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RESPONSE SURFACE METHODOLOGY: OPTIMISATION OF ANTIOXIDANT ACTIVITIES FROM *EUCALYPTUS MARGINATA* L. BY PRODUCTS UNDER MACERATION AND ULTRASOUND-ASSISTED EXTRACTION

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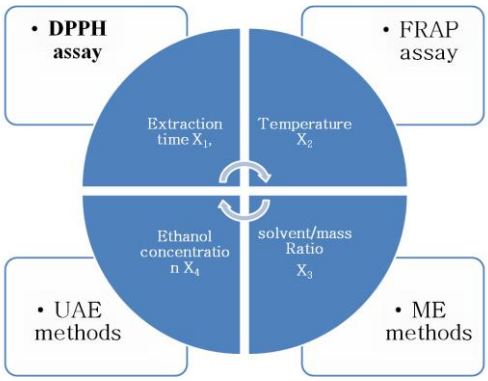
This study was designed to optimize maceration (ME) and ultrasound-assisted extraction (UAE) parameters (extraction time X_1, temperature X_2, liquid-to-solid ratio X_3 and Ethanol concentration X_4) of antioxidant activity of *Eucalyptus marginata* L. leaves' using response surface methodology (RSM). The antioxidant activity was evaluated using the DPPH assay and ferric reducing antioxidant power (FRAP). The optimal conditions for ME and UAE of antioxidant compounds were: X_1 (min) = 88 and 50, X_2 (°C) = ~75 and ~79, X_3 (ml/g) = 40 and 39.5 and X_4 (% of ethanol) = 59.65 and 58.48, respectively. Consequently, these optimized ME and UAE methods have shown a potential application for the efficient extraction of polyphenolic antioxidants from *Eucalyptus marginata* L. by-products in several industries including nutraceutical and pharmaceutical.



INTRODUCTION

In humans, antioxidant mechanisms counteract reactive oxygen species (ROS). These include enzymes, exogenous scavengers such as vitamins (E and D) and polyphenols, which inhibit the propagation of oxidative chain reactions. It is therefore important to find more sources rich in antioxidants and detect the best conditions to extract the best quantity and quality of these natural scavengers. It has been shown that, for example, the extraction conditions, the duration and the solvent *ratio* (volume) can influence the quality and amount of antioxidants. Vegetables, fruits, cereals, spices, and medicinal plants such as *Eucalyptus marginata* Donn ex Sm are natural sources of antioxidants.[1,2] The former are health-promoting and are in demand by the food, cosmetics and pharmaceutical industries because of their antimicrobial, hypoglycaemic, anti

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inflammatory and antitumor properties. Therefore, *Eucalyptus* can be an alternative low-cost source of bioactive compounds, and efficient extraction of important phenols from the plant could feed and enhance many industries.The extraction of phenolic compounds from the Eucalyptus genus has been studied over the last few decades, however extraction technics have been limited to traditional extraction methods such as maceration and Soxhlet. These techniques are time consuming and require large amounts of solvents.[3]

The main aim of the present study is to compare the suitability of two extraction methods, Ultrasound assisted extraction (UAE) and maceration and to determine the optimal conditions that lead to the highest level of phenolics and the best antioxidant activity of *E. marginata* leaves extracts. A new mathematical procedure consisting in the central composite design (CCD) according to the Response Surface Methodology (RSM) was used. The relevant parameters of these green extraction techniques including the the extraction time, temperature, the liquid to solid *ratio* and solvent concentration were then evaluated. Finally, the extracts were characterized in terms of their antioxidant profile.

MATERIALS END METHODS

Plant material and sample preparation

Eucalyptus marginata L. leaves were collected in January 2020, from Souiniet arboreta, from northern eastern region of Tunisia (35°54 N and 8°48 E. 492 m) which covers a total area of 135 ha. A voucher specimen (LGVR 2020) was deposited at the Laboratory of forest resources Management and Valorization, Tunisia. Harvest was performed with pruning shears on various mature trees randomly selected at different heights and the four cardinal points. Transport of samples was performed in ventilated plastic boxes. Subsequently, healthy leaves were air-dried at room temperature and were finely grounded using an electric grinder to obtain a fine powder that was kept in closed containers (vials) until analysis.

Maceration and ultrasound-assisted extraction

Maceration extraction. 2.5 grams of *E. marginata* leaves powders were extracted by maceration according to the experimental design and under continuous agitation.

Ultrasound-assisted extraction. Ultrasound-assisted extraction was performed with an ultrasonic apparatus (BANDELIN HD3200) equipped with a BANDELIN SONPULS Noise protection box LS: frequency, 20 kHz; Temperature monitoring and measurement varied between 0–120 °C; Pulsation: ON cycles 0.2–600 s, OFF cycles 0.3–600 s; Amplitude control: 10–100%. Therefore, power amplitude has been adjusted to be equal to 25% while, a continuous mode has been chosen. The *E. marginata* leaves powders was placed in a Beaker (100 mL) and mixed with an appropriate amount of the extraction solution in order to obtain a liquid-to-solid ratio varying between 15 and 40 mL/g. The extraction time ranged from 10–60 min while, ultrasonic temperature oscillated between 20 to 95 °C and Ethanol concentration = 0–100%. The resulting extracts were evaporated at 35 °C to dryness then stored at 4 °C until use. For both extraction methods, the resulting extract was then filtered through Whatman no. 4 paper and evaporated under vacuum at 40 °C on a rotary evaporator. Each sample was kept in the refrigerator at +4 ° C in closed dark vials.

