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ORIGINAL PAPER

LC–ESI–MS/MS analysis, biological effects of phenolic compounds extracted by microwave method from Algerian *Zizyphus lotus* fruits

Farida Berkani[1] · Farid Dahmoune[2,3] · Nabil Kadri[2,3] · Maria Luísa Serralheiro[4,5] · Asma Ressaissi[4] · Amina Abbou[1] · Mouna Kaci[6] · Smail Meziane[6] · Sabiha Achat[3] · Nourelimane Benzitoune[1] · Meriem Adouane[1] · Khodir Madani[7] · Lotfi Mouni[1]

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Abstract

Jujube fruit is a considerable source of antioxidants that could be used as ingredients against several diseases. The optimization of microwave-assisted extraction (MAE) of bioactive compounds from *Z. lotus* pulp and peel (*Zlp*) was achieved using response surface methodology. The effect of the extraction parameters (microwave power "MW", extraction time, ethanol/water solvent and liquid–solid ratio) on the total phenolic compounds (TPC) was well described by second-degree regression model. The liquid chromatography-high resolution tandem mass spectrometry (LC–MS–MS) was done. Best results were obtained at 600 W MW power, 180 s irradiation time, 51% ethanol concentration and 47:1 mL/g solvent-to solid ratio obtaining 7473.38 ± 740.55 mg GAE/100 g of TPC, 1019.96 ± 75.03 mg CE/100 g of TFC, 14,253.11 ± 2453.86 mg CE/100 g of TTC. LC–MS–MS determined that from all phytochemicals, 44.51% of the extract was flavonoids which represent 80.75% of all secondary metabolites from which 5-hydroxy-7-*O*-nerylflavanone is found to be the most abundant one. The extracts revealed the presence of 34 bioactive compounds, from which 10 phenolic compounds have never been previously identified from *Zizyphus* plant. The *Zlp* extract exhibited a greatest antioxidant effects by PAOT, DPPH, and FRAP activity; as well as, a lowest cytotoxic effects against both HepG2 and MCF-7 cells. In a concentration of 1 mg/mL, *Zlp* produced a strong AChE inhibition. This research revealed that MAE is more rapid and an effective method to extract TPC recovery which can be used in food matrixes and/or in nutraceutical formulations for its good biological effect.

Keywords *Zizyphus lotus* · Bioactive compounds · Microwave assisted extraction · RSM optimization · Biological activities

Introduction

Reactive oxygen species (ROS) play an important role in progression of degenerative diseases such as cancer, Alzheimer, inflammation, autoimmune, and cardiovascular diseases [1, 2]. This seems to be attributed to the strong oxidative stress, caused by unbalance between antioxidants and oxidants, including ROS [3, 4]. The antioxidant nutrients are considered a sophisticated system developed by our organism against oxidative stress, thus reducing the risk of chronic

✉ Farida Berkani
berkanifarida95@gmail.com

1 Laboratoire de Gestion et Valorisation des Ressources Naturelles et Assurance Qualité, Faculté des Sciences de la Nature et de la vie et des Sciences de la Terre, Université de Bouira, 10000 Bouira, Algeria

2 Département de Biologie, Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre, Université de Bouira, 10000 Bouira, Algeria

3 Laboratoire de Biomathématiques, Biophysique, Biochimie, et Scientométrie (L3BS), Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaïa, Algeria

4 Faculty of Sciences, BioISI—Biosystems & Integrative Sciences Institute, University of Lisbon, 1649-004 Lisbon, Portugal

5 Departamento de Química e Bioquímica, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal

6 Institut Européen des Antioxydants, University of Nancy, 18 rue Victor de Lespinats, 54230 Neuves-Maisons, France

7 Centre de Recherche en Technologies Agro-alimentaires, Route de Targua Ouzemmour, 06000 Béjaia, Algérie

diseases [5]. In most food industries, synthetic antioxidants have been used as a solution against ROS that causes lipid peroxidation resulting in product alterations (color and flavor) [6]. Propyl gallate (PG), butylatedhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and butylatedhydroxyanisole (BHA) are the main food antioxidants (Biglari etc.). However, the uses of these synthetic antioxidants have been substituted by natural antioxidants due to their toxic and tumorigenic effects [7]. Beside, medicinal plants are well known as veritable source of antioxidants thanks to phenolic compounds that preserve the quality of products and inhibit oxidative mechanism. More recently, an increasing demand on the uses of safe antioxidants obtained from plant and food by-products as additives and health-promoting ingredients in food, pharmaceuticals, and cosmetics have been mentioned [3, 8, 9].

Zizyphus is a fruit tree, which belongs to the family of Rhamnaceas. This tree is originated from tropical regions such as Asia and America, in Europe and also in all the North of Africa as Algeria. Furthermore, the forms of this tree vary with the soil and climate. Since millennia, the consumption of its fruits is very vast [4]. The edible fruit of jujube (*Zizyphus lotus* L.) is present in abundance in the Mediterranean region (Morocco, Algeria and Tunisia) with various traditional therapeutic use [10]. It is known in some regions of Algeria as '*Sedra*'. In others, It's called '*Nbag*' [4, 11]. More recently, the use of certain traditional medicines as drugs has increased very significantly and considered as a natural alternative to synthetic drugs. Aromatic plant products have played an important part in human culture and folklore medicine [12]. In addition, several scientific studies have shown the state of many biological properties from this plant and its constituents through in vitro and in vivo studies, jujube has been a dietary food, it appears in the list A of the medicinal plants of French pharmacopeia [13]. *Zizyphus lotus* L. fruits (*ZLp*) is a famous Algerian species used in several traditional treatments for its antioxidant, anti-inflammatory, anti-diabetic and hypoglycemic activities. The different part from this medicinal plant offers a variety of specific therapeutic properties (root, leaf, stem bark and fruit) due to its diversity of bioactive compounds present in the plant, especially polyphenols that are known by neutralization of free radicals with a significant antioxidant capacity [4, 14, 15]. Hence it is more interesting to see the compounds responsible for this biological activity extracted from jujubes.

Bioactive compounds extraction from *ZLp* has been investigated in the last decades focusing mainly on conventional solvent extraction (CSE). Alternatively, modern techniques such as microwave-assisted extraction (MAE), the use of supercritical fluid (SFE), accelerated solvent extraction (ASE) and ultrasound assisted extraction (UAE) have been widely used to promote extraction in terms of reducing energy and time with obtaining a higher bioactivity which is not the case for traditional techniques that are found to consume energy, time, solvent and participate to the degradation of bioactive compounds [3, 4, 16–18]. Among these techniques, UAE seems an efficient technique in term of profitability and economic in relation to the shortened time and to the power reduced of the extraction, thanks to its physiochemical effect of absorption assigned to the cavitation phenomenon induced with the ultrasound waves in the extraction medium [19]. On the other hand, MAE is a new method based on the heating of the sample matrix which can accelerate the vaporization of solvent, raising the intracellular pressure and causing the rupture of the cell walls to release the bioactive compounds [20]. The use of microwaves for extracting plant constituents has been found to improve three times the yield of total phenols extracted compared to other extraction methods. This green technology known for its extraction efficiency is still in their infancy [17, 21, 22]. As a result, alongside the majority of previous research focused on various bioactive compounds found in this plant, mainly polyphenols, polysaccharides, proteins and lipids. The ecofriendly-extraction of polyphenols from jujube by green technology based on MAE could be one of the solutions deemed to be a promoter for the valorization of vegetable plants for the protection of the environment and the consumer [22]. This green extraction methods applied to plants will meet the challenges of the twenty-first century on recovering bioactive compounds without spoiling the environment through the use of organic chemical solvents.

To the best of our knowledge, no study has been reported on the effect of microwaves and ultrasound in the extraction of phenolic compounds from *Zlp* comparatively to the conventional organic solvent process. This present work is motivated by the increased need of the agro-food industries to develop the production of new functional and nutraceutical ingredients by optimizing the extraction conditions, as well as the increased consumers demand for natural food products with important nutritional values. In addition, there is no research available in the literature concerning the identification of phenolic profile from *Zlp*. Therefore, the present work deals with (i) the determination of optimum MAE by RSM using as factors: irradiation time, ethanol concentration, solvent-solid ratio in terms of the TPC recovery, (ii) identification of the compounds present in *Zlp* by liquid chromatography high resolution mass spectrometry (LC-HRMS/MS) and (iii) evaluation of some biological activities of the optimum extract.

