodate test described by Bossu et al. (2006) method. Five milliliter of aqueous solution KIO_3 (2.5% v/v) was heated for 7 min at 30 °C, then 1 mL of the aqueous solution (diluted 5 times), was added. The mixture was then placed in a water bath at 30 °C for 2 min before the absorbance was read at 550 nm (Jenway 6300 Spectrophotometer). A calibration curve was obtained using a tannic acid solution (5000 mg/L) prepared by solubilization of 0.25 g of tannic acid in 50 mL of methanol (80%). The analytical standard solutions of tannic acid were prepared by aqueous dilution. Results were expressed as follows: milligram tannic acid equivalent (TAE) per gram of dry bark (mg TAE/g bark).

2.5.4. Determination of Stiasny number

The reactivity of the extracts to formaldehyde was determined by measuring the Stiasny number as described by Voulgaridis

et al. (1985). A solution of extract at a concentration of 4 g L^{-1} was prepared. 25 mL of this solution was put in a round bottom flask and 5 mL of formaldehyde 37% and 2.5 mL of HCl 10 M were added. The mixture was heated under reflux for 30 min. The residue was filtered through a sintered glass n°4. The precipitate was washed with water and dried at 105 °C until constant weight. The reactivity was calculated with the formula (Eq. (2)):

$$\%SN = \frac{(A * 100)}{B}. \quad (2)$$

SN: Stiasny number. A: dry weight of the precipitate. B: dry weight of extract.

2.6. Optimization of the results by the response surface methodology (RSM)

After elimination of low or no-influence factor(s), we aimed to optimize extraction process: time extraction, solvent proportion, microwave power and modeling of the responses. RSM was applied to optimize the extraction conditions of polyphenols and tannins extracted by microwave. For this objective, a face centered composite design (FCCD) was used to determine the optimal conditions and the relevance of the three factors was evaluated: time extraction (X_1), solvent proportion (X_2) and microwave power (X_3). The responses measured were the following: polyphenols content (Y_1) and condensed tannins content (Y_2). The range of independent variable and their levels was presented in Table 1, which was based on the results of preliminary experiments. The best solvent extraction was methanol. All experimental data of FCCD are shown in Table 2; the whole design consisted of 20 experiments point carried out in random order. For three variables ($n = 3$) and two levels (low (−) and high (+)), the total number of experiments was 20 determined by the expression: 2^n ($2^3 = 8$: full factor points) + $2n$ ($2 * 3 = 6$: axial points) + 6 (center points: six replications). The software design expert (JMP version 11) was employed for experimental design, data analysis and model building. The experimental data were replicated three times to evaluate the statistical quality of the results. Experiments were randomized to maximize the effects of unexplained variability in the observed response due to extraneous factors. The suggested model for the quadratic model of the variables Y according to the independent variables was indicated in the following Eq. (3):

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3. \quad (3)$$

where Y is the predicted response: the yield of polyphenols or condensed tannins. A_0 is the offset term, a_1, a_2 and a_3 are the linear effect a_{11}, a_{22} and a_{33} are the quadratic effect and a_{12}, a_{13} and a_{23} are the interaction effect. X_1, X_2 and X_3 are the studied factors.

2.7. RP-HPLC characterization

The composition of the proanthocyanidins in the extracts was determined by RP-HPLC. The extracts were dissolved in methanol (10 g L^{-1}). An aliquot (400 µL) was added to 400 µL of acidified methanol (3.3% HCl) and 800 µL of cysteamine hydrochloride (50 g L^{-1} in methanol). The mixture was put in a water bath at 40 °C for 30 min. The

Table 1 Study domain for the studied factors.

Coded level	X_1: time extraction (min)	X_2: solvent proportion (%)	X_3: microwave power (W)
Lower: −1	1	20	150
Central: 0	3	50	250
Higher: 1	5	80	350

Table 2 Experimental design in coded and reels variables for face centered composite design.

N° of experiments	Matrix of the coded factors			Matrix of the reels factors			Comment
	X_1	X_2	X_3	X_1: time extraction (min)	X_2: proportion of the solvent (%)	X_3: microwave power (W)	
1	−1	−1	−1	1	20	150	Full factorial
2	1	−1	−1	5	20	150	Full factorial
3	−1	1	−1	1	80	150	Full factorial
4	1	1	−1	5	80	150	Full factorial
5	−1	−1	1	1	20	350	Full factorial
6	1	−1	1	5	20	350	Full factorial
7	−1	1	1	1	80	350	Full factorial
8	1	1	1	5	80	350	Full factorial
9	**0**	**0**	**0**	**3**	**50**	**250**	Center point
10	**0**	**0**	**0**	**3**	**50**	**250**	Center point
11	**0**	**0**	**0**	**3**	**50**	**250**	Center point
12	**0**	**0**	**0**	**3**	**50**	**250**	Center point
13	**0**	**0**	**0**	**3**	**50**	**250**	Center point
14	**0**	**0**	**0**	**3**	**50**	**250**	Center point
15	0	0	−1	3	50	150	Axial point
16	0	−1	0	3	20	250	Axial point
17	−1	0	0	1	50	250	Axial point
18	0	0	1	3	50	350	Axial point
19	1	0	0	5	50	250	Axial point
20	0	1	0	3	80	250	Axial point

Bold values represent the replication six times of the center points.

proanthocyanidins were depolymerized by thiolysis with cysteamine as described by Torres and Selga (2003). The samples were analyzed by RP-HPLC-DAD (Ultimate 3000, Thermo Scientific), equipped with a acclaim 120 C18, 250 × 4.6 mm, 5 μm column. Elution: [A] 0.1% (v/v) aqueous TFA, [B] acetonitrile. The solvent gradient was as follow: 0–12.6 min, 97.7A/2.3B (v/v); 12.6–15.6 min, 97A/3B; 15.6–18 min, 92A/8B; 18–28 min, 84A/16B; 28–58 min, 100 B; 58–83 min, 100 B. Detection was done at 220, 254, 272 and 280 nm. The chromatograms were analyzed with Chromeleon software.

2.8. Statistical analysis

All the experiments were replicated three times to evaluate the statistical quality of the results. The data were expressed as mean ± SD values. The treatment of the results for experimental designs was carried out using software of experimental design JMP 11. The extraction yield, the polyphenolic content and the condensed tannins content were compared using ANOVA test, Fisher Snedecor test, and Student test and the difference of mean test used when required. All the statistical analyses were carried out at P values < 0.01 significance level.

3. Results and discussions

3.1. Screening factors

The results of preliminary essays: the evaluation of the effect of progression time extraction on the yields, the choice of the adequate power extraction and extraction solvent were the follows:

3.1.1. Effect of time extraction

The polyphenols contents, condensed and hydrolysable tannins affected by different extraction time (1 min, 2 min and 3 min) are shown in Table 3, when other three factors (microwave power, extraction solvent and ratio of liquid to solid) were fixed at 250 W, water and 20 mL/g. Significant difference in yield obtained with progression microwave time extraction was confirmed by ANOVA test and Student test (Table 3). More the time extraction increases, more significant becomes difference in phenolic contents and the better the yield. Progression time extraction improved the polyphenols and condensed tannins contents. The polyphenols content crossed from 407 mg GAE/g bark at 1 min in 436 mg GAE/g bark for 3 min. Significant difference for cyanidin content was showed between the yields obtained at 1 min, 12.42 mg Cya/g bark and the yields obtained at 2 and 3 min, 13.47 mg Cya/g bark and 13.76 mg Cya/g bark, respectively, Student test. The cyanidin content does not significantly change between the extract at 2 and 3 min, Student test, 13.47 mg Cya/g bark and 13.76 mg Cya/g bark, respectively. For hydrolysable tannins, it seems that the progression time extraction improved the contents of the extracts with microwave. We obtained 0.71 mg TAE/g bark; 0.88 mg TAE/g bark and 1.24 mg TAE/g bark, respectively, for 1–3 min. The experimental result showed that the progression time extraction improved the polyphenols contents, condensed and hydrolysable tannins. Highest polyphenols yield, 436 mg GAE/g bark, condensed tannins (cyanidin content), 13.76 mg Cya/g bark, and hydrolysable tannins, 1.24 mg TAE/g bark, extracted with microwave at 250 W, were obtained at 3 min using water.