Box-Wilson central composite design

According to the principle of the Box-Wilson central composite design, liquid-to-solid ratio, ultrasonic time, and ultrasonic power were identified to have strong effects on the response in preliminary one-factor-at-a-time experiments. In addition, a three-factor, 3-level Box-Wilson central composite design was used. A total of 27 experiment runs were generated by design expert software.

The independent variables were respectively, the extraction time (min, X_1), temperature (°C, X_2), liquid-to-solid ratio (ml/g, X_3) and ethanol concentration (%, X_4) while the antioxidant activities, evaluated using DPPH ($Y(DPPH)_{ME}$ and $Y(DPPH)_{UAE}$) and FRAP ($Y(FRAP)_{ME}$ and $Y(FRAP)_{UAE}$) methods, were the reponses (dependants variables). The range of independent variables was chosen based on the result of various initial trials. Here, all the variables including liquid-to-solid ratio, extraction time, and temperature and ethanol concentration were studied at three different levels: low (−1), medium (0), and high (+1). Various formulations to express the antioxidant capacity as function of the independent variables were selected as shown in

Table 2. A second-order polynomial equation has been used to predict the optimum conditions of extraction process and to construct the response surfaces (RSM).

$$Y = b0 + \sum_{i=1}^{n} biXi + \sum_{i=1}^{n-1} \sum_{j=2}^{n} bijXiXj + \sum_{i=1}^{n} biiXi^2$$

where Y is the dependent variable (response: $Y_{DPPH/ME}$; $Y_{DPPH/UAE}$; $Y_{FRAP/ME}$ and $Y_{FRAP/UAE}$), b_0 is the fixed coefficient, bi is the coefficient of linear effect, bij is the coefficient of interaction effect, bii the coefficients of quadratic effect and n is the number of variables.

Table 1

The operating conditions according to an experiment plan

N°	X_1 ME t (min)	X_1UAE t (min)	X_2 T (°C)	X_3 R (mL/g)	X_4 Ethanol (%)
1	30	10	25	20	20
2	30	10	25	20	60
3	30	10	25	40	20
4	30	10	25	40	60
5	30	10	75	20	20
6	30	10	75	20	60
7	30	10	75	40	20
8	30	10	75	40	60
9	90	50	25	20	20
10	90	50	25	20	60
11	90	50	25	40	20
12	90	50	25	40	60
13	90	50	75	20	20
14	90	50	75	20	60
15	90	50	75	40	20
16	90	50	75	40	60
17	60	30	50	30	40
18	60	30	50	30	40
19	60	30	50	30	40
20	17.58	17.2	50	30	40
21	102.42	58.2	50	30	40
22	60	30	14.65	30	40
23	60	30	85.35	30	40
24	60	30	50	15.9	40
25	60	30	50	44.1	40
26	60	30	50	30	16.56
27	60	30	50	30	96.56

Maceration extraction, UAE: Ultrasound-Assisted extraction, t: time expressed in min, T: Temperature expressed in °C, R: Liquid-to-solid ratio expressed in mL/g.

Antioxidant activity

DPPH radical scavenging activity: The free radical-scavenging capacity was measured using the DPPH method described in previous studies. IC_{50} was expressed as microgram per milliliter.[4,5] All tests were carried out in triplicate.

Ferric reducing antioxidant power (FRAP) assay: FRAP assay was estimated following the procedure described by Rigane *et al.*[6] Results are expressed as milligram BHT equivalents (BHTE) per gram of dry weight. All tests were carried out in triplicate.

Table 2

Independent variables used in the Box-Wilson central composite design for the optimization of antioxidant activities of *Eucalyptus marginata* L. Leaves extract

	Factors	Unit	Symbol	Factor levels		
				-1	0	1
	Extraction time	Min	X_1	30	60	90
	Temperature	°C	X_2	25	50	75
ME	***Liquid-to-solid ratio***	mL/g	X_3	20	30	40
	Ethanol	%	X_4	20	40	60
	Extraction time	Min	X_1	10	30	60
UAE	***Temperature***	°C	X_2	20	50	75
	Liquid-to-solid ratio	mL/g	X_3	20	30	40
	Ethanol	%	X_4	20	40	60

ME: Maceration extraction, **UAE**: Ultrasound-assisted extraction.

RESULTS AND DISCUSSION

Preliminary experiments

In the present study and in order to optimize the extraction process to obtain the best antioxidant activity among the various extractions performed, the response surface method was also used in the same way as the method.[7]

Effect of extraction time: Extraction time is one of the most important factors affecting the extraction yield of antioxidant molecules, different extraction times affect the contact of the solvent with the solid,[8] and finally the isolation of polyphenols. In this experiment, different extraction times: 10, 20, 30, 40, 50, 60 min, were evaluated, maintaining the rest of the parameters. Fig. 1A shows that when using maceration as extraction method, 60 min is the most effective time for the best antioxidant potential: in this condition, we noticed that for DPPH assay, IC_{50} = 23.44 µg/mL while $FRAP_{ME}$ = 76.05 mg BHTE/g DW. However, the extraction time is declined for the DPPH when the extraction has been made by UAE. The best antioxidant potential of *E. marginata* leave extracts has been found within only 30 min for DPPH and FRAP assays, respectively (20.18 µg/ml and 120.56 mg BHTE/g DW). Nevertheless, no significant difference was observed between the extraction time for FRAP assay in UAE and maceration. In a previous study, the highest antioxidant potential of *E. robusta* leaf was obtained with UAE under the extraction time (90 min).[9] However, in the present work, ultrasonic cavitation for a longer period (60 min) resulted a decrease in the extract scavenger activity. In this case, the antioxidant activity determined by DPPH assay of the extracts obtained after 60 min of UAE was 37% lower than that of the 30 min extraction showing a 15% reduction for the DPPH assay when the extraction was made by maceration. It has been reported by Tomšik *et al*.[10]; that high ultrasonic potency may lead to low phenolic recovery, probably caused by the degradation of certain sensitive bioactive compounds. Based on these results seems to be the appropriate technique to extract antioxidants from *E. marginata* leaves. UAE has been proposed as a novel green method for phenolic extraction. It is considered to be one of the most efficient, inexpensive and simplest existing extraction systems.[10]