Materials and methods

Raw material

The fruits material of *Zlp* were collected from Djelfa, Algeria (34° 40′ 30″ N, 3° 15′ 30″ E). The pulp and peels were

dried at 40 °C for 24 h in a ventilated oven (Memmert, Modell 100–800, Schwabach, Germany) and ground by an electrical grinder (WH model 8100 Basic, Beijing, China) and the powder was passed through a standard sieve, and only the fraction with particle size < 125 µm was used. The powder was stored in closed airtight bottles and kept in the dark conditions (4 °C) until analysis.

Experimental work

Before MAE optimization, a preliminary study based on single-factor experiments was performed to evaluate the influences of process parameters (Table 1). When one variable was not studied, it was kept constant. The experimental level was defined according to the preliminary experiment results. Each factor was evaluated at different levels within the following intervals: 300–700 W, 30–300 s and 10–70% for microwave power, extraction time and ethanol concentration respectively. However, pH and particle size were fixed in all experiments at 4 and 125 µm respectively.

Based on the experimental results of the preliminary single-factor study, major significant influence factors were selected. Then, a RSM based on a central composite design (CCD) for MAE was conducted to optimize the extraction processes (Tables 2). Regression analysis of the data to fit a second-order polynomial equation was carried out according to the following general equation (Eq. 1) which was, then, used to predict the optimum conditions of the extraction process.

$$Y = B_0 + \sum_{i=1}^{k} BiXi + \sum_{i=1}^{k} BiiX^2 + \sum_{i>1}^{k} BiiXiXj + E, \quad (1)$$

where Y represents the response function (in this case, the TPC); B_0 is a constant coefficient; *Bi*, *Bii*, and *Bij* represent linear, quadratic, or interaction regression coefficients,

Table 1 Central composite design with the observed responses for phenolic compounds yield from *Zlp* using microwave-assisted extraction (MAE)

Run	X_1— Irradiation time (s)	X_2— Microwave power (W)	X_3— Solvent-to solid ratio (mL/g)	X_4— Ethanol concentration (% v/v)	TPC yield (mg GAE/100 g DM)	
					Experimental	Predicted
1	180 (+1)	300 (− 1)	50 (+1)	30 (− 1)	7094.41	7054.59
2	120 (− 1)	700 (+1)	20 (− 1)	30 (− 1)	5816.37	5914.58
3	150 (0)	700 (+1)	35 (0)	50 (0)	8215.96	7654.08
4	120 (− 1)	300 (− 1)	20 (− 1)	70 (+1)	3896.71	3918.11
5	120 (− 1)	700 (+1)	50 (+1)	30 (− 1)	7198.74	7410.62
6	120 (− 1)	700 (+1)	50 (+1)	70 (+1)	7355.24	7498.36
7	180 (+1)	700 (+1)	50 (+1)	70 (+1)	7094.41	7059.09
8	120 (− 1)	700 (+1)	20 (− 1)	70 (+1)	4267.08	4158.29
9	120 (− 1)	300 (− 1)	50 (+1)	70 (+1)	6729.26	6636.12
10	120 (− 1)	300 (− 1)	20 (− 1)	30 (− 1)	5252.99	5139.71
11	120 (− 1)	300 (− 1)	50 (+1)	30 (− 1)	6168.49	6013.69
12	150 (0)	500 (0)	50 (+1)	50 (0)	7381.32	7294.14
13	180 (+1)	300 (− 1)	50 (+1)	70 (+1)	7003.12	6992.36
14	120 (− 1)	500 (0)	35 (0)	50 (0)	6846.63	6842.05
15	150 (0)	500 (0)	20 (− 1)	50 (0)	4689.61	5021.49
16	180 (+1)	500 (0)	35 (0)	50 (0)	6727.96	6977.24
17	180 (+1)	700 (+1)	20 (− 1)	30 (− 1)	5884.19	5828.72
18	150 (0)	500 (0)	35 (0)	50 (0)	6965.31	7114.52
19	150 (0)	500 (0)	35 (0)	50 (0)	6919.66	7114.52
20	150 (0)	500 (0)	35 (0)	70 (+1)	6079.81	6281.56
21	150 (0)	500 (0)	35 (0)	30 (− 1)	7147.88	7190.83
22	150 (0)	300 (− 1)	35 (0)	50 (0)	6426.70	7233.28
23	180 (+1)	700 (+1)	20 (− 1)	70 (+1)	3145.53	3387.77
24	150 (0)	500 (0)	35 (0)	50 (0)	8192.69	7114.52
25	180 (+1)	300 (− 1)	20 (− 1)	70 (+1)	4303.59	3943.11
26	180 (+1)	300 (− 1)	20 (− 1)	30 (− 1)	5905.05	5849.37
27	180 (+1)	700 (+1)	50 (+1)	30 (− 1)	7589.98	7656.01

TPC total phenolic content, *GAE* gallic acid equivalent

Table 2 Estimated regression coefficients for the quadratic polynomial model and the analysis of variance (ANOVA) for the experimental results of TPC from *Zlp*

Parameter	DF	Estimated coefficients	Sum of squares	F-value	Prob > F
		7114.5237	41,407,285	12.9234	< 0.0001*
X_1	1	67.59694	82,248	0.3594	0.5600
X_2	1	210.39819	796,813	3.4817	0.0867
X_3	1	1136.3241	23,242,185	101.5561	< 0.0001*
X_4	1	− 454.6311	3,720,410	16.2562	0.0017*
Quadratic					
X_1^2	1	− 204.8755	107,933	0.4716	0.5053
X_2^2	1	329.16208	278,608	1.2174	0.2915
X_3^2	1	− 956.7013	2,353,570	10.2839	0.0075*
X_4^2	1	− 378.3236	368,045	1.6082	0.2288
Interaction					
X_1X_2	1	− 198.8785	632,842	2.7652	0.1222
X_1X_3	1	82.811685	109,724	0.4794	0.5019
X_1X_4	1	− 171.1659	468,764	2.0483	0.1779
X_2X_3	1	155.51643	386,966	1.6908	0.2179
X_2X_4	1	−133.6724	285,893	1.2492	0.2856
X_3X_4	1	461.0678	3,400,436	14.8581	0.0023*
Lack of fit	10		1,703,270.2	0.3266	0.9082
Pure error	2		1,043,056.2		
Total error	12		2,746,326.3		
R^2				0.937801	
r^2 adjusted				0.865235	
CV%		7.58			
Corr.Total	26		44,153,611		

X_1: Irradiation time

X_2: Microwave power

X_3: Solvent-to-solid ratio

X_4: Ethanol concentration

*means that the concerned factor is significant

respectively. *Xi* and *Xj* represent the coded independent variables.

In order to define the interaction of the independent variables and their effects, the 3D response surface plots have been used from the mathematical equation of fitted the polynomial model. The factor levels were coded as − 1 (low), 0 (central point or middle) and 1 (high), respectively. Analysis of variance was performed for the response variable (TPC recovery) using the full model where *p*-values (distributed into linear and interaction factors) indicated whether the terms were significant or not. To control the appropriateness of the proposed model from RSM and giving the MAE optimal conditions, a comparison with the experimental results was established. The efficiency of the MAE method (optimum conditions) was compared with UAE and CSE based in respect to TPC recovery (response) and biological activities.

Microwave-assisted extraction (MAE)

The TPC was extracted from *Zlp* powders using a modified microwave oven apparatus (2450 kHz, Samsung Model, Malaysia). This microwave was equipped with a numerical digital control system for power adaptable (from 100 to 900 W) and irradiation time. One gram of plant material powder was extracted into 500 mL flask containing aqueous ethanol solvent. Different conditions of extraction were occurred by varying the four variables or parameters studied as ethanol concentration (30–70%), irradiation time (120–180 s), microwave power (300–700 W) and solid-to-solvent ratio (1:20–1:50 g/mL) (Table 1). After irradiation, the flask was taken out and cooled in an ice water bath (4 °C). The mixtures were filtered by Whatman No.1 paper and collected in a volumetric flask than centrifuged at 1000×*g* for 5 min. The extract was stored at 4 °C until use. Each trial was carried out in triplicate.