3.1.2. Effect of microwave power

Different microwave power was set at 150, 250, 300, 450 and 600 W to investigate the effect of microwave power on the polyphenols content, condensed tannins (cyanidin content) and hydrolysable tannins when to other reaction conditions were set as follows: extraction time 1 min, solvent extraction water; ratio of liquid to solid 20 mL/g. The effect of progression power extraction on yields extracts is shown in Table 3. The progression power extraction at 300 W improved the polyphenols content, condensed tannins (cyanidin content); (ANOVA test and Student test, Table 3), but not significantly change was observed for hydrolysable tannins in this interval of power extraction. In the other hand, the polyphenols content and condensed tannins (cyanidin content) started to

Table 3 Result of screening factors: time extraction, microwave power and solvent nature on phenolic contents.

Factor studied	Extraction condition	Polyphenolic contents (mg GAE/g bark)	Condensed tannins: butanol test (mg Cya/g bark)	Hydrolysable tannins (mg TAE/g bark)
Time extraction	Water 250 W-1 min	406.64 ± 6.53[a,c]	12.42 ± 0.45[b,c]	0.71 ± 0.31[b,c]
	Water 250 W-2 min	418.74 ± 5.10[a,c]	13.47 ± 0.46[b,c]	0.88 ± 0.31[b,c]
	Water 250 W-3 min	435.47 ± 8.77[a,d]	13.76 ± 0.25[b,d]	1.24 ± 0.31[b,d]
Microwave power	Water 1 min-150 W	298.77 ± 0.63[a,d]	12.42 ± 0.45[a,d,c]	0.11 ± 0.04[a,d]
	Water 1 min-250 W	406.64 ± 6.53[a,d]	14.28 ± 0.27[a,d]	0.71 ± 0.24[a,c]
	Water 1 min-300 W	534.21 ± 1.43[a,d]	18.26 ± 0.07[a,d]	0.71 ± 0.25[a,c]
	Water 1 min-450 W	434.11 ± 0.82[a,d]	14.70 ± 0.34[a,d,c]	3.88 ± 0.25[a,c]
	Water 1 min-600 W	437.92 ± 0.62[a,d]	14.76 ± 0.17[a,b,c]	3.53 ± 0.25[a,c]
Solvent nature	1 min-150 W Ethanol	312.26 ± 1.09[a,d]	18.50 ± 0.06[a,d]	0.03 ± 0.01[a,d]
	1 min-150 W Ethyl acetate	114.08 ± 1.20[a,d]	5.02 ± 0.40[a,d]	0.36 ± 0.02[a,d]
	1 min-150 W Water	298.77 ± 0.63[a,d]	47.64 ± 0.45[a,d]	0.09 ± 0.02[a,d]
	1 min-150 W Methanol	441.63 ± 0.30[a,d]	19.09 ± 0.21[a,d]	0.08 ± 0.01[a,d]

[a] High significant variance at the level 99%. $F_{0.01}$ (2;6) = 10.92 for time extraction. $F_{0.01}$ (4;10) = 5.99 for microwave power. $F_{0.01}$ (3;8) = 7.59 for solvent nature; ANOVA test.
[b] Non significant variance at the level 99%. $F_{0.01}$ (2;6) = 10.92; ANOVA test.
[c] Difference does not reliably change between (1–2 min; 2–3 min) and (450 W–600 W) at the level 99%; $T_{0.01;\,4}$ = 3.75; Student test.
[d] Difference does reliably change between (1–3 min), microwave power and solvent nature at the level 99%. $T_{0.01;\,4}$ = 3.75; Student test.

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decrease when the power exceeds 300 W, but hydrolysable tannins yield increased. Significant difference in cyanidin content obtained at different power extraction is illustrated in Fig. 2. Lower polyphenols content and condensed tannins obtained at high power (450 and 600 W) was probably due to the liquefaction of condensed tannins, it was a degradation of the condensed tannins structures to produce some galloyl groups at high pressure and temperature. This will explain the increase in the yield of hydrolysable tannins at these powers. Best polyphenols yields, 534 mg GAE/g bark, condensed tannins (cyanidin content), 18.26 mg Cya/g bark, extracted with microwave during 1 min were obtained at 300 W. For hydrolysable tannins, 3.88 mg TAE/g bark, the best was obtained at 450 W.

3.1.3. Effect of solvent nature

The polyphenols contents, condensed and hydrolysable tannins affected by different extraction solvent (ethanol, methanol, water and ethyl acetate) are shown in Table 3, when other three factors (microwave power, extraction time and ratio of liquid to solid) were fixed at 150 W, 5 min and 20 mL/g. The significant influence of the solvent extraction on the phenolic contents was confirmed by the ANOVA test and Student test with a confidence level of 99%, Table 3. The use of different solvent extraction gives a big difference on the phenolic contents. The difference in yield observed was due to the different nature of the phenolic compounds extracted by each solvent (Ignat et al., 2011).

Effective solvent to extract polyphenols was methanol followed by the ethanol and finally the water. Methanol extraction gives a wide range of phenolic compounds including: anthocyanins, phenolic acids, catechins, flavanones, flavanols and procyanidins (Ross et al., 2009; Bleve et al., 2008; Caridi et al., 2007; Mattila and Kumpulainen, 2002). On the other hand, the ethanol extracted: anthocyanins, flavanols and free phenolic acids (Wang and Huang, 2004; Altiok et al., 2008; Bleve et al., 2008; Balas and Popa, 2007) and water extracted the procyanidins and flavanols (Diouf et al., 2009).

The difference in yield, for condensed tannins, may be attributed to the difference of polymerization degree for the tannins extracted by different solvents (Khoddami et al., 2013). Highest cyanidin content was obtained with water followed the content obtained with methanol, ethanol and finally the content obtained with ethyl acetate. These differences can be due to the hydroxyl groups which improve the tannins polycondensation and consequently, the amount of tannin dimmers and trimmers extracted from the bark of *A. mollissima* is large (Venter et al., 2012). Highest hydrolysable tannins were obtained using ethyl acetate followed by ethanol. The result of this study showed that the effectiveness of extraction was related to the choice of extraction solvent and phenolic compound desired; polyphenols, condensed tannins or hydrolysable tannins.

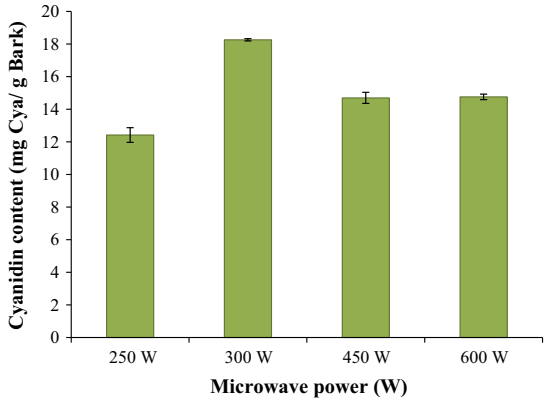

Figure 2 Evaluation of progression power extraction on the yield of condensed tannins.

The best solvent to extract polyphenols content and gives highest content, 441.63 mg GAE/g bark was methanol. For condensed tannins, 47.64 mg Cya/g bark, the best was water. For hydrolysable tannins, 0.36 mg TAE/g bark was ethyl acetate. The results of this preliminary study proved the influence of the nature of the solvent in phenolic compounds extractions.

3.2. FCCD response surface analysis

The yield extraction, polyphenols contents, condensed tannins and hydrolysable tannins affected by different conditions of FCCD are shown in Table 4, when three factors (microwave power, time extraction and methanol proportion) were changed according to the Hadamard matrix represented in Table 2. All experimental data of FCCD are shown in Tables 2 and 4, the whole design consisted of 20 experiments point carried out in random order. Optimization of MAE conditions was reported according to the proportion solvent used. The significant influence of the extraction condition on the yield extracts were confirmed by the ANOVA and Student test with a confidence level of 99%, Table 4.