Effect of extraction temperature: As reported in many published papers, extraction temperature has a significant factor to extract biological compounds.[11–13] When following parameters has been fixed: $t_{/ME}$= 60 min, $t_{/UAE}$= 30 min, R= 30 ml/g and ethanol (%) equal to 40, our research team concluded that the antioxidant activities increased significantly (p<0.05) with increasing of extraction temperature from 20 until 50 ° C to reach its maximum at 50 °C (Fig. 1B). However, when the temperature of the two studied methods was more than 90 °C, the antioxidant activities decreased dramatically to not exceed 60 µg/ml and 80 mg BHTE/g DW, for DPPH and FRAP assays, respectively. The obtained results were consistent with those reported previously by Xu and his co-workers at 2016[2] and Dzah *et al*.,[14] who mentioned that extraction at high temperatures increased the rate of phenol oxidation and the sensitivity of polyphenols to temperature influences directly the antiradical activities. In addition, Vázquez *et al.*[15] estimated that the highest and the most suitable temperature to get high phenol content was 50 °C.

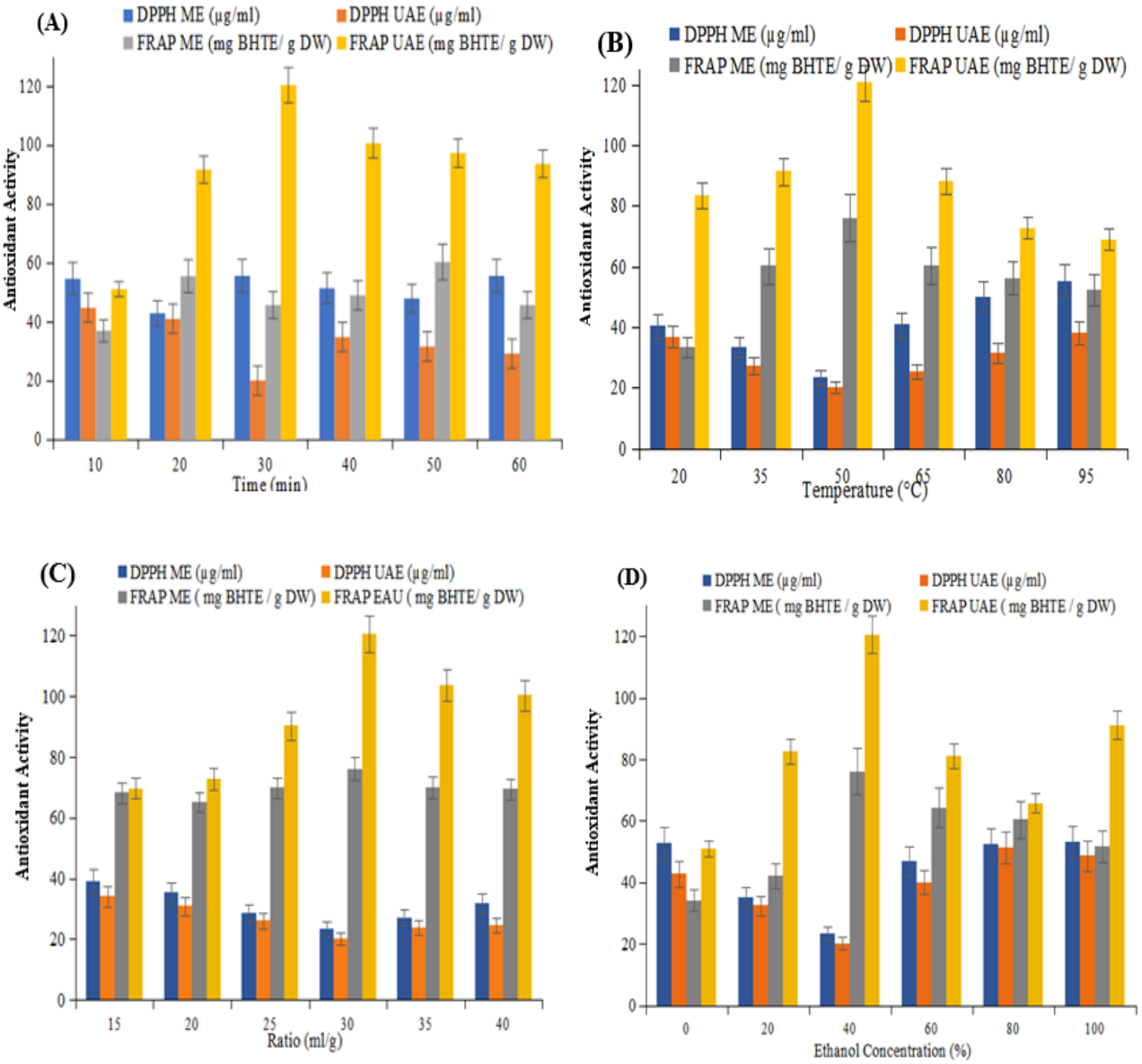

Fig. 1 – Preliminary experiments to select the relevant variables and instrumental parameters to centre their experimental domain previous to the **RSM** application: impact of extraction time (min), temperature (°C), liquid-to-solid ratio (ml/g) and ethanol concentration (%, v/v).