UAE and CSE methods

The extraction of TPC from *Zlp* was carried out in an ultrasonic bath UAE (J P.SELECTA 195 W 50/60HZ, Spain) according to the method optimized by Hammi et al. [23] and by conventional solvent extraction by the method described by Dahmoune et al. [8].

Analytical determinations

Total phenolic content (TPC)

Total phenolic content in *Zlp* was determined according to the Folin–Ciocalteu method of Hammi et al. [23]. A calibration curve was established to calculate the TPC concentration using gallic acid as standard and the results were expressed as mg of gallic acid equivalent per 100 g of dry matter (mg GAE/100 g DM).

Total flavonoids content (TFC)

The total flavonoid content was determined according to the Ghafar et al. [24] method. The TFC results were expressed as mg quercetin equivalents per 100 g of dry matter (mg QE/100 g DM). Samples were measured in triplicate.

Total condensed tannins content (TTC)

The total condensed tannins content was determined using the method of Berkani et al. [3], based on the condensation of phenolic compounds with vanillin in acid medium. The TTC was expressed as mg catechin equivalent per 100 g of dry matter (mg CE/100 g DM). Samples were measured in triplicate.

Compound identification by liquid chromatography–high resolution tandem mass spectrometry (LC–HRMS/MS)

The identification of phenolic compounds from *Zlp* was performed by means of liquid chromatography-high resolution tandem mass spectrometry (LC–MS–MS), as described by Guedes et al. [25].

Biological activities

Total antioxidant power by PAOT technology The total antioxidant activity of *Zlp* was determined according to Kaci et al. [26] by using PAOT (Pouvoir AntiOxydant Total) Liquid® technology (WO 2020/109736A1). Results were expressed as PAOT Score per gram of extract (PAOT Score/g) [27], according to the following formula:

$$\text{Antioxidant activity} = \left(\frac{EP_{product10} - EP_{control0}}{EP_{control0}}\right) \times 100, \quad (2)$$

where $EP_{control0}$ is the electrochemical potential at time 0. $EP_{product10}$ is the electrochemical potential obtained after 10 min registration in presence of tested antioxidants or studied supplements samples.

Scavenging activity against DPPH· radical The free radical-scavenging ability of *Zlp* was expressed as the percentage of inhibition of DPPH· radical. The free radical-scavenging activity was measured according to the colorimetric method used by Achat et al. [28] where the % scavenging activity was determined according to Eq. 3.

$$\%\ \text{Scavenging activity} = \left(\frac{A_{control} - A_{extract}}{A_{control}}\right) \times 100, \quad (3)$$

where $A_{control}$ is the absorbance of DPPH and solvent. $A_{extract}$ is the absorbance of DPPH and sample extract. Trolox and BHA were used as positive controls

The effective sample concentration required to inhibit 50% of the DPPH radical (EC_{50} value) is obtained by linear regression analysis of the dose–response curve plot between% inhibition and concentrations.

Ferric-reducing antioxidant power (FRAP) activity The ability of the extracts tested to reduce ferric iron (Fe^{3+}) present in potassium ferricyanide to ferrous iron (Fe^{2+}) in presence of an antioxidant that has the power to give out electrons, was determined by Hammi et al. [29]. A calibration curve was plotted using gallic acid as standard and the results are expressed in terms of antioxidants having an iron reduction capacity equivalent to that of equivalents in gallic acid of 1 mg per 100 g of dry matter (mg GAE/100 g DM).

Anticholinesterase activity Anticholinesterase activity (AChE) was determined using AChE assay, an enzyme localized in the neurosynaptic gaps and neuromuscular junctions, used in the treatment of Alzheimer disease according to Berkani et al. (2021). All tests were done in triplicate and the percentage inhibition was calculated as:

$$I\,(\%) = 100 - \left(\frac{Vsample}{Vcontrol}\right) \times 100, \quad (4)$$

where I is the percent inhibition of acetylcholinesterase. Vsample: the initial velocity of the jujube extract containing reaction and Vcontrol: the initial velocity of the control reaction.

Anti-proliferative activities on HepG2 and MCF-7 cells The MTT viability test against the human tumor cell lines HepG2 (ATCC#HB-8065), from human hepatocellular liver carcinoma cell lines, and MCF-7 HTB-22), from breast cancer were used for evaluating *Zlp* cytotoxicity according to the method described by Berkani et al. (2020b). At different concentrations of extract, the assays were done in 8 × 12 replicates and the cell viability percentage was calculated by the following equation:

$$Viability\,(\%) = \left[\frac{(Abs\,595 - Abs\,630\,of\,experimental\,wells)}{(Abs\,595 - Abs\,630\,of\,control\,wells)}\right] \times 100 \quad (5)$$

Statistical analysis

Each extraction trial and all the analyses were carried out in triplicate and all the data in this paper have been reported as means ± S.D. Influence of each factor on the TPC yields in the single-factor experiment for the MAE was statistically assessed by ANOVA and Tukey's post hoc test with a 95% confidence level. Data obtained from the CCD trials for the MAE were statistically analyzed using ANOVA for the response variable in order to test the model significance and suitability. The $p < 0.05$ and $p < 0.01$ were taken as significant and highly significant level, respectively. The JMP (Version 10.0, SAS) software was used for the analysis of all experimental results and the construction of CCD. Component Analysis (PCA) was applied to show the possible correlation between phenolic compounds extracted by different methods and antioxidant activity.

Results and discussion

Optimization of MAE conditions

Modeling and fitting the model using response surface methodology

Based on the preliminary study (data not shown), the effects of extraction parameters such as nature of solvent, microwave power, extraction time, concentration of solvent and solvent-to-solid ratio were systematically were chosen. Then, the extraction efficiency of the microwave process was estimated by measuring bioactive compounds. The experimental design matrix and the response *Zlp* extracts obtained in the trials of the CCD from 27 experiments are presented in Table 1. The regression coefficients were determined using least squares as a technique of individual linear, quadratic and model interaction [30]. As shown in the Table 2, for the TPC response, ethanol concentration and solvent-to-solid ratio have significant linear effects ($p < 0.05$), while no significant effect of irradiation time (X_1) was found. However, TPC shows significant quadratic effects for the solvent-to-solid ratio (X_3^2) with a negative effect. On the other hand, the irradiation power (X_2^2) showed no significant effect on TPC yields. Regarding the effect of interaction between the variables, a significant effect was observed between the solvent-to-solid ratio (X_3) with ethanol concentration (X_4) (Table 2). Three second order polynomial models were fitted to generated data to describe the significance and adequacy of the model and the empirical relationship between the TPC and operational conditions. The below predictive equations in term of significant independent variables were obtained (Eq. 4). Table 2 reports the statistical analysis of the regression model.

$$Y(TPC) = 7114.52 + 1136.32X_3 - 454.63X_4 - 956.7013X_3^2 + 461X_3X_4 \quad (6)$$

Very high F-value and a very low p-value ($p < 0.0001$), indicating that these models were highly significant and it can be used to optimize the extraction variables (Table 2). In addition, the determination coefficients for TPC yields was for $R^2 = 0.93$ and Adj. $R^2 = 0.86$, were reasonably close to 1, with low value of CV (< 10%) (Table 2). This indicates a high degree of correlation between the observed and predicted values, which implied that only 3, 6 and 1% respectively of these variations could not be justified by the models. Also, other parameters were insignificants as the lack of fit ($p > 0.05$) for the TPC response which provide also the validity of the experimental model. The coefficient of variation (CV) was within the acceptable range (˂ 10%) [31, 32]. This indicates that variation in the mean value is small and satisfactorily develops an adequate response model.

Analysis of response surfaces

The response surfaces of regression models were plotted by their three-dimensional profile in order to study the effects of interaction between independent variables on the TPC extraction yields (Fig. 1A–F). According to Hayat et al. [33], maintaining two independent variables at their zero levels and using the z-axis against two independent variables, a response can be drawn. The surface response (3D) is considered as the diagram permitting to represent the regression equation.