The progression time extraction to 1 min at 5 min for the extracts obtained using 20% or 80% of methanol, gives the twice polyphenolic contents extracted at lower microwave power, 150 W, 347 mg GAE/g bark, 441 mg GAE/g bark respectively compared with the content obtained at 1 min, 195 mg GAE/g bark. This results confirm that progression time extraction improve significantly the polyphenols yield. Lower difference in polyphenolic contents was observed for the extracts obtained at 250 W during 3 min, using 20%, 50% and 80% of methanol. For this microwave power, highest polyphenols contents was obtained using 20% of methanol, 240.31 mg GAE/g bark, followed by the yield obtained using 50% of methanol, 285.18 mg GAE/g bark, and finally the yield obtained using 80% of methanol, 216.41 mg GAE/g bark. This result confirms that reducing methanol proportion to 20% gives the best polyphenols contents. Significative difference in polyphenols contents was proved for the extract obtained at 350 W during 1 min and 5 min using 80% of methanol, Student test, Table 4. The highest polyphenols contents, 441.63 mg GAE/g bark, was obtained at 150 W during 5 min using 80% of methanol, respectively.

The proanthocyanidin contents were significantly improved with progression time extraction to 1 min at 5 min, or with progression methanol proportion to 20% at 50%, for the extract at lower microwave power, 150 W. The cyanidin content crossed from 22.25 mg Cya/g bark at 1 min in 43.06 mg Cya/g bark for the extract during 5 min at 150 W using 20% of methanol and it crossed to 44.44 mg Cya/g bark using 50% of methanol. For condensed tannins extracted at 250 W during 3 min, the progression methanol proportion gives lower cyanidin content, 74.04 Cya/g bark; 26.50 Cya/g bark and

Table 4 Colorimetric assays results of extracts according to the conditions of experimental design.

Identification	Yield extraction polyphenols (%)[a,b]	Yield extraction tannins (%)[a,b]	Yield extraction oligomers (%)[a,b]	Total polyphenolic (mg GAE/g bark)[a,b]	Hydrolysable tannins (mg TAE/g bark)[a,b]	Condensed tannins: butanol test (mg Cya/g bark)[a,b]
1 min; 20%; 150 W	26.60 ± 2.13[a]	22.91 ± 1.08[a]	2.58 ± 0.27[a]	195.66 ± 0.33[a]	0.266 ± 0.001[a]	22.25 ± 0.30[a]
5 min; 20%; 150 W	33.82 ± 1.17[a]	26.53 ± 0.88[a]	6.76 ± 0.18[a]	347.55 ± 0.32[a]	0.277 ± 0.001[a]	43.06 ± 0.23[a]
3 min; 50%; 150 W	**42.38** ± 0.89[a]	30.21 ± 0.53[a]	8.89 ± 0.34[a]	309.13 ± 0.61[a]	0.413 ± 0.001[a]	**44.44 ± 0.14**[a]
1 min; 80%; 150 W	30.16 ± 1.20[a]	23.52 ± 0.13[a]	4.65 ± 0.33[a]	173.59 ± 0.30[a]	0.158 ± 0.001[a]	14.28 ± 0.27[a]
5 min; 80%; 150 W	38.3 ± 1.33[a]	30.28 ± 0.27[a]	7.94 ± 0.50[a]	**441.63** ± 0.30[a]	0.085 ± 0.001[a]	19.09 ± 0.21[a]
1 min; 50%; 250 W	24.9 ± 1.89[a]	21.27 ± 0.08[a]	3.39 ± 0.25[a]	123.48 ± 0.47[a]	0.184 ± 0.001[a]	17.04 ± 0.28[a]
3 min; 50%; 250 W	**49.62** ± 0.49[a]	39.99 ± 0.23[a]	9.49 ± 0.37[a]	235.64 ± 0.35[a]	0.684 ± 0.001[a]	26.22 ± 0.14[a]
3 min; 50%; 250 W	49.95 ± 0.43[a]	39.78 ± 0.23[a]	9.32 ± 0.19[a]	234.94 ± 0.45[a]	0.675 ± 0.001[a]	25.67 ± 0.29[a]
3 min; 50%; 250 W	49.42 ± 0.35[a]	39.88 ± 0.19[a]	9.41 ± 0.15[a]	235.18 ± 0.15[a]	0.678 ± 0.001[a]	26.50 ± 0.37[a]
3 min; 50%; 250 W	49.38 ± 0.51[a]	39.96 ± 0.21[a]	9.45 ± 0.14[a]	233.24 ± 0.39[a]	0.686 ± 0.001[a]	27.06 ± 0.04[a]
3 min; 50%; 250 W	49.36 ± 0.13[a]	39.71 ± 0.33[a]	9.29 ± 0.32[a]	236.12 ± 0.09[a]	0.674 ± 0.010[a]	26.36 ± 0.24[a]
3 min; 50%; 250 W	49.77 ± 0.34[a]	39.89 ± 0.38[a]	9.34 ± 0.35[a]	234.89 ± 0.19[a]	0.680 ± 0.001[a]	25.86 ± 0.29[a]
5 min; 50%; 250 W	37.77 ± 2.09[a]	20.54 ± 1.28[a]	16.67 ± 0.66[a]	254.27 ± 0.23[a]	0.426 ± 0.001[a]	37.04 ± 0.28[a]
3 min; 20%; 250 W	35.67 ± 1.09[a]	16.68 ± 1.43[a]	17.37 ± 0.58[a]	240.31 ± 0.47[a]	0.385 ± 0.001[a]	**74.04** ± 0.14[a]
3 min; 80%; 250 W	46.16 ± 2.13[a]	30.13 ± 1.53[a]	15.64 ± 0.86[a]	216.41 ± 0.36[a]	0.388 ± 0.001[a]	21.04 ± 0.58[a]
1 min; 20%; 350 W	20.24 ± 1.28[a]	10.76 ± 1.35[a]	9.08 ± 0.07[a]	264.43 ± 0.31[a]	0.404 ± 0.001[a]	15.00 ± 0.14[a]
5 min; 20%; 350 W	23.75 ± 1.67[a]	5.27 ± 1.26[a]	17.69 ± 0.17[a]	269.35 ± 0.43[a]	0.452 ± 0.001[a]	11.80 ± 0.20[a]
3 min; 50%; 350 W	28.13 ± 1.56[a]	15.39 ± 1.20[a]	11.93 ± 0.13[a]	250.84 ± 0.60[a]	0.806 ± 0.002[a]	16.13 ± 0.14[a]
1 min; 80%; 350 W	**39.86** ± 0.13[a]	10.42 ± 1.32[a]	25.16 ± 0.09[a]	332.36 ± 0.30[a]	0.225 ± 0.001[a]	8.08 ± 0.21[a]
5 min; 80%; 350 W	**42.03** ± 0.53[a]	7.75 ± 1.57[a]	31.29 ± 0.07[a]	401.88 ± 0.61[a]	0.314 ± 0.001[a]	22.41 ± 0.21[a]

[a] High significant variance at the level 99%. $F_{0.01}$ (14.30) = 2.74; ANOVA test.
[b] Difference does reliably change between extract at the level 99%.
$T_{0.01;\ 4}$ = 3.75; Student test.
Bold values represent the higher values of extractibles.

21.04 Cya/g bark respectively using 20%; 50% and 80% of methanol. Higher microwave power, 350 W, affects proanthocyanidin contents. Lower condensed tannins obtained at 350 W, 15 Cya/g bark, was probably due to the liquefaction of condensed tannins, it was a degradation of the condensed tannins structures to produce some galloyl groups at high pressure and temperature. This will explain the increase in the yield of hydrolysable tannins at this power. Highest cyanidin content, 74.04 Cya/g bark, was obtained at 250 W using 20% of methanol and during 3 min. For hydrolysable tannins, the progression power extraction improves their contents. Highest hydrolysable tannins contents, 0.806 mg TAE/g bark, was obtained at 350 W using 50% of methanol and during 3 min.