As previously reported by Wang *et al.*[16] and Gaffoor *et al.*[17] the effect of ultrasound is known as acoustic cavitation, which is generated in the solvent by the passage of ultrasonic waves. Ultrasound also offers a mechanical effect allowing greater penetration of solvent into the sample matrix increasing the contact surface area between the solid and the liquid phase to the solvent. On the other hand, Rostango and his co-workers at 2003[18] showed that the increase in the pressure and temperature caused by the compressed leads to the collapse of the bubble. Ultrasound also may produce some chemical effects due to the production of radicals within the cavitation bubbles. In addition, the ultrasonic extraction can be carried out at lower temperature in order to avoid thermal damage to extracts and loss of volatile components during boiling.[19]

Effect of liquid-to-solid ratio: According to Yang *et al.*[20] the liquid-to-solid *ratio* is an important factor in the extraction of antioxidants. Different liquid-to-solid ratios have been tested ranging from 15 to 40 ml/g, while other parameters have been adjusted as follow: Extraction time= 60 and 30 min for ME and UAM, respectively; T = 50 °C and Ethanol (%) = 40. As illustrated in Fig. 1C, for both extraction methods, the highest antioxidant activities have been shown for a liquid-

to-solid ratio equal to 30 ml/g. On the other hand, liquid-to-solid ratio <30 ml/g or >30 ml/g, produced no significant differences in the antioxidant activities measured by DPPH and FRAP assays for both extracted methods (p > 0.05). Therefore, the obtained results were in accordance with those presented by Ince *et al.*[21] who reported that the effect of the studied parameter was not significant, when the liquid-solid ratio was insufficient or excessive.

Effect of ethanol concentration: Most natural antioxidants are easily dissolved in in low-polarity organic solvents such as ethanol, acetone, and methanol with different percentages of water concentration.[4] Albuquerque *et al.* at 2016,[22] mentioned that the solvent could play an important role in the separation of compounds. Therefore, and considering many published results[23,24] our research team have been decided to study the effect of ethanol % on the antioxidant activity of *E. marginata* leaves extracts. Furthermore, ethanol % in water varied between 0 to 100 % while the extraction time for both studied methods was fixed to be 60 and 30 min for ME and UAM, respectively; T = 50 °C and R = 30 ml/g. For both extracted methods, the obtained results are illustrated in Fig. 1D. The antioxidant activities increased with an increasing ethanol % (0–40%). The best suitable ethanol concentration in water was 40 %. Moreover, antioxidant activities decreased significantly ($p<0.05$) when the ethanol % was more than 40 %. As reported previously, ethanol was used as the most extraction solvent for many reasons such as: low price, low toxicity, easiness of recycling, and good polarity to extract the phenolic compounds while water is acting as a plant swelling agent. Moreover, water has a high dielectric constant, which leads to different polarities of the extraction solvent.[25]

Optimization of extraction procedure

Model fitting: The effect of independent variables (extraction time, temperature, liquid-to-solid ratio and ethanol concentration) on the antioxidant activities of *E. marginata* leaves were investigated using the CCD. The DPPH and FRAP were the reponse variables for both methods (ME and UAE). The analysis of Variance ANOVA was used to determine the quadratic model of experiments. On the other hand, the reliability of the model was determined by the coefficients of determination R^2 as well as the adjusted coefficients of determination R^2_{adj} and the lack of the fit of the proposed model where a p-value of more than 0.05 was not significant.[26,27] The R^2 of the model define the correlation between experimental and predicted data, if the difference between R^2 and R^2_{adj} was less, the model is better statistically analyzed.

According to this study and second-order polynomial regression and the coded coefficients were obtained for each response DPPH and FRAP respectively for ME and UAE. In our study, the R^2 were higher than 80% (Table 3), suggesting that the models described well the preference and the behavior of these analysis. Moreover, for all the studied samples, the R^2_{adj} were higher than 0.5, which indicated a best predictive comparison. A model will fit the experimental data when a significant regression and a non-significant have been found (p<0.05). RSM defines not only the effect of independent factors but also their interactions.[13, 28] The model showed to be suitable to well describe the relationship between the responses and the independent variables (X_1, X_2, X_3 and X_4).

Three-dimensional analysis of correlation between parameters: Three-dimensional response surface plots were constructed to study the effects of interactive operational parameters on the anti-free radical activity DPPH and FRAP for the two techniques as it was shown in Figs. 2, 3 and Table 3. The 3D surface was developed to elucidate the interactions between the independent variables. These 3D plots allow better understanding of the main effects of the independent variables on desired responses.