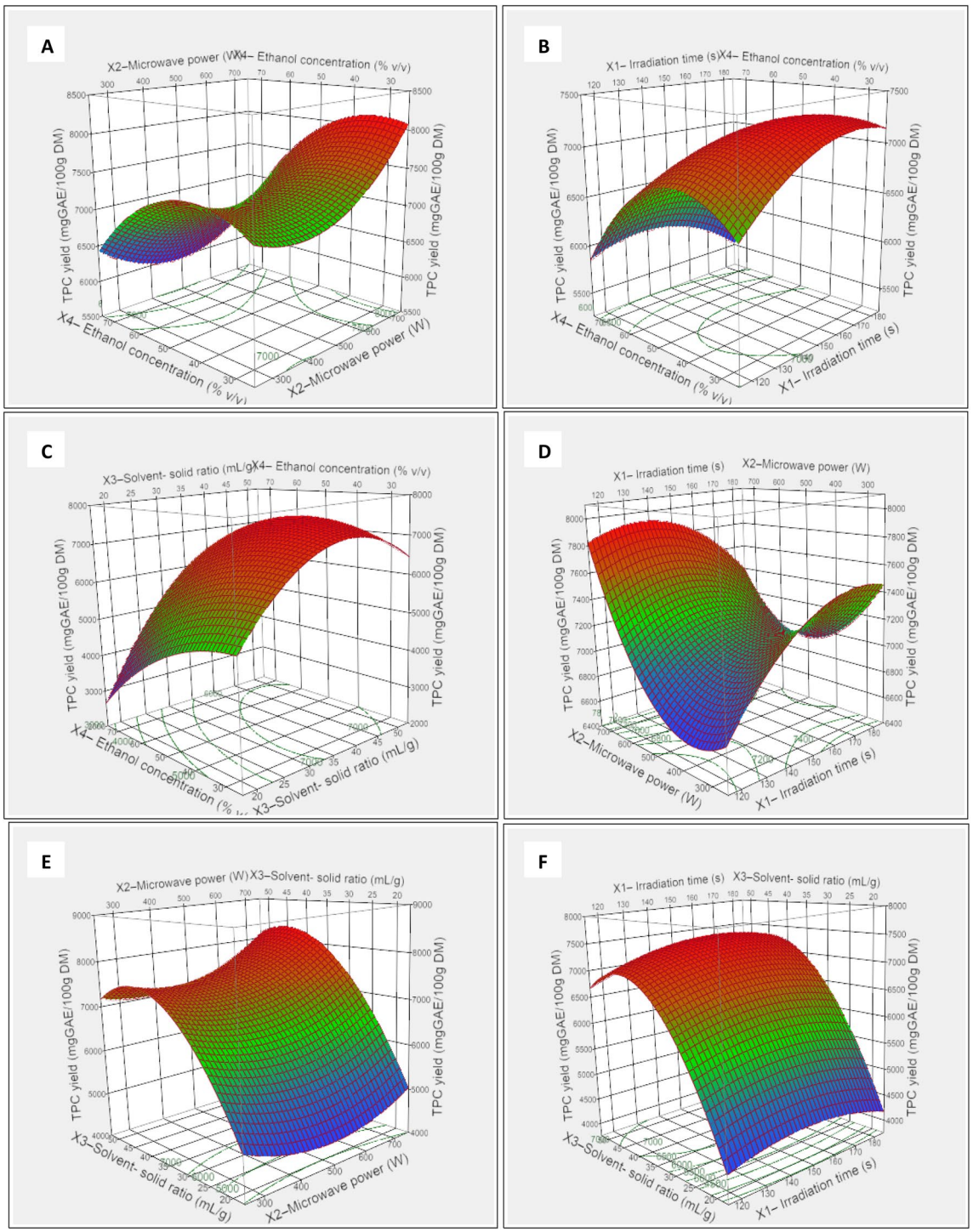

Fig. 1 Response surface analysis for the TPC yields from *Zlp* with MAE with respect to ethanol concentration and solvent-to-solid ratio (**A**); ethanol concentration and irradiation time (**B**); ethanol concentration and microwave power (**C**); /irradiation time and microwave power (**D**); microwave power and solvent-to-solid ratio (**E**); irradiation time and solvent-to-solid ratio (**F**)

Response surface analysis of TPC yield

The effect of ethanol concentration on each of the factors (solvent-to-solid ratio, microwave power and irradiation time) was shown in Fig. 1. Figure 1A shows that the TPC could be greater than 6000 mg GAE/100 g for a low solvent-to-solid ratio with ethanol concentration between 30 and 40%. At 50% ethanol concentration, the TPC yield

increased up to 7000 mg GAE/100 g with the significant increase in extraction time up to 180 s, whereas, above this extraction time with additional ethanol concentration, the extraction yield decrease gradually (Fig. 1B). As shown in the Fig. 1B, the TPC content increases considerably and could reach more than 8000 mg GAE/100 g at higher power with an increase in ethanol concentration up to 50%. But TPC decreases progressively when the microwave power decreased (500 W) and ethanol concentration increases over 50%. Indeed, as shown in Table 2, the yield of TPC depends mainly on ethanol concentration as its linear and interaction effect (positive effect) with solvent-to-solid ratio were highly significant ($p < 0.01$). Figure 1 (D, E) describe the interactions between the irradiation power and each of the other two factors (irradiation time and solvent-to-solid ratio) on the TPC recovery. Figure 1D shows that the amount of TPC increased with increasing microwave power and irradiation time during the initial extraction followed by a decrease at mean values. The graphs suggested that the irradiation power has an interaction effect with the extraction time and linear effect ($p < 0.01$) on the TPC yield (Table 2). Our results are in agreement with those found by different authors who underlined that all factors influencing the output of phenolic compounds extraction from medicinal plant tissues [34, 35]. Figure 1E clearly shows that with a significant increase in the solvent-to-solid ratio from 30 to 47 mL/g, the extraction yields increased approximately to 7000 mg GAE/100 g with a slight decrease when power increased. This trend could be explained by the total absence of inertia effect between the extraction power and the solvent-to-solid ratio, but we can say that the yield of TPC from *Zlp* depends mainly on the solvent-to-solid ratio, since its quadratic and linear effects were very significant ($p < 0.01$). Figure 1F depicts the effect of the solvent- solid ratio with the irradiation time, the TPC content is maximized (6500 mg GAE/100 g) at a high solvent-to-solid ratio with an extraction time of less than 150 s, but it decreases considerably when the solid to liquid ratio begins to decrease.

Validation and verification of predictive model

The MAE optimal conditions obtained for TPC recoveries were: 51% ethanol, 600 W microwave power, 180 s irradiation time and 47 mL/g solvent-to-solid ratio. To ensure the equation of the model and the adequacy of the chosen experimental model compared to the predicted model, the optimal response values were tested at the selected optimal values. The experimental values of extraction yield for the TPC was very close to the predicted values. According to Zhang et al. [30] the adequacy of the regression response of the model compared to that of the experimental model is necessarily due to a very strong correlation between the actual results and the expected results to express the desired optimization, so we can say that the model has been validated.

Comparison between MAE, UAE and CSE

The efficiency of TPC yields using MAE was compared with other methods such as UAE and CSE (Table 3). The results indicate that MAE showed a significantly higher TPC extraction capacity (7473.38 ± 740.55 mg GAE/100 g) (p < 0.05) compared to UAE (6841.41 ± 4.61 mg GAE/100 g) and CSE (4818.72 ± 7.02 mg GAE/100 g). This may be due to the long extraction time and the amplitude of the UAE which had a negative influence on the recovery of the phenols. In the work of Al-Saeedi et al. [12] who reported a TPC of 64.89 ± 0.44 mg GAE/100 g from the fruit of Oman *Zizyphus jujuba* (*Zj*) which is significantly lower than our results using the methanol as extraction solvent. In addition, Spanish jujube fruit has a content of 1442 to 3432 mg GAE/100 g according to Wojdyło et al. [36]. Similarly, Cosmulescu et al. [37] have shown that the TPC content was between 475.3 and 1634.4 mg GAE/100 g from the fruit of *Zj* by a conventional extraction method, these quantities are lower than our results found from *Zlp*.