The yield extraction of polyphenols, tannins and oligomers affected by different conditions of FCCD presented in Table 4, confirm the results of colorimetric essays of phenolic compounds. The results shown lower yield extract at higher microwave power, 350 W; 18.45%, and highest yield at lower microwave power, 150 W; 39.82%. This result can explain the lower polyphenols contents and condensed tannins at 350 W.

The highest polyphenols content, 441.63 mg GAE/g bark, was obtained at 150 W during 5 min using 80% of methanol, respectively. For condensed tannins, highest cyanidin content, 74.04 Cya/g bark, was obtained at 250 W using 20% of methanol and during 3 min. For hydrolysable tannins, the highest, 0.806 mg TAE/g bark, was obtained at 350 W using 50% of methanol and during 3 min.

3.3. Modeling of extraction process of polyphenols

The modeling results of polyphenols were carried out using software JMP 11. It is used for treatment of the results of experimental designs. The estimation of the coefficients was carried out with the least squares method. All the experiments carried out were repeated three times to evaluate the quality of the established model.

3.3.1. Evaluation of the quality of the model

The evaluation of the estimated quality of the quadratic model chosen to model extraction condition of polyphenols was carried out using statistical tools: the linear regression and the adjusted regression.

The linear regression of the model, permitted to evaluate graphically the quality of the established model. It is represented by the linearity of the points measured by the real model according to the points considered by the quadratic model. The goodness of the model is judged if the linear of regression coefficient is equal to or higher than 0.80. The linear analysis of regression was established by the Experimental

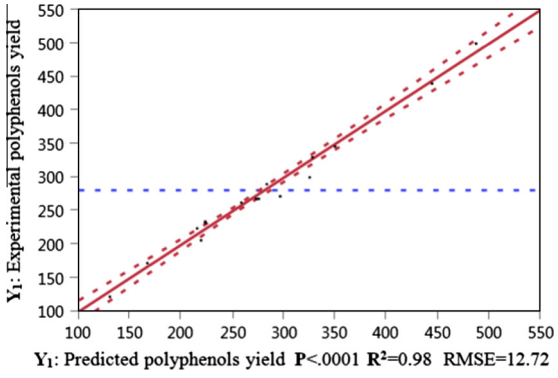

Figure 3 Linear regression of the quadratic model of polyphenols.

Table 5 Evaluation quality of quadratic model chosen to model the extraction conditions of phenolic compounds.

	Variable studied	Polyphenols	Condensed tannins
Regression analysis	R square (R^2)	0.984	0.994
	Adjusted R square (R_A^2)	0.981	0.992
ANOVA test	F value	267.11	688.41
	P value	**<.0001***	**<.0001***
Validation model	F value	11701.46	260.31
	P value	<.0001*	**<.0001***

* High significant at the level 99%.
Bold values represent the high significant value at the level 99%.

design JMP 11 software are shown in Fig. 3. The coefficient of the linear regression R^2 was equal to 0.98. This coefficient was very close to 1, therefore the choice of quadratic model to modeling extraction process of polyphenols was better.

Adjusted regression of the model permits to evaluate the quality of adjustment of the model established and also it takes into account the degrees of freedom of the model. It was determined by experimental design JMP 11 software. The result of the adjusted regression was represented in Table 5. The coefficient of adjusted regression R_A^2 was equal to 0.98. This showed that more than 98% of the variation observed was explained by the direct effects of the factors. This coefficient was very close to 1, therefore the quality of adjustment of the quadratic model choose was better. The results of the linear and adjusted regression R^2 and R_A^2, 0.98 and 0.98 respectively, indicated a coefficient very closed to 1. These results proved that the quadratic model was the best choice for the modeling extraction process of polyphenols.

3.3.2. Validation of the quadratic model for the modeling of polyphenols results

The validation of the quadratic model of polyphenols was carried out using variance analysis of the model (ANOVA) and the method of lack of adjustment analysis, also known as the bias analysis. *The ANOVA test* permitted to evaluate the variance of the established model compared to the variance of the residue, using the «***Fisher Snedecor***» test. The results of this analysis are shown in Table 5. The result was regarded significant if «$F_{exp} \gg F_{\alpha}$, v_{mod}, v_{res}». The analysis of the result give an experimental factor $F_{exp} = 267.11$. The theoretical value determined according to Fisher Snedecor table, (for $v_{model} = 9$, $v_{residue} = 38$ and for a confidence level = 99%), was $F_{theo} = F_{\alpha}$, v_{mod}, $v_{res} = F_{0,01,\ 9,\ 38} = 2.92$. This Factor was very lower than experimental factor, $F_{exp} = 267.11 \gg F_{theo} = 2.92$, therefore the condition of Fisher Snedecor test was validated and the regression was thus significant with a confidence level of 99%.

The lack of adjustment analysis permits to evaluate the variance of the residue compared to the experimental error. It is calculated from points in the center, using the «***Fisher Snedecor***» test. The model is validated; if the p value was lower than 0.01. The results of this analysis, determined by software JMP 11, were represented in Table 5. The results of the lack of adjustment analysis, indicate that the p value of the experimental factor was largely lower than 0.0001. This result showed that the error between the established and theoretical model was thus not significant for a confidence level of 99% and the result was very significant. The results of ANOVA test and the lack of adjustment analysis showed that the quadratic model established to model the extraction conditions of polyphenols by microwave was validated.

3.3.3. Determination of the polyphenols equation model

After validating the established quadratic model of polyphenols, the equation of this model we can be determined. The coefficients are estimated by the method of least squares. They are said significant if the p value was lower than 0.01. The significant effects are shown in Fig. 4. Under the linear term of three factors (time extraction, methanol proportion and microwave power), the quadratic effect of these factors and their interactions were very significant, P value <0.0001, to improve the yields of polyphenols extracted by microwave. The equation of quadratic model used for the modeling extractions conditions of polyphenols, determined according to the results of the JMP 11 software, was represented in the following equation:

$$Y_1 = 223.10 + 72.82 * X_1 + 40.76 * X_2 + 20.76 * X_3 + 36.51 * X_{12} - 29.18 * X_{13} + 29.97 * X_{23} - 20.54 * X_1^2 + 32.61 * X_2^2 + 80.58 * X_3^2.$$

The established equation showed that the polyphenols content (Y_1) was significantly affected by the synergistic effect of linear term of time extraction (X_1) and methanol proportion (X_2). So,

Terme	Estimation	Standard deviation	t value	t value	Prob.>|t|	Comment
X_1: time (min) (1.5)	72.82	2.32	31.36		<.0001*	Significant
X_3: power (W)*X_3: power (W)	80.59	4.52	17.82		<.0001*	Significant
X_2: solvent (%)(20.80)	40.76	2.32	17.55		<.0001*	Significant
X_1: time (min)* X_2: solvent (%)	36.51	2.59	14.07		<.0001*	Significant
X_2: solvent (%)*X_3: power (W)	29.97	2.59	11.55		<.0001*	Significant
X_1: time (min)* X_3: power (W)	-29.18	2.59	-11.24		<.0001*	Significant
X_3: power (W)(150.350)	20.76	2.32	8.94		<.0001*	Significant
X_2: solvent (%)*X_2: solvent (%)	32.61	4.52	7.21		<.0001*	Significant
X_1: time (min)* X_1: time (min)	-20.54	4.52	-4.54		<.0001*	Significant

* High significant at the level 99%.

Figure 4 Significant effects coefficients of established equation model of polyphenols.

to maximize the polyphenols yield, the maximum value for these factors must be taken. In other words, to maximize the polyphenols yield extracted by microwave, it is necessary to carry out the extractions during 5 min with 80% of methanol at moderate power extraction 150 W or 250 W. But it is necessary to make a consideration the interaction of progression time extraction and high power extraction, which could cause a degradation of the structure of the extracts, hence the interest to carry out the extraction at a low power of extraction at 150 W.