Effects of variables interaction on the $DPPH_{/ME}$ and $DPPH_{/UAE}$: The ANOVA of antioxidant activities for the DPPH assay demonstrated that the response of $Y_{DPPH/ME}$ and $Y_{DPPH/UAE}$ were significantly with a low p-Values with a determination coefficients R^2 equal to 0.929 and 0.970 for ME and UAE respectively (Fig. 2). These results were similar to those reported by Junyusen *et al.*[29] who proved that the ANOVA results of the fitted quadratic model indicated that the model terms were significant. In addition, non-significant difference between the model and the experimental data suggested a satisfactory fit (the high $R^2 = 0.983$ and $R^2_{adj} = 0.961$) which indicated a good agreement between the experimental and predicted data. Fig. 2A illustrate the interaction effect between the extraction time X_1 and temperature X_2 given at 30 ml/g of ratio (X_3) and 40% of ethanol (X_4). The yield of antioxidant

potential was increased with increases in X_1 and X_2 until the maximum was reached. It decreased as X_2 increased which is explained by the degradation polyphenols and some biological products by the high extraction temperature. However, high temperatures along with high extraction times can promote the oxidation of phenolics, diminishing extract antioxidant properties as it was mentioned by Khoddami *et al*.[30] and Fernández-Agulló *et al*.[31]

The Fig. 2B shows the importance of correlation t (X_1) and R (X_3) on the IC_{50} and the levels were slightly decreased as the time increases because an extended time extraction could be attributed to a high temperature which could be caused structure compounds degradations.[11]

On the other hand, from Fig. 2C, we can say that the highest antioxidant activities obtained through DPPH assay was achieved by the correlation X_1X_4 which showed that the increase of ethanol concentration had a significant impact on the antioxidant but an increase in the ethanol concentration results a decrease of antioxidant activity. Our study was similar to that presented by Ezzoubi and his research team[32], who suggested that the UAE-RSM approach was effective in order to increase the extraction of phenolic compounds and consequently the antioxidant activities of the obtained extracts when the optimum conditions were 40% of ethanol concentration and 30 ml/g of liquid-to-solid ratio. Moreover, the X_2X_4 and X_3X_4 had an influence significant which demonstrated that the temperature and the ethanol had a dominant effect on the antioxidant activity (Fig. 2D and 2E).

Effects of variables interaction on the FRAP$_{ME}$ and FRAP$_{UAE}$. The ranges of treatment time, temperature, liquid-to-solid ratio and ethanol concentration have given the highest of extracted biological product such as TPC which present an antioxidant potential.[33,34] Figs. 3A, 3B, and 3C show that correlation between X_1X_2, X_1X_3, and X_1X_4 which had a positive significant difference on the $Y_{FRAP/ME}$ and $Y_{FRAP/UAE}$ while X_2X_3 and X_3X_4 presented a linear non-significant impact on FRAP responses. Indeed, to the ANOVA results the $R^2_{(FRAP/ME)}$ = 0.886 and $R^2_{(FRAP/UAE)}$ = 0.810 which mean that more than 80% were predicted with the mathematical model used which check its validity but R^2_{adj} was equal to 0.587 far from 1, hence this model was not significant enough for FRAP antioxidant activity.

Table 3

ANOVA for the DPPH and FRAP reponse obtained by the two extraction methods

Reponse	***Regression***		***R^2***	***R^2 adj***
	F-Value	***P-Value***		
Y DPPH $_{ME}$ = *23.204 – 4.665 X_1 + 4.233 X_2 – 1.499 X_3 – 0.604 X_4 + 5.918 X_1^2 + 8.999 X_2^2 + 7.174 X_3^2 + 8.237 X_4^2 + 0.026 X_1X_2 + 0.634 X_1X_3 + 1.346 X_2X_3 + 0.109 X_1X_4 – 0.169 X_2X_4 + 0.649 X_3X_4*	*3879.71*	*<0.001*	*0.929*	*0.845*
Y FRAP $_{ME}$ = *78.922 + 6.082 X_1 + 4.048 X_2 + 1.885 X_3 + 1.918 X_4 – 5.681 X_1^2 - 10.213 X_2^2 - 5.606 X_3^2- 8.55 X_4^2 - 0.898 X_1X_2 + 0.363 X_1X_3 – 1.223 X_1X_4 + 0.202 X_2X_3 + 1.096 X_2X_4 + 1.096 X_2X_4 – 0.086 X_3X_4*	4272.24	<0.005	0.886	0.753
Y DPPH UAE = *21.264 – 6.386 X_1 – 0.092 X_2 –2.429 X_3 – 3.491 X_4 + 7.630 X_1^2 + 4.450 X_2^2 + 3.404 X_3^2 + 3.127 X_4^2 + 0.062 X_1X_2 + 0.471 X_1X_3 – 1.178 X_1X_4 – 0.104 X_2X_4 + 1.002 X_2X_4 – 0.834 X_3X_4*	2436.38	< 0.001	0.970	0.936
Y FRAP UAE = *107.326 + 6.146 1 – 5.105 X_2 + 16.506 X_3 + 6.845 X_4 – 30.399 X_1^2 - 8.685 X_2^2 - 6.885 X_3^2 - 4.559 X_4^2 + 0.239 X_1X_2 + 2.134 X_1X_3 + 1.885 X_2X_3 + 0.844 X_1X_4 + 0.217 X_2X_4 + 0.925 X_3X_4*	1938.60	< 0.002	0.810	0.583

X_1: time expressed in min, X_2: Temperature expressed in °C, X_3: Solvent-to-material ratio expressed in ml/g, X_4: Ethanol (%)

Table 4

Optimum extraction conditions and antioxidant activities obtained maceration and ultrasound after optimization

Extraction	**Time (min)**	**Temperature (°C)**	**Ratio (mL/g)**	**Ethanol (%, v/v)**	**DPPH**	**FRAP**
ME	88	74.42	40	59.65	23.204	78.922
UAE	49	78.922	39.5	58.48	21.264	116.901

ME: Maceration extraction, **UA**E: Ultrasound-assisted extraction.
DPPH assay was expressed as µg/ml and **FRAP** assay was expressed as mg BHTE/g DW.