However, the TFC extracts obtained by MAE, UAE (1019.96 ± 75.03; 1047.46 ± 1.89 mg QE/100 g respectively) were significantly higher ($p < 0.05$) than those obtained by CSE (934.41 ± 6.05 mg QE/100 g). We suggest there, that the recovery of flavonoids was influenced negatively by longer extraction time. Our results are superior to those obtained by Al-Saeedi et al. [12] of Oman variety fruit of *Zj* 27.43 ± 0.18 mg QE/100 g using chloroform as extraction solvent, likewise for those obtained by Koley et al. [38] who reported TFC content from 8.36 to 21.97 mg QE/100 g from *Zj*. The flavonoid extract of *Zj* showed a significantly lower content than ours, ranging from 19.9 to 48.5 mg QE/100 g [37]. Moreover, the TTC extracts by MAE were very significantly higher ($p < 0.01$) (14,253.11 ± 2453.86 mg CE/100 g) than those obtained by UAE and CSE respectively (10,339.50 ± 0.1; 4629.68 ± 0.08 mg CE/100 g). Gao et al. [39] Compared proanthocyanidin extracts from several *Zizyphus* varieties, among those containing the highest content about 413.7 ± 23.1 mg/100 g DM from *Zj cv. Zaowangzao* which is significantly lower than our extracts obtained from *Zlp*.

It is more interesting to mention that all the factors studied have a significant influence on the jujube yield and therefore on the antioxidant activity which was improved by MAE with the evaluation of each parameter and the interaction effects that may exist. In which this work has combined the three responses studied to have similar optimal conditions which will allow future readers to deduce that the methodology used for MAE from *Zlp* extracts made it possible not only to improve the TPC

Table 3 Comparison of extraction yield of bioactive compounds from *Zlp* by microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), and conventional solvent extraction (CSE)

	MAE	UAE	CSE
Extraction method/Factors			
Irradiation time (min)	3	25	120
Ethanol concentration (%)	51	50	50
Microwave power (w)	600	–	–
Temperature (°C)	–	63	60
Solvent solid/Ratio (mL/g)	47	67	50
Results of bioactive compounds			
Recovery of total phenolic TPC (mg GAE/100 g)	7473 ± 740^{a}	6841.41 ± 4.61^{b}	4818.72 ± 7.02^{c}
Recovery of total flavonoids TFC[a] (mg QE[b]/100 g)	1019.96 ± 75.03^{a}	1047.46 ± 1.89^{a}	934.41 ± 6.05^{b}
Recovery of condensed tannins TTC[c] (mg EC[d]/100 g)	$14{,}253.11 \pm 2453.86^{a}$	$10{,}339.50 \pm 0.1^{b}$	4629.68 ± 0.08^{c}
Results of biological activities			
PAOT Score/g	339 ± 0.30^{a}	156.5 ± 0.02^{b}	113 ± 0.01^{c}
DPPH scavenging EC_{50} (µg/mL)	0.24 ± 0.01^{a}	0.55 ± 0.03^{b}	1.05 ± 0.12^{c}
FRAP activity (mg $_{GAE}$/100 g)	3305.18 ± 9.75^{a}	1284.53 ± 10.99^{b}	1284.53 ± 10.99^{b}
AChE[i] assay % (10 mg/mL)	25.00 ± 3.81^{a}	19.66 ± 0.16^{b}	–
HepG2 cells IC_{50} (mg/mL)	$< 0.02 \pm 0.77^{a}$	$< 2.04 \pm 1.81^{b}$	–
MCF-7 cells IC_{50} (mg/mL)	$< 1.36 \pm 5.48^{a}$	$< 3.99 \pm 3.36^{b}$	–

Results are expressed as means standard deviation (±)

[a] *TFC* total Flavonoids content

[b] *QE* quercitin equivalent

[c] *TTC* total condensed Tannins content

[d] *CE* catechin equivalent

yields but also knowing that of flavonoids and tannins for their possible individual or collective use. In addition, the polyphenols are varied because of their capacity to react with each other and may be with other substances. Thus, there has been an hydrolysis or a polymerization of these molecules in smaller or larger having a strong antioxidant activity [40]. According to some authors Zhang et al. [30], MAE gives a very good yield in a minimum of irradiation time, herein its advantageous compared with other methods. Moreover, the increase of the interaction of the cells of the matrix leads to the transfer of the tissues so facilitates the interaction of electromagnetic fields. The energy transfer from the inside to the outside world will allow the promotion of the solubility of the solvent.

Identification of phenolic compounds from *Zlp* extract

In order to identify the bioactive compounds from our samples, LC–HRMS/MS was carried out, LC–HRMS in the negative and positive mode was performed and the compounds are indicated in Table 4. Compounds were tentatively identified under the analytical conditions by Data Analysis program from Bruker, and the chemical structures were suggested by pubchem, metlin and published reference on the same plant species or genus. In order to visualize the relationship between the compounds by knowing the intensities and antioxidant activities, the heatmap represented in Fig. 3 was used. This visual representation allows confirming which of compounds are more active in terms of antioxidant effect. Table 4 indicates the presence of 34 compounds based on their occurrence in plants from literature and by taking into account the exact mass and the fragmentation pattern and availability of reference standards and to the best of our knowledge have not been previously reported in *Zlp*.

Identification of secondary metabolites

The following three major peaks obtained at 1.96, 2.32 min detected with a deprotonated molecule at m/z 549.1590 and 169.0126 were assigned as caffeic derivative glycosylated and gallic acids. These compounds were analogous to those previously identified in *Zizyphus* species [41, 42].

Flavan-3-ols and proanthocyanidin were characterized in this study. Compounds observed at R_t of 1.21 and 1.74 min with m/z 525.1609 ($[M+H]^+$), 455.0950 ($[M-H]^-$) with different ion fragments were proposed to be barbatoflavan and epigallocatechin 3-*O*-vanillate,

Table 4 Identification proposal of the phenolic compounds present in the *Zlp* by LC–MS/MS in ESI negative and positive mode

R_t (min)	Intensity	$[M—H]^-/[M+H]^+$	Fragments ion (m/z); intensity (%)	Error (mg/kg)	Molecular formula	Tentative identification	References
1.05^-	29,390	387.1089	341.1061 (42.9); 119.0321 (40.9); 101.0219 (23.2); 161.0427 (13.5); 149.0425 (11.2); 83.01 (3.2)	2.5	$C_{21}H_{16}N_4O_4$	Indole derivative	125554521 (pubchem)
1.11^-	17,000	179.0535	59.0105 (100); 71.0110 (90.1); 96.9570 (39.9)	14.6	$C_6H_{12}O_6$	Glucose	5793 (pubchem)
1.21^+	52,178	525.1609	354.1041 (100); 273.0782 (98); 245.0469 (37.7); 145.0473 (17.6);	− 1.2	$C_{24}H_{28}O_{13}$	Barbatoflavan	47366 (metlin)
1.49^-	7764	475.1235	133.0110 (100); 115.0009 (28.8); 71.0098 (19.9); 56.9939 (1.7)	2.3	$C_{23}H_{24}O_{11}$	Luteolin 5,3′-dimethyl ether 7-glucoside	49394 (metlin)
1.58^-	14,314	431.1347	89.0208 (100); 119.0311 (4.1); 341.1040 (8.1)	0.1	$C_{22}H_{24}O_9$	Isosakuranetin-7-*O*-rhamnoside	52823 (metlin)
1.74^-	9174	455.0950	112.9824 (100); 68.9919 (51.8)	7.4	$C_{23}H_{20}O_{10}$	Epigallocatechin 3-*O*-vanillate	47348 (metlin)
1.96^-	6618	549.1590	179.0529 (84.4); 383.1130 (31.8); 323.0925 (18.7); 161.0429 (19.4); 341.1037 (11.9)	19.8	$C_{26}H_{30}O_{13}$	Caffeic acid derivative glycosylated	985394 (metlin)
1.97^+	11,640	139.0486	121.0380 (100); 93.0434 (72.5)	19.4	$C_6H_6N_2O_2$	4-Methylpyrimidine-2-carboxylic acid	723920 (metlin)
1.98^-	36,208	191.0170	87.0054 (100); 85.0257 (47.9); 111.0066 (37.2); 57.0306 (17.7)	8.52	$C_6H_8O_7$	Citric acid	Berkani et al. [4]
2.01^-	2632	117.0158	73.0260 (100); 45.9963 (96.0); 68.9922 (94.7)	20.80	$C_4H_6O_4$	Succinic acid	Berkani et al. [4]
2.32^-	290	169.0126	NF	3.25	$C_7H_6O_5$	Gallic acid	Benabderrahim et al. [41] Zhao et al. [42]
3.74^+	4362	195.1203	45.0326 (100); 109.9576 (24.9); 135.0422 (13.1); 107.0483 (9.4)	19	$C_8H_{12}N_4O$	Pirimidine derivative	926880 (metlin)
5.67^+	148,518	393.2040	NF	5.17	$C_{25}H_{28}O_4$	5-Hydroxy-7-*O*-nerylflavanone	52674 (metlin)
6.11^+	5386	465.1873	407.1842 (16.4)	5.1	$C_{18}H_{24}N_8O_7$	Tetrapeptide (Asp-Gly-His-His)	121608 (metlin)
6.12^+	9780	476.3004	45.0328 (100); 133.0834 (26.5)	3.8	$C_{21}H_{41}N_5O_5S$	Tetrapeptide (Leu-Cys-Lys-Leu)	176048 (metlin)
6.43^+	60,668	407.2194	254.9096 (0.3); 365.1489 (0.3); 372.3353 (0.3)	6	$C_{26}H_{30}O_4$	5-Methoxy-7-prenyloxy-8-C-prenylflavanone	52663 (metlin)
6.50^+	13,542	611.1521	303.0459 (100); 304.0494 (20.4); 465.0962 (11.8)	14.0	$C_{27}H_{30}O_{16}$	Quercetin-3-*O*-robinobioside	Wojdylo et al. [36]