3.4. Modeling of the extraction process of condensed tannins

To evaluate the quality of the established model chosen for the extraction conditions of condensed tannins we carried out the same approach used for the modeling of the extraction conditions of polyphenols.

3.4.1. Evaluation of the quality of the model chooses for condensed tannins

The linear regression of the quadratic model of condensed tannins, represented in Fig. 5, indicated a regression coefficient $R^2 = 0.99$. This coefficient was very close to 1, therefore the choice of quadratic model to modeling extraction process of condensed tannins was better. The result of the *adjusted regression* of the quadratic model of condensed tannins was represented in Table 5. The coefficient of adjusted regression R_A^2 was equal to 0.99. This showed that more than 99% of the variation observed was explained by the direct effects of the factors. This coefficient was very close to 1, therefore the quality of adjustment of the quadratic model choose was better. The results of the linear and adjusted regression R^2 and R_A^2, 0.99, indicated a coefficient very closed to 1. These result proved that the quadratic model was the best choice for the modeling extraction process of condensed tannins.

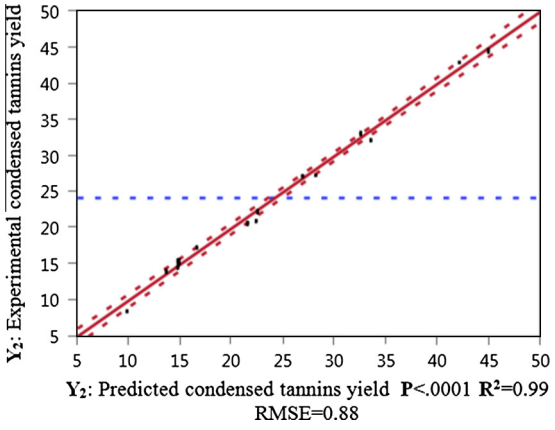

Figure 5 Linear regression of the quadratic model for condensed tannins.

3.4.2. Validation of the quadratic model for the modeling of the results of condensed tannins

The validation of the quadratic model of condensed tannins was carried out using ANOVA test and the lack of adjustment analysis. The results of these tests are shown in Table 5. The results of ANOVA test give an experimental factor ($F_{exp} = 688.31$) higher than the theoretical factor ($F_{theo} = 2.92$) determined using Fisher Snedecor table, $F_{exp} = 688.31 \gg F_{theo} = F_{0,01,\ 9,\ 38} = 2.92$, therefore the condition of Fisher Snedecor test was validated and the regression was thus significant with a confidence level of 99%. The results of lack of adjustment analysis indicate that the p value of the experimental factor was largely lower than 0.0001. Therefore the error between the established and theoretical model was thus not significant for a confidence level of 99% and the result was very significant. These results showed that the quadratic model established to model the extraction conditions of condensed tannins by microwave was validated.

3.4.3. Determination of the equation of the condensed tannins model

After validating the established quadratic model of condensed tannins, the equation of this model was determinate. The significant effects and their coefficients are shown in Fig. 6. Under the linear term of three factors (time extraction, methanol proportion and microwave power), the quadratic effect of time extraction were very significant, P value < 0.0001, to improve the yields of condensed tannins extracted by microwave. On the other hand, the antagonistic effect of time extraction and methanol proportion (X_{12}), was not significantly affected condensed tannins yield, p value = 0.966 larger than 0.01. Therefore, we can neglect this coefficient from the equation of modeling of condensed tannins. The equation of quadratic model used for the modeling extractions process of condensed tannins, determinate according to the results of

Terme	Estimation	Standard deviation	t value	t value	Prob.>|t|	Comment
X_2: solvent (%) (20.80)	-6.18	0.16	-38.44		<.0001*	Significant
X_3: power (W) (150.350)	-6.10	0.16	-37.96		<.0001*	Significant
X_2: solvent (%)*X_2: solvent (%)	10.51	0.31	33.59		<.0001*	Significant
X_1: time (min)* X_1: time (min)	-9.51	0.31	-30.40		<.0001*	Significant
X_1: time (min) (1.5)	3.87	0.16	24.09		<.0001*	Significant
X_3: power (W)* X_3: power (W)	-7.40	0.31	-23.66		<.0001*	Significant
X_2: solvent (%)*X_3: power (W)	3.72	0.18	20.73		<.0001*	Significant
X_1: time (min)* X_3: power (W)	-0.48	0.18	-2.66		0.0113*	Significant
X_1: time (min)* X_2: solvent (%)	-0.007	0.18	-0.04		0.9666	Not Significant

* High significant at the level 99%.

Figure 6 Significant effects coefficients of established equation model of condensed tannins.

the JMP 11 software, was represented in the following equation:

$$Y_2 = 28.20 + 3.87 * X_1 - 6.10 * X_2 - 6.10 * X_3 - 0.007 * X_{12} + 0.48 * X_{13} + 3.72 * X_{23} - 6.52 * X_1^2 + 10.51 * X_2^2 - 7.40 * X_3^2.$$

The established equation showed that the condensed tannins content (Y_2) was significantly affected by the quadratic term of time extraction (X_1^2) methanol proportion (X_2^2), synergistic effect of linear term of time extraction (X_1), methanol proportion (X_2), microwave power (X_3) and also the antagonistic effect of linear term of these factors (X_{23}). So, to maximize condensed tannins yield, the maximum value of time extraction, minimum value of methanol proportion and microwave power will be taken. In other words, to maximize condensed tannins yield extracted by microwave, it is necessary to carry out extractions during 5 min with 20% of methanol at moderate power extraction 150 W or 250 W.

3.5. Optimization process of phenolic compounds

After validation of the established model of polyphenols and condensed tannins process, the optimal extraction conditions were determinate using iso-response curves and response surface curves.

3.5.1. Optimization extractions process of polyphenols

The iso-response curves is a graphically method that permits to illustrate the result according to two factors chooses, time extraction, power extraction or methanol proportion. It is a graphical representation at two dimensions, 2D. This method permits to visualize optimal conditions and determine the best yield extract for three factors when one factor was fixed. It permits also, to compare the estimated result with the real result obtained with the experiments. The optimal condition to extract polyphenols, determined using JMP 11 Software was represented in Fig. 7. The result of iso-response curves indicated a maximum polyphenols content, 444.3 mg GAE/g bark, under the conditions as follows: microwave power, 150 W, time extraction, 5 min and methanol proportion, 80%.

The response surface curve was a three dimensional graphical method that permits to illustrate the progression of the response according to the progress of three factors. The addition of the 3rd factor at iso-response curve, 2D, constitutes the response surface curve measured, 3D. The progression polyphenols yield according to the progress of three significant factors: time extraction, microwave power and methanol proportion, was determined using response surface curve presented in Fig. 8. The exploiting method was carried out using JMP 11 Software. The result of response surface indicated the highest polyphenols content, 481.4 mg GAE/g bark, at 157 W during 1 min and using 80% of methanol.

3.5.2. Optimization extractions process of condensed tannins

The optimization extraction condition of condensed tannins, was carried out using iso-response curve (Fig. 9) and response surface curves are represented in Fig. 10. The result of iso-response of the quadratic model of condensed tannins indicated highest cyanidin content, 74.49 mg Cya/g bark at 182 W during 3.7 min and using 20% of methanol. On the other hand, the result of response surface indicated a highest cyanidin content, 79 mg Cya/g bark at 152 W during 1.04 min and using 20.5% of methanol.

RSM used in this study to optimize extraction process of polyphenols and condensed tannins permitted to reduce power extraction at 150 W, solvent proportion at 20% of methanol and time extraction at 1–3 min. The use of experimental design permits to reduce the number of experimental trial, to model extraction process of phenolic extract and also to define optimal conditions given highest yield, using lower solvent proportion and shorter time extraction.