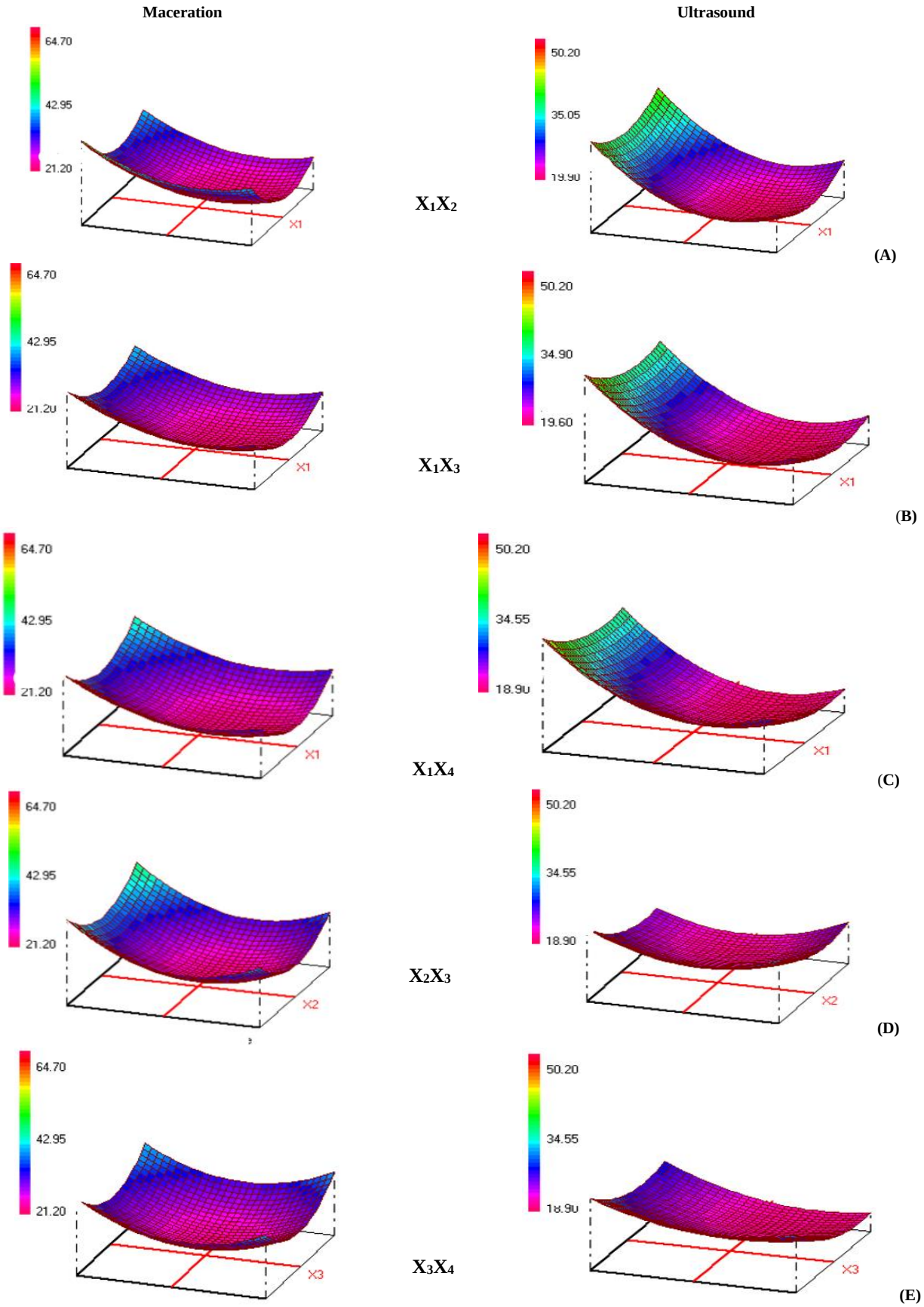

Fig. 2 – Response surface plots (3D) of antioxidant activity content DPPH (µg/mL) at optimum extraction time (min), temperature (°C), liquid-to-solid ratio (ml/g) and ethanol concentration (%, v/v) for ME and UAE.