Table 4 (continued)

R_t (min)	Intensity	$[M—H]^-/[M+H]^+$	Fragments ion (m/z); intensity (%)	Error (mg/kg)	Molecular formula	Tentative identification	References
6.57^+	11,738	301.1146	106.0636 (100); 132.0428 (50.4); 225.0994 (61.0); 197.1041 (35.5)	− 1.1	$C_{11}H_{16}N_4O_6$	5-Oxoprolylaspar-aginyl glycine	487255 (metlin)
6.59^+	5296	467.2203	331.0835 (1.2); 290.5074 (0.9)	− 4.6	$C_{20}H_{30}N_6O_7$	Tetrapeptide (Val-Pro-His-Asp)	244401 (metlin)
6.84^+	6326	619.3095	103.0375 (100); 191.0891 (30.7); 235.1127 (18.2)	16.6	$C_{28}H_{42}N_8O_8$	Pentapetide (Pro-Tyr-Pro-Arg-Ser)	264985 (metlin)
7.25^+	7332	417.2332	45.0328 (100); 89.0586 (42.4); 133.0838 (25.3); 177.1098 (12.9); 144.0754 (12.6)	5.4	$C_{17}H_{30}N_4O_7$	Tetrapeptide (Leu-Val-Asp-Ala)	182319 (metlin)
7.33^+	11,710	317.1460	106.0638 (100); 132.0424 (18.2)	− 5.6	$C_{12}H_{20}N_4O_6$	Tripeptide (Ser-Pro-Asn)	19282 (metlin)
8.06^+	3365	301.1136	106.0638 (100)	− 2.2	$C_{10}H_{20}O_{10}$	Dioside	70700334 (pubchem)
8.41^+	13,106	505.2559	419.2203 (2.3); 113.0570 (2.3); 124.0784 (1.5)	− 3	$C_{19}H_{36}N_8O_6S$	Tetrapeptide (Ala-Met-Gln-Arg)	107754 (metlin)
8.48^+	4558	433.1185	61.0274 (100);132.0427 (50.3); 208.0571 (18.6); 269.0881 (17); 311.0968 (13.1); 327.0893 (12.2)	− 12.87	$C_{21}H_{20}O_{10}$	Apigenin-7-*O*-glucoside	Khan et al. [49]
9.54^+	25,954	225.0990	132.0428 (100); 104.0485 (17.7)	− 4.5	$C_8H_{16}O_7$	Glycoside	102183608 (pubchem)
10.15^+	8300	255.1559	209.1506 (100); 123.1152 (47.8); 191.1413 (44.7); 109.0998 (28.9); 167.1052 (22.8)	2	$C_{14}H_{22}O_4$	9-(3-Hydroxy-5-oxocyclopent-1-en-1-yl)nonanoic acid	689561 (metlin)
10.91^+	4476	301.0728	182.0871 (57.3)	− 7.09	$C_{16}H_{12}O_6$	6-Methylluteolin	49158 (metlin)
10.99^+	3366	255.0957	181.0607 (26.5); 204.9606 (23.3)	2.1	$C_{11}H_{14}N_2O_5$	Glycine derivative	500366 (metlin)
11.17^+	7960	255.1568	209.1511 (100); 123.1152 (36.5); 191.1404 (27.9); 109.1010 (17.9); 167.1043 (21.3)	− 1.6	$C_{10}H_{18}N_6O_2$	Glycine derivative	347687 (metlin)
14.99^+	6378	291.2494	45.0327 (100); 161.0930 (15.9); 151.0927 (10)	31.7	$C_{13}H_{10}N_4O_3$	Guanidine derivative	104888008 (pubchem)
15.10^-	11,328	353.1957	96.9565 (61.5); 122.9722 (2.9)	2.0	$C_{19}H_{32}O_7$	Blumenol C-glucoside	95146 (metlin)
16.50^+	49,376	595.4105	309.1994 (100); 310.2025 (21.6); 221.1492 (1.1)	− 6.6	$C_{30}H_{58}O_{11}$	Stearyllisomaltse	9894835 (pubchem)
16.53^+	98,018	287.2188	69.0689 (100); 111.1153 (54.1); 199.1664 (4.3)	13.9	$C_{16}H_{30}O_4$	3,6-Dihydroxy-4-ethylidene-6-ethyldecanoic acid ethyl ester	10803152 (pubchem)

Peaks with a minus/plus superscript were analyzed in negative/positive mode. Compounds are indicated by retention time (R_t) order and numbered according to the appearance in each chromatogram, negative $[M-H]^-$ or positive mode $[M+H]^+$

NF not fragmented

respectively [43, 44]. However, compounds with retention times of 5.67, 6.43 min showed a MS^2 fragmentation characteristic to flavanones with m/z of 393.2040, 407.2194 at positive mode were tentatively identified as 5-hydroxy-7-*O*-nerylflavanone and 5-methoxy-7-prenyloxy-8-C-prenylflavanone, respectively. In addition, a flavonoid with methoxy groups was at $R_t = 10.91$ with a [M + H] + m/z of 301.0728 which was assigned as 6-methylluteolin [45]. The flavonoid aglycones were in accordance to those identified in previous reports from other plant materials that showed a significant antioxidant properties [46]. To the best of author's knowledge, these compounds are reported from *Zizyphus* genus for the first time.

Four compounds were characterized as flavanones glycosides in this study. Peaks observed at 1.49, 1.58 min, with $[M\text{-}H]^-$ ion at m/z of 475.1235 and 431.1347, were tentatively identified as luteolin 5,3′-dimethyl ether-7-glucoside and isosakuranetin-7-O-rhamnoside, respectively [47, 48]. Our study is the first detecting the presence of these compounds from *Zizyphus* genus. However, two known flavones, including quercetin-3-*O*-robinobioside ($R_t = 6.50$ min), apigenin-7-*O*-glucoside ($R_t = 8.48$ min) were found with positive mode in the samples. These compounds were in accordance with previous funding from jujube samples [15, 49].