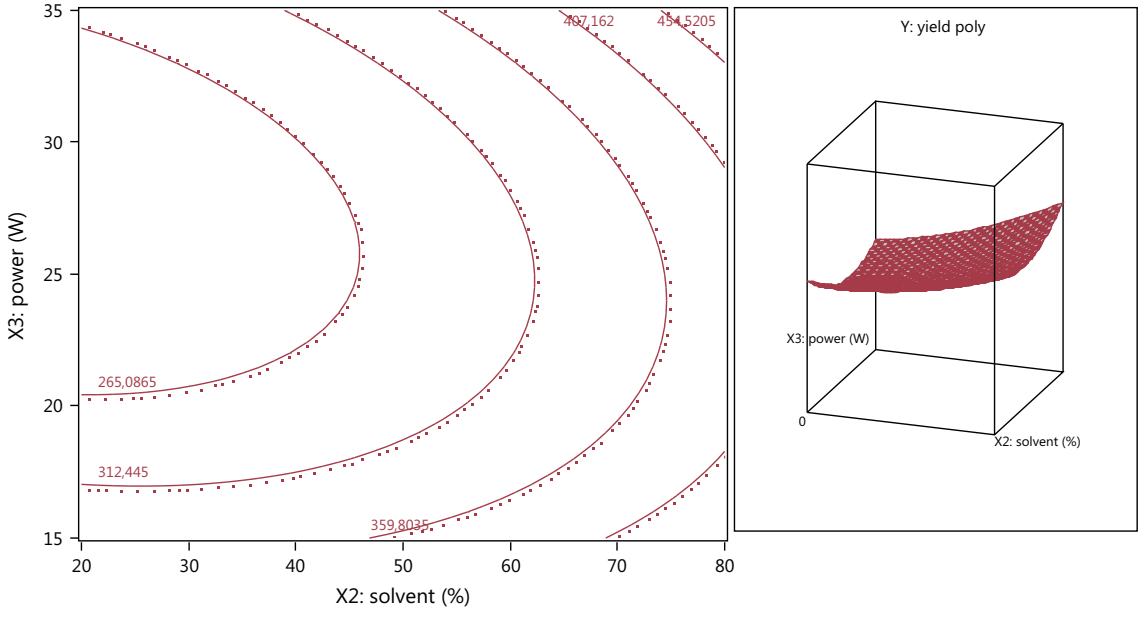

Figure 7 Iso-response result of quadratic model for polyphenols determined by JMP 11.

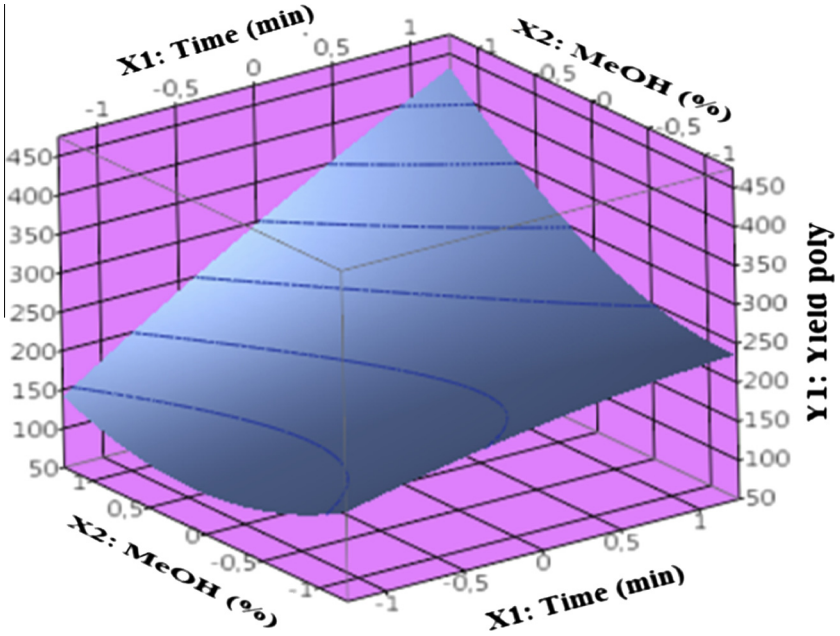

Figure 8 Response surface result of quadratic model for polyphenols.

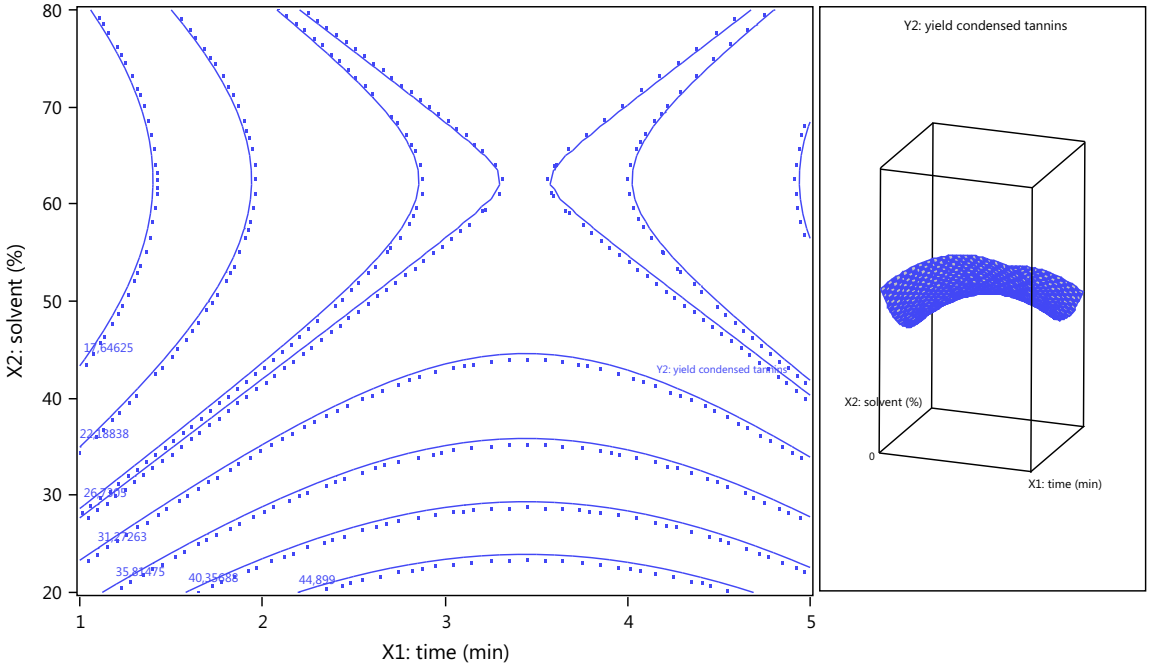

Figure 9 Iso-response result of quadratic model for condensed tannins determined by JMP 11.

3.6. Confirmation experiment

To verify the quality of the result obtained using RSM, optimal conditions to extract polyphenols and condensed tannins determined by experimental data and modeling data were evaluated using the confirmation experiment. RSM gives two optimal extraction conditions, according to the tools used, iso-surface curve or response surface curve. Experimental data dive one optimal. The total polyphenolic contents and condensed tannins were determined for the different extraction conditions. The result of this test is show in Table 6. The response values of the confirmation experiment trial present the same values compared to predicted optimal conditions (80.16 mg Cya/g bark and 79 mg Cya/g bark respectively for condensed tannin; 478 mg GAE/g bark and 481 mg GAE/g bark for polyphenols contents, Table 6). No significant difference was observed between experiment trial and predicted values, ANOVA test and Student test(Table 6). Hence the RSM

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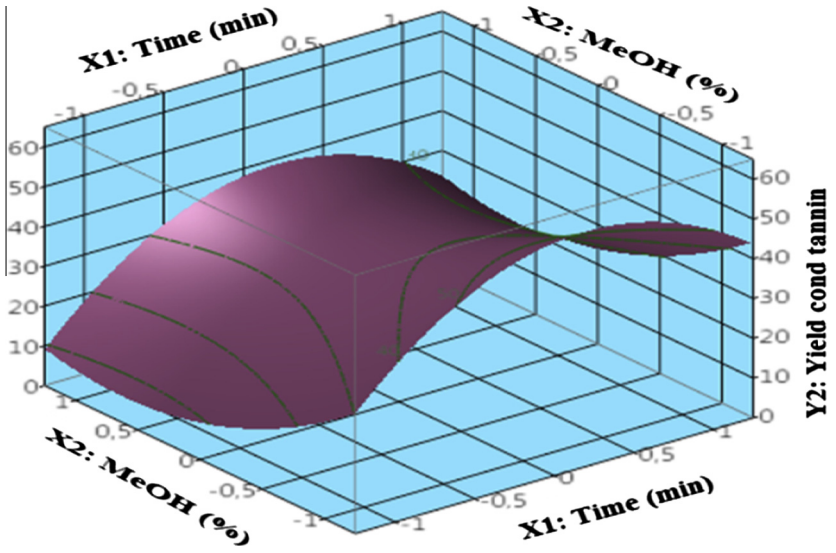

Figure 10 Response surface result of quadratic model for condensed tannins.