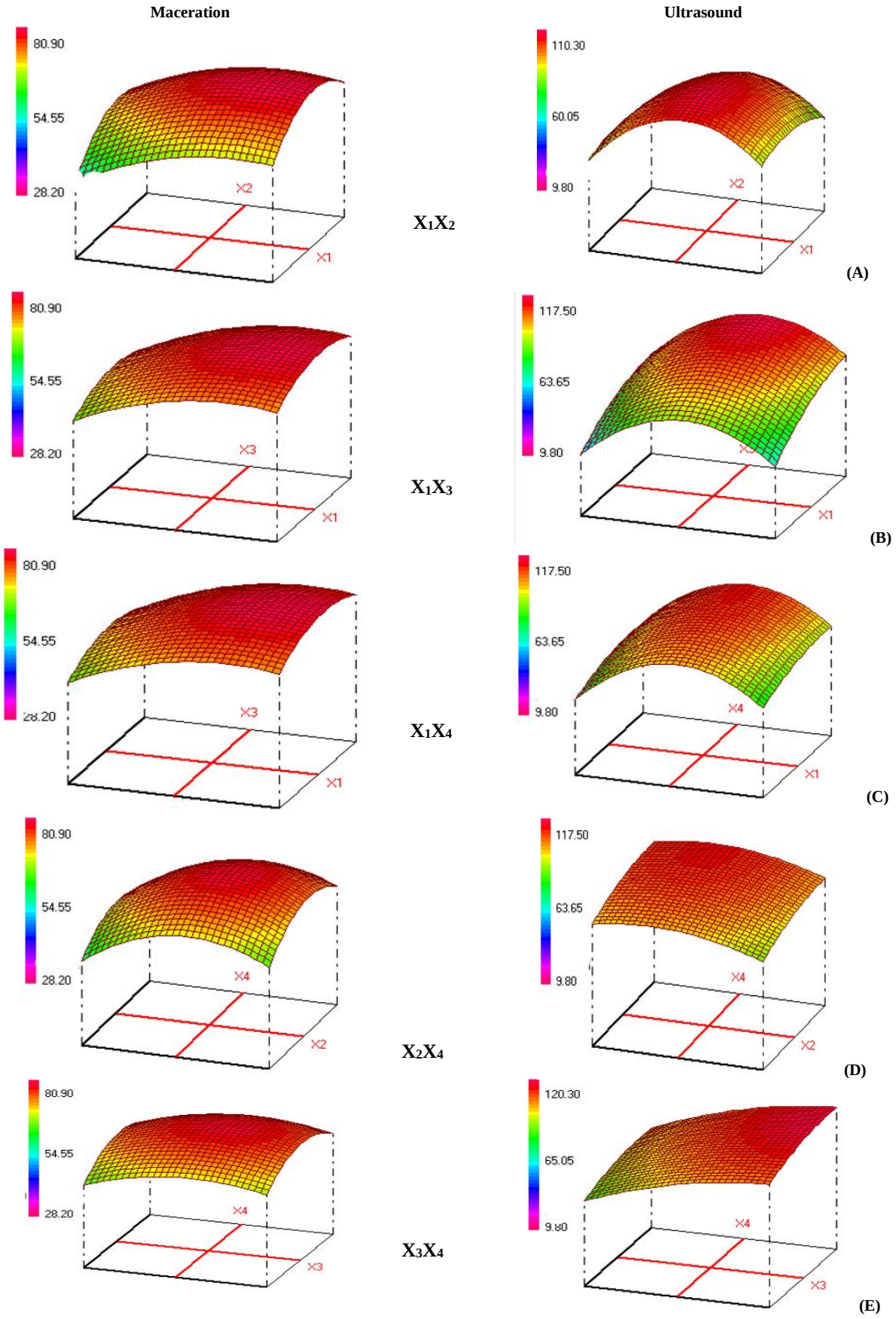

Fig. 3 – Response surface plots (3D) of antioxidant activity content FRAP (mg BHTE/g DW) at optimum extraction time (min), temperature (°C), liquid-to-solid ratio (mL/g) and ethanol concentration (%, v/v) for ME and UAE.

Verification of the model

In this study, the value of antioxidant potential measured using DPPH and FRAP methods of extracts obtained by maceration and ultrasound-assisted extraction under RSM-CCD optimization were equal, respectively 23.204 µg/ml and 21.264 µg/ml and 78.922 mg BHTE and 116.901 mg BHTE/g DW. The last results were close to the experimental values with some difference in the parameters conditions which are explained by the uncertain between the operating conditions and the materials used (Table 4). In addition, our study demonstrated that the mathematical system used here is more significant especially for the IC_{50} obtained by ultrasound (R^2 = 0.970) which provided that 97% were predicted by the model. It is noted that the extraction by ultrasound was much better and more useful than maceration. In fact, the difference on the antioxidant activity between the two assays was too significant and important as the experimental conditions were almost the same as indicated in Table 4. These results were explained by the economic effect, which is based on the reduction of the extraction time, also in order to avoid the enormous use of solvents and this taking into account the principles of green chemistry.

CONCLUSIONS

In the present study, the conditions for enhanced extraction of antioxidant compounds from *Eucalyptus marginata* L. leaves by ME and UAE were optimized using a Central Composite Design based on the response surface methodology (RSM-CCD). Based on the single-factor-test, Central Composite Design was used to evaluate and optimize the extraction variables (extraction time, temperature, liquid-to-solid ratio and ethanol concentration) for the antioxidant activities. These results indicated that the UAE-RSM approach using the following parameters: extraction time of 49 min, temperature of about 79 °C, liquid-to-solid ratio of 39.5 ml/g and ethanol concentration equal to 58.48% was effective for maximizing the antioxidant compounds extraction. The information gained from this study could be useful for the further exploitation and application of the materials containing phenolic compounds.

LIST OF ABBREVIATIONS

ME : Maceration extraction; ***UAE***: Ultrasound-assisted extraction; X_1: Extraction time; X_2: Temperature; X_3: Liquid-to-solid ratio; X_4: Ethanol concentration; ***RSM***: Reponse Surface Methodology; ***DPPH***: 2,2-diphenyl-1-picrylhydrazyl; ***FRAP***: Ferric reducing antioxidant power; ***ROS***: Reactive oxygen species; ***CCD***: Central composite design; IC_{50}: Inhibition capacity; ***BHT***: Butyl hydroxy toluene; ***DW***: Dry Weight; ***ANOVA***: Analysis of variance; R^2: Coefficient of determination; R^2adj: Adjusted coefficient; ***P-Value***: Lack of fit; ***3D***: Three dimensional analysis of correlation