Figure 3 shows clearly that both glycoside and aglycone flavonoids are 44.51% of all phytochemicals and 80.75% of all secondary metabolites detected in our samples. 5-hydroxy-7-*O*-nerylflavanone is found to be the most abundant flavonoid from our samples. We can say that the antioxidant activities of *Z. lotus* extracts will be mainly due to flavonoids group by knowing the intensities of each compound present in extracts as we mentioned them at different color in heatmap. Alkaloid group was characterized in this study and observed at 1.05 min with m/z of 387.1089. This compound was assigned as indole derivative. This last is detected also from other plants and it is known as one of antimalarial, antioxidant and anti-inflammatory agents [50]. In addition, peak observed at $R_t = 15.10$ min with a $[M\text{-}H]^-$ ion at m/z 353.1957 was attributed to blumenol C-glucoside. This compound showed a good pharmacological effect in several plant material like major class of terpenes, thus it is demonstrated by Amat-ur-Rasool et al. [51] from Indian *Bombax ceiba* that is a good antioxidant. Others suggested that it is one of inhibitors on pro-inflammatory cytokines [52].

Identification of primary metabolites

Peaks observed at 1.11, 8.06, 9.54 and 16.50 min with positive and negative modes at m/z of 179.0535, 301.1136, 225.0990 and 595.4105 were attributed to glucose, dioside glycoside and stearyllisomaltse, respectively. These compounds are not present with high intensities in our sample and some of them are in accordance to other data detecting sugars from *Zizyphus mauritana* fruits [53].

Recently, several studies have given a considerable interest to the bioactive peptides derived from plant materials which have a large biological effect on human health. Otherwise, different parts of jujubes such as fruits, roots, seeds and leaves have been used against the treatment of several diseases [54, 55]. However, several small peptides are detected in *Zlp* extracts. Peaks observed at R_t of 3.74, 14.99 min with m/z of 195.1203, 291.2494 were tentatively assigned as pyrimidine and guanidine derivative. In addition, three glycine derivatives were tentatively identified at 6.57, 10.99 and 11.17 in positive mode with m/z of 301.1146, 255.0957 and 255.1568, respectively. Furthermore, five different tetrapeptides were identified tentatively in positive mode at different retention times 6.11, 6.12, 6.59, 7.25 and 8.41 min. These compounds were assigned with different molecular formula $C_{18}H_{24}N_8O_7$, $C_{21}H_{41}N_5O_5S$, $C_{20}H_{30}N_6O_7$, $C_{17}H_{30}N_4O_7$ and $C_{19}H_{36}N_8O_6S$, respectively. However, one pentapeptide was detected at 6.84 min with m/z of 619.3095, and one other tripeptide was observed at 7.33 min with m/z of 317.1460. These compounds were in accordance to those identified in previous reports from *Zizyphus* species and others data isolated bioactive compounds from plants [56, 57]. Figure 3 showed the presence of 14.04% peptides from total compounds present in our *Zlp* extracts and we mentioned 31.30% from total primary metabolites. Several studies have been characterized the antioxidant potential of peptides from several plants as demonstrated from *Zj* fruits [58]. Thus, *Zlp* have a non-negligible amount of peptides which may participate in the studied antioxidant activities.

The examination of chromatograms obtained at negative and positive modes indicated the presence of some organic acids at 1.97, 1.98, 2.01, 10.15 and 16.53 min with m/z of 139.0486, 191.0170, 117.0158, 255.1559 and 287.2188, respectively were assigned tentatively as 4-methylpyrimidine-2-carboxylic, citric, succinic, 9-(3-hydroxy-5-oxocyclopent-1-en-1-yl)-nonionic and 3,6-dihydroxy-4-ethylidene-6-ethyldecanoic ethyl ester acids. In addition to other substances, these acids have found to exert antioxidant and anti-Alzheimer effects from several plants [59, 60].

Antioxidant activities

In order to evaluate the antioxidant activities of *Zlp* extract obtained by the three methods studied, several tests were used such as PAOT, $DPPH^{\cdot}$ and FRAP assays (Table 3).

The greater antioxidant capacity of the phenolic extracts was attributed to a significantly lower EC_{50} ($p < 0.01$). The MAE extract showed the higher antioxidant (0.24 ± 0.01 µg/mL) than UAE extract (0.55 ± 0.03 µg/mL) and CSE (1.05 ± 0.12 µg/mL). Our results are in the same range to our previous works on *Zl* seeds by UAE (0.39 µg/mL) [3] and

lower antioxidant capacity than that of MAE obtained from jujube seeds (0.067 µg/mL) [4]. These results are in accordance with Ghazghazi et al. [61] and works who have used MAE for *Zj* from Tunisia and Oman. Similarly our results obtained by UAE and CSE are greater than those of Hammi et al. [23] reported an EC_{50} of 0.2895 mg/ml for Tunisian variety extract but close to those of MAE extract. Moreover, the iron-reducing power of the *Zlp* extracts revealed a highly significant effect by the MAE extract (3305.18 ± 9.75 mg GAE/100 g), compared with the UAE and CSE extracts (1284.53 ± 10.99 and 672.86 ± 4.87 mg GAE/100 g, respectively). Our results are much higher than those of Wang et al. [62] (471.6 ± 30.8 mg CE/100 g DM) obtained from *Zj cv. Zaowangzao*. In the meantime, the PAOT technology evaluating the antioxidant effect which is used for the first time in jujube plant was well marked by the extracts from MAE (339 ± 0.30 PAOT Score/g) than the other two methods (Table 3). Thus, we confirmed the highest correlation between phenolic compounds and antioxidant effect where MAE showed the highest level. Our results are in accordance to [63] demonstrating the higher correlation between bioactive compounds and both PAOT and DPPH activities in wine samples. In addition to the remarkably antioxidant compounds present in *Zlp* which are mainly responsible for antioxidant effect (Pearson test n = 0.94) in comparison to some standard antioxidant showed in that work such as trolox, myrecitin and epicatechin (544.16 ± 16.81, 677.78 ± 7.41 and 730.2 ± 93.73 mg PAOT/L, respectively). As well as, by comparing to other source of antioxidants such as Arkovital® Pur'Energie and synthetic supplement showing a PAOT Score of 0.034 ± 0.0009 and 0.045 ± 0.0037 mg GAE, respectively [64]. These results are very lower to that found in jujube extracts by comparing to equivalent of gallic acid where the highest PAOT Score was obtained by MAE (0.012 ± 0.0004 GAE/g) followed by UAE and CSE (0.004 ± 0.0000 and 0.002 ± 0.0004 GAE/g of extract, respectively). This means that MAE method is more efficient for the recovery of phenolic compounds than the herein tested UAE and CSE methods. The present study confirmed the results obtained by the other antioxidant activities that microwave extract presented a significant and high antioxidant and chelating activities than UAE and CSE extracts.

The antioxidant capacity by PAOT, DPPH scavenging and FRAP assays of the extracts was evaluated by PCA analysis in order to demonstrate the relationship between these last and phenolic antioxidants. PCA was applied to the jujube extracts (MAE, UAE and CSE) for phenolic compound (TPC, TFC and TTC) and antioxidant activity, where the two chosen factors justified 100% of total variance. The resulting plots allowed selecting the better extraction method of *Zlp* antioxidant compounds, and clearly divided the samples into three groups, depending on the extraction method as shown in Fig. 2. The first group which explains 90.18% of the total variance showed a negative correlation with PC1,