Table 6 Confirmation experiment of optimal extraction conditions of phenolic compounds.

Extractable nature	Extraction condition	Experimental result (Test points)[a,b]	Previous result (RSM)[a,b]
Total polyphenolic content (mg GAE/g bark)	150 W; 80%; 5 min	441.63 ± 0.13	444.3
	157 W; 80%; 1 min	478.13 ± 0.61	481.4
	350 W; 80%; 5 min	400.18 ± 0.26	401.9
Condensed tannins cyanidin content (mg Cya/g bark)	152 W; 20.5%; 1 min	80.16 ± 0.86	79
	182 W; 20%; 4 min	77.88 ± 1.88	76
	250 W; 80%; 3 min	76.03 ± 0.26	74

[a] Non significant variance at the level 99%. $F_{0.01}$ (4;10) = 5.99; ANOVA test.
[b] Difference does not reliably change between previous and experimental result at the level 99%. $T_{0.01;\,4}$ = 3.75; Student test.

used for the optimization process of polyphenols and condensed tannins is a very useful tool for predicting the response values, reducing time extraction, microwave power and methanol proportion of phenolic compounds extracted.

3.7. Stiasny number

Optimal conditions to extract condensed tannins determined by experimental data and modeling data were chosen to evaluate tannin reactivity with formaldehyde. Three conditions were compared with Brazilian commercial Tannin; the first was an extraction at 182 W using 20% of methanol during 3.7 min, the second was an extraction at 152 W using 20.5% of methanol during 1 min and the third was an extraction at 250 W using 20% of methanol during 3 min. The Stiasny number gives us the reactivity of our extracts to formaldehyde, this information can help us to determine if the extracts can be used as adhesives (Chupin et al., 2013). The results of Stiasny number determined for the different extraction conditions are show in Table 7. According to Yazaki and Collins (1994), at least 65% Stiasny number is needed for a highest quality adhesive. The Stiasny number relieved from this study is between 89.9% and 96.3%. Thus, the extracts obtained in different operating conditions could be used as adhesives. The highest reactivity was calculated for Moroccan tannins obtained at various microwave power 152 W, 182 W and 250 W using 20% of methanol (respectively 96.3%, 90.8% and 89.9%). The Stiasny number decreases for high microwave power. Similar results are obtained by Kueny and Lecoanet (2012) and Hoong et al. (2009), 92.2% and 94.2% respectively for *Tanazanian mimosa* and *Acacia mangium*. However, Moroccan *A. mollissima* tannins seem to be more reactive than Brazilian commercial tannin (respectively 96.3% and 92.8%), Table 7.

Table 7 Tannin reactivity with formaldehyde: Stiasny number.

Identification extraction condition	Stiasny number (%)	Total polyphenolic (mg GAE/g bark)	Condensed tannins cyanidin content (mg Cya/g bark)
1 min; 20.5%; 152 W	96.29 ± 1.34	557.88 ± 2.58	80.16 ± 0.86
3.7 min; 20%; 182 W	90.76 ± 1.32	501.82 ± 1.14	77.88 ± 1.88
3 min; 20%; 250 W	89.98 ± 1.80	498.18 ± 1.15	76.31 ± 0.35
Commercial *mimosa* tannin	92.81 ± 2.34	463.13 ± 1.39	77.03 ± 0.26

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3.8. RP-HPLC result

The composition of the proanthocyanidins extracted from *A. mollissima* using microwave was determined by RP-HPLC. For a better peaks separation, the proanthocyanidins are depolymerized by thiolysis according to the protocol Torres and Selga (2003). The elution gradient used was a binary system (Solvent A and B). The solvent A was an aqueous solution with lower proportion of acetic acid, and solvent B was an acetonitrile. Small proportion of acetic acid (0.1%) was added in solvent A, to prevent the ionization of phenolic and carboxylic groups and also to improve the resolution and reproducibility of the analysis. The acetonitrile was defined as the most suitable solvent for elution of phenolic compounds absorbed on C_{18} (Garcia Pérez, 2008). For the best determination of phenolic compounds, detection was done at multiple wavelengths: 220, 254, 272 and 280 nm and the chromatograms were analyzed with Chromeleon software.

The RP-HPLC results of proanthocyanidins extracted from *A. mollissima* show two mains comments:

- The thiolysis allows good separation of proanthocyanidins peaks of *A. mollissima*.
- A Brazilian *Acacia* present a similar composition of proanthocyanidins that of Moroccan *Acacia*. Catechin, gallic acid and ellagic acid are detected in all the chromatograms. Ellagic acid is the main condensed tannin present in the extracts followed by catechin and then gallic acid.

Highest intensity of ellagic acid was observed for Moroccan tannins extracted by microwave, 650 mAU, compared with Brazilian tannin obtained from Acacia, 500 mAU, Figs. 11a and 11b. Lower intensity of gallic acid is detected in the chromatograms of Moroccan tannins extracted by microwave, which can be due to a slight degradation of condensed tannins at high-temperature.

3.9. Greenness of analytical procedures

The first principle of green chemistry is to eliminate or reduce the solvents used (Gauszka et al., 2012). In our study, we reduce the methanol proportion from 80% to 20%. Methanol is a non-green solvent but in the other hand, it gives the highest yield in polyphenols and condensed tannins. The methanol extract a wide range of phenolic compounds: Cyanidin, catechin, phenolic acids, flavanones, procyanidins, and flavanols but water extracted only flavanols and procyanidins (Ignat et al., 2011). The mixture of methanol and water gives a best yield of phenolic extracts compared to the use of each solvent apart. The use of RSM replace initial reactive with less toxic reagents. 60% of the solvent is replaced by water.

The second principle of green chemistry is the use of renewable resources (Gauszka et al., 2012). In this research, the synthetic phenol is replaced by the use of vegetable tannins obtained of renewable resources: "The bark of Moroccan *Acacia mollissima*". The third principle is to reduce energy consumption (Gauszka et al., 2012). The optimization extraction process permits to reduce microwave power from 800 W to 150 W. The use of lower microwave power consumes less energy and also the third principle is respected.