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REFERENCES

1. Taxonomic-references: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=502477#null. Taxonomic Serial No: 502477
2. D. P. Xu, J. Zheng, Y. Zhou, Y. Li, S. Li and H. B. Li, *Int. J. Molec. Sci.*, **2016**, *17*, 16-64.
3. B. Gullón, P. Gullón, T. A. Lú-Chau, M. T. Moreira, J. M. Lema and G. Eibes, *Ind. Crops Prod*., **2017**, *108*, 649–659.
4. O. Khedher, G. Rigane, R. Ben Salem and Y. Moussaoui, *Chem. Africa*, **2020**, *3*, 813–820, https://doi.org/10.1007/s42250-020-00170-3.
5. M. Ben Hmed, G. Rigane, R. Ben Salem, N. Zouari and S. Cherif, *Rev. Roum. Chim.*, **2020**, *65*, 173–178. https://DOI: 10.33224/rrch.2020.65.2.06.
6. G. Rigane, S. Mnif and R. B. Salem, *Rev. Roum. Chim.*, **2018**, *63*, 5–10.
7. I. A. Mohamed Ahmed, F. Al-Juhaimi, A. R. Adisa, O. Q. Adiamo, E. E. Babiker, M. A. Osman and M. A. Elkareem, *J. Food Sci. Technol*., **2020**, *57*, 3071–3080.
8. H. Majid and F. V. Silva, *Food and Bioproducts Processing*, **2020**, *124*, 445–454.
9. D. J. Bhuyan, Q. V. Vuong, A. C. Chalmers, I. A. van Altena, M. C. Bowyer and C. J. Scarlett, *Sep. Sci. Technol.*, **2017**, *52*, 100–112.
10. A. Tomšik, B. Pavlić, J. Vladić, M. Ramić, J. Brindza and S. Vidović, *Ultrasonics sonochemistry*, **2016**, *29*, 502–511.
11. Z. S. Zhang, L. J. Wang, D. Li, S. S. Jiao, X. D. Chen and Z. H. Mao, *Separation and Purification Technology*, **2008**, *62*, 192–198.
12. G. Zhang, Y. Chen, K. Tariq, Z. An, S. Wang, F. Q. Memon and H. Si, *Czech J. Food Sci*., **2020**, *38*, 115–122.
13. A. Ciric, B. Krajnc, D. Heath, N. Ogrinc, *Food and Chem. Toxicol.*, **2020**, *135*, 110-976.
14. C. S. Dzah, Y. Duan, H. Zhang, C. Wen, J. Zhang, G. Chen and H. Ma, *Food Biosc*., **2020**, *35*, 100–547. https://doi.org/10.1016/j.fbio.2020.100547.
15. M. B. Vázquez, L. R. Comini, R. E. Martini, S. N. Montoya, S. Bottini, J. L. Cabrera, *Ultrasonics Sonochemistry*, **2014**, *21*, 478–484.
16. J. Wang, B. Sun, Y. Cao, Y. Tian and X. Li, *Food Chem.*, **2008**, *106*, 804–810.
17. K. Ghafoor, Y. H. Choi, J. Y. Jeon and H. Jo, *J. Agricultural and Food Chem.*, **2009**, *57*, 4988–4994.

18. M. A. Rostagno, M. Palma and C. G. Barroso, *J. Chromatography A*, **2003**, *1012*, 119–128.
19. J. Wu, L. Lin and F. T. Chau, *Ultrasonics sonochemistry*, **2001**, *8*, 347–352.
20. L. Yang, Y. L. Cao, J. G. Jiang, Q. S. Lin, J. Chen and L. Zhu, *J. Separation Sci.*, **2010**, *33*, 1349–1355.
21. A. Ince, S. Şahin and S. G. Şümnü, *Turkish J. Agriculture and Forestry*, **2013**, *37*, 69–75.
22. B. R. Albuquerque, M. A. Prieto, M. F. Barreiro, A. Rodrigues, T. P. Curran, L. Barros, I. C. F. R Ferreira, Ind. Crops and Products, **2017**, *95*, 404–415.
23. G. Rigane, S. B. Younes, H. Ghazghazi and R. B. Salem, *Int. Food Research J.*, **2013**, *20*, 30–01.
24. M. Boukhris, M. S. Simmonds, S. Sayadi and M. Bouaziz, *Phytotherapy research*, **2017**, *27*, 1206–1213.
25. Y. Zhao, Y. Hou, G. Tang, E. Cai, S. Liu, H. Yang and S. Wang, *J. Anal. Methods in Chem.***, 2014,** Article ID 864654, https://doi.org/10.1155/2014/864654.
26. D. M. Neiva, J. Gominho and H. Pereira, *BioResources*, **2014**, *9*, 2907–2921.
27. N. W. Ismail-Suhaimy, S. S. A. Gani, U. H. Zaidan, M. I. E. Halmi and P. Bawon, *Plants*, **2021**, *10*, 682–686.
28. J. L. Pilkington, C. Preston and R. L. Gomes, *Ind. crops and products*, **2014**, *58*, 15–24.
29. T. Junyusen, P. Liplap and P. Junyusen, *Agriculture and Natural Resources*, **2020,** *54*, 309–316.
30. A. Khoddami, M. A. Wilkes and T. H. Roberts, *Molecules*, **2013**, *18*, 2328–2375.
31. A. Fernández-Agulló, M. S. Freire, C. Ramírez-López, J. Fernández-Moya and J. González-Álvarez, *Biomass Conversion and Biorefinery*, **2021**, *11*, 609–618.
32. Y. Ezzoubi, M. Fadil, D. Bousta, A. El Ouali Lalami, M. Lachkar and A. Farah, *J. Chem.*, **2021,** https://doi.org/10.1155/2021/8830902.
33. A. Ghasemzadeh and H. Z. Jaafar, *The Sci. World J.*, **2014**, https://doi.org/10.1155/2014/523120.
34. A. A. Kamarudin, N. M. Esa, N. Saad, N. H. Sayuti and N. A. A. Razak, *Ind. Crops and Prod.*, **2020**, *144*, 112–064. https://doi.org/10.1016/j.indcrop.2019.112064.