The greatest progress in making analytical procedures greener has been the use of microwave assisted extraction

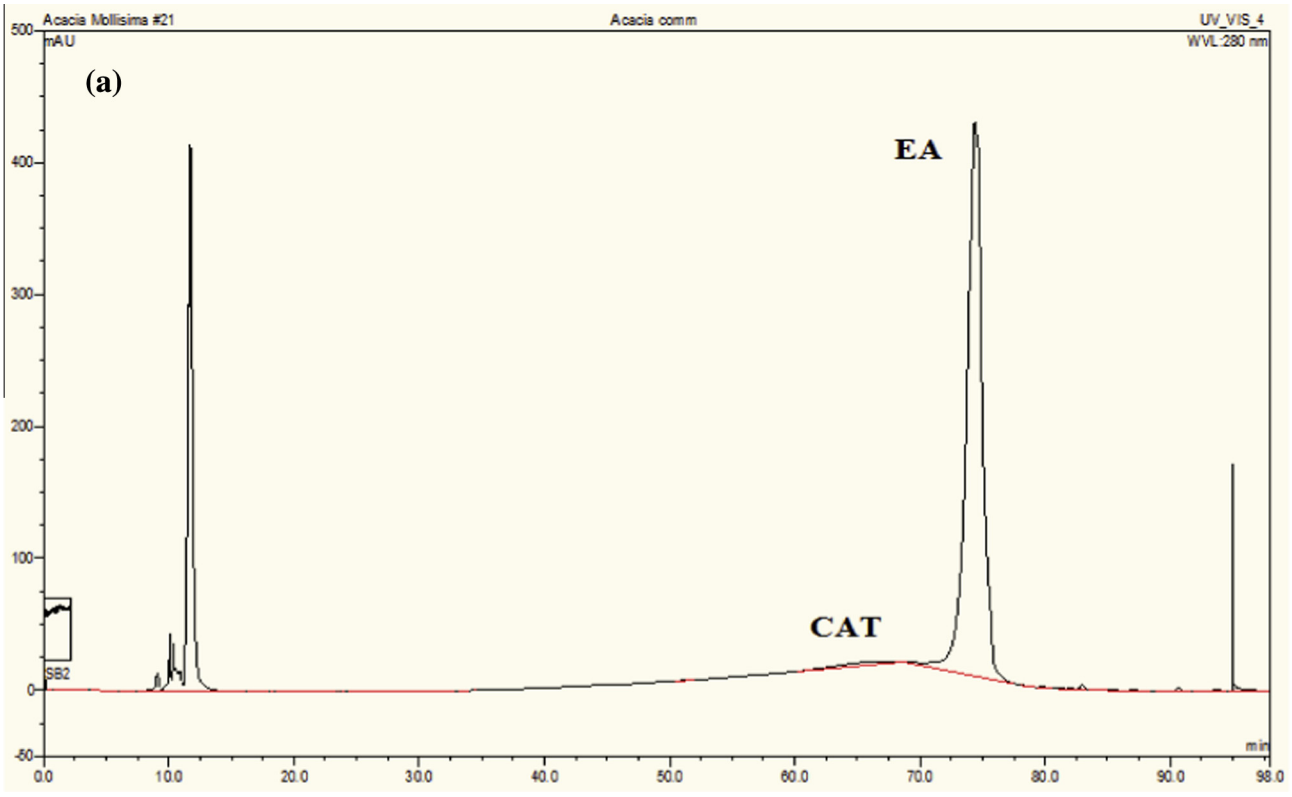

Figure 11a RP-HPLC chromatogram of commercial *mimosa* tannins after thiolysis at 280 nm (Cat: catechin; EA: ellagic acid).

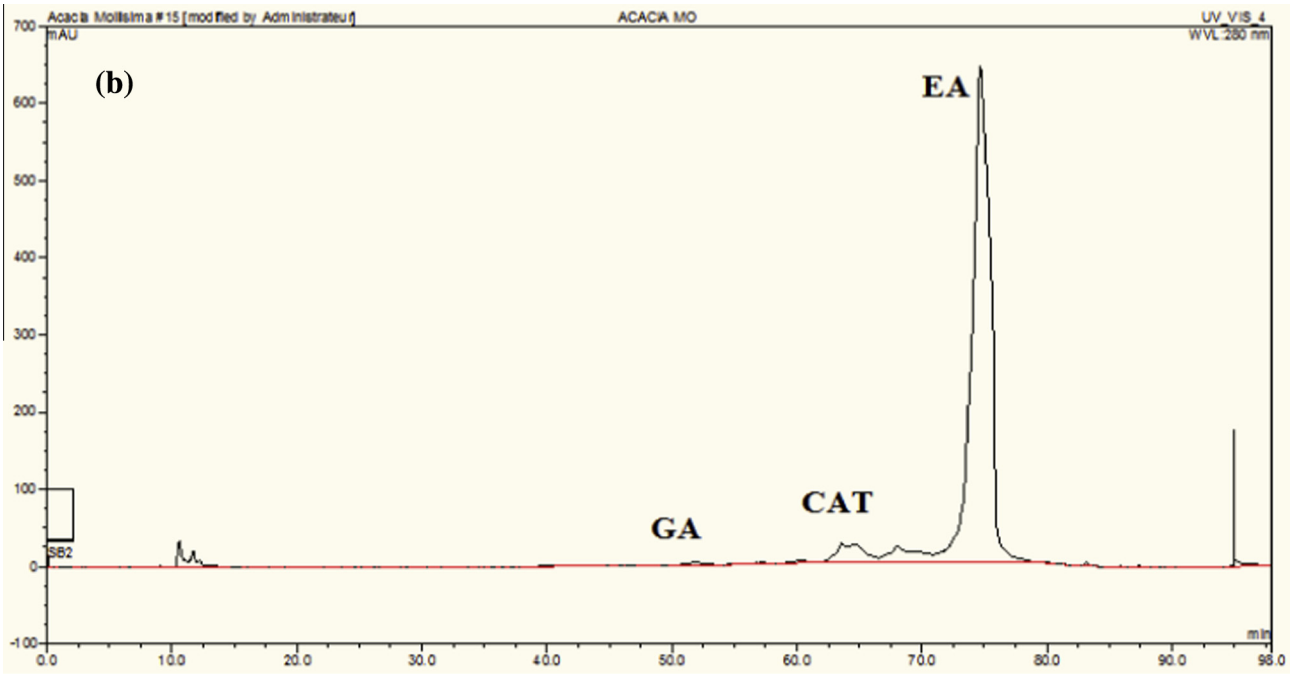

Figure 11b RP-HPLC chromatogram of Moroccan tannins extracted at 152 W using 20% of methanol after thiolysis at 280 nm (Cat: catechin; EA: ellagic acid; GA: gallic acid).

(MAE) to extract phenolic compound: "polyphenols and tannins". After extraction phenolic compound, the waste can be valorized to extract lignin and cellulose or to elaborate a green carbon. The waste resulting after extraction cannot affect the health of consumer or the environment.

The goal of green analytical chemistry is to use analytical procedures that generate less hazardous waste and that are safer to use and more benign to the environment. The spectrophotometer UV–Vis and HPLC are classified green analytical procedures (Keith et al., 2007). In our study, these analytical procedures are used to determine the polyphenols and tannins contents and also to determine the composition of proanthocyanidin extracted. All the aspects developed in this study, confirm that the method used in this study is green and ecological.

4. Conclusion

In this study, we evaluated the effect of time extraction, methanol proportion and microwave power, to improve the polyphenols contents and condensed tannins, extracted by microwave from Moroccan barks of *A. mollissima*. These tests were carried out according to the conditions determined by the experimental design. The optimization of the extraction conditions of these various components was carried out using RSM. Progression time extraction and methanol proportion improve significantly the polyphenols contents and condensed tannins. Lower polyphenols content and condensed tannins were obtained at high power (450 and 600 W). The optimal conditions to obtain the highest polyphenols content, 481.4 mg GAE/g bark are the following: methanol proportion, 80% (v/v), microwave power, 157 W, time extraction, 1 min. For condensed tannins, the green conditions to obtain the highest cyanidin content, 80.16 mg CYA/g bark and the highest reactivity to formaldehyde, 96.3%, are the following: methanol proportion, 20.5% (v/v), time extraction, 152 W, time extraction, 1 min. The main condensed tannins units present in the extracts were ellagic acid units. Catechin and gallic acid were also detected but in lower quantities. This study has permitted to define the optimal extraction process for each chemical component of Moroccan barks of *A. mollissima*, according to the proportion of solvent using experimental design. RSM used in this research permitted to define the green extraction process which gives a highest yield and reduces power extraction at 150 W, solvent proportion of 20% and time extraction of 1–3 min.

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