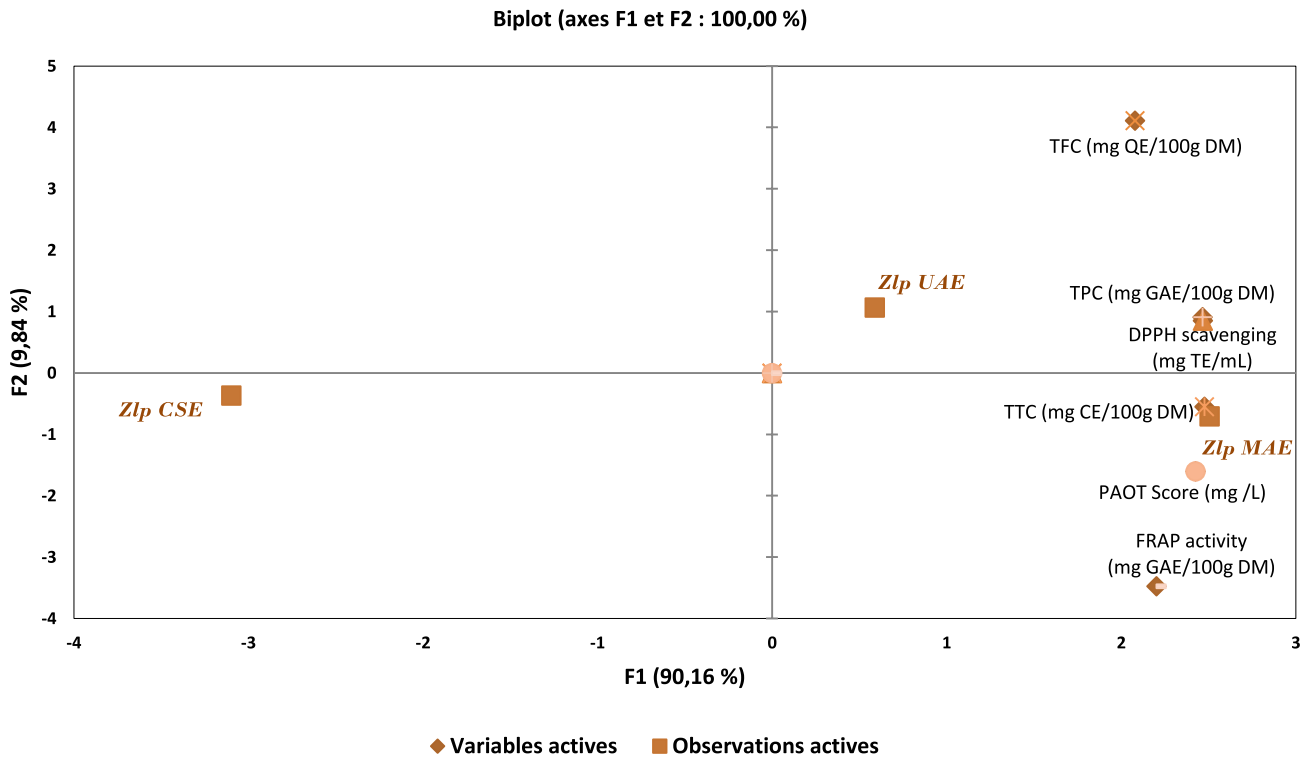

Fig. 2 Principal component analysis of TPC for *Zlp* with MAE, UAE and CSE

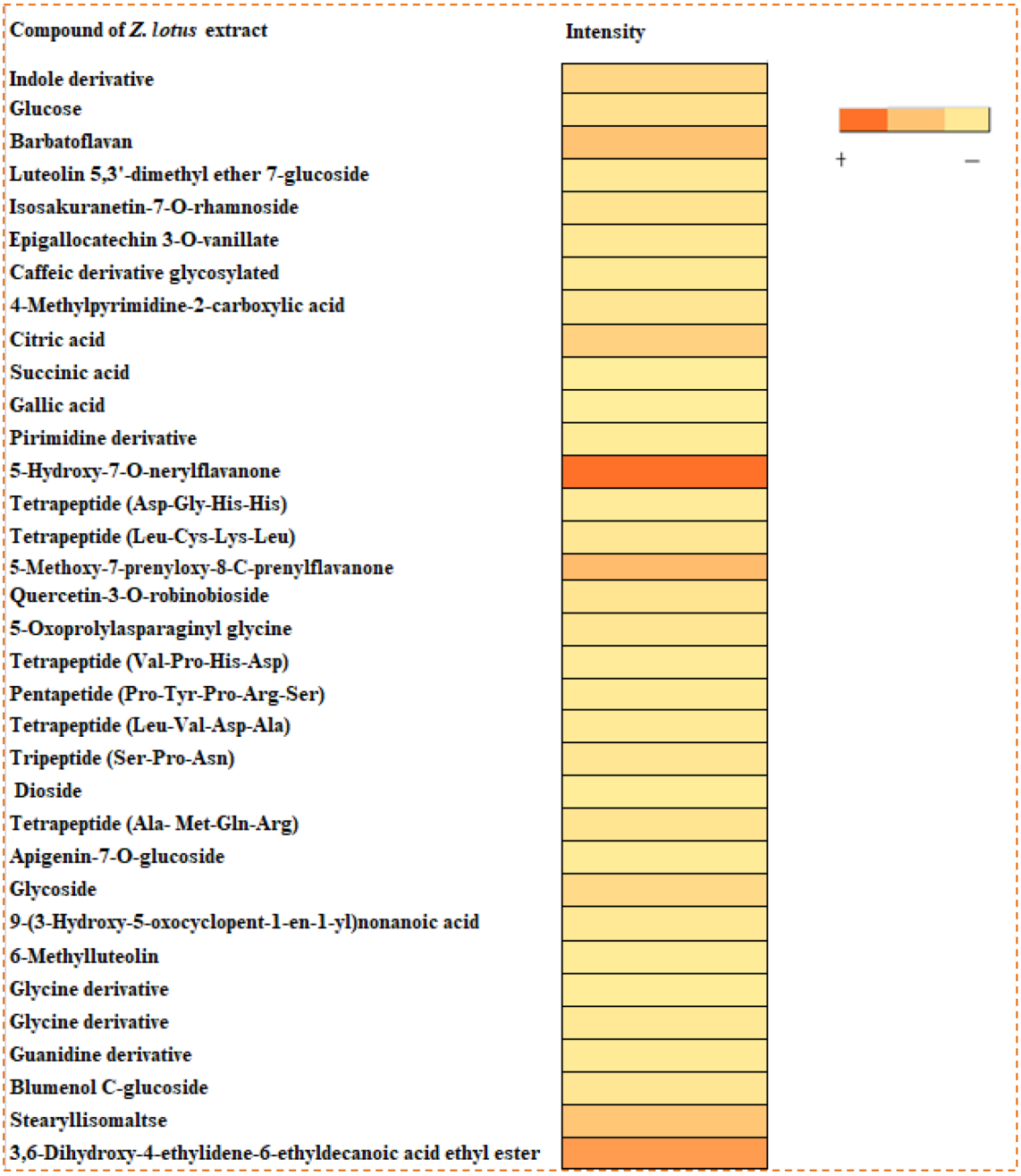

Fig. 3 Heatmap of the chemical profile and of *Zlp* extracts. Mean values refer to colors from minimum displayed in light orange to maximum represented with dark orange (Color figure online)

thus confirming that MAE was the best extraction method for phenolic compounds with potent antioxidant activity. The highest correlation was found between antioxidant activity (PAOT, FRAP) and TTC, as well as between DPPH and TPC. Thus, our results suggest that phenolic compounds might have a good influence on the antioxidant capacity of the *Zlp*. In addition to UAE which showed a positive correlation with PC2. The best correlation between the UAE and both TPC and TFC were observed in this group with a remarkably higher correlation with DPPH assay. While, the third group (PC3) explains only better 9.84% of the experimental variability, which could essentially be associated to the CSE method and TFC.

The mechanism responsible may be due to the bioactive compounds present in jujube extract, such as phenolic and organic acids, flavonoids aglycones and glycosides, peptides and some of sugars as we have seen by LC–MS/MS analysis. Figure 3 demonstrated that most of these phytochemicals

with high intensities are polyphenols (55%) known which could provide hydrogen and electron to the radicals [65]. Their activities could be attributed to the presence of a high number of hydroxyl groups which are present in their aromatic rings [66]. As well as, the increasing of these activities due to the content of other compounds is associated to the strong reducing of the oxidized state which causes an increase in antioxidant capacity. Nevertheless, *Zlp* extract showed another combination of phenolic compounds with other primary metabolites, mainly peptides and derivative that are complexing together in plant extracts and increase the antioxidant effect [58]. Furthermore, from a heatmap we clearly notice that combination between glycoside and aglycone flavonoids (80.75%) are the most determinant of the antioxidant activity in jujube extracts where 5-hydroxy-7-*O*-nerylflavanone was the most abundant. It should be emphasized that the proposed approach is limited for the identification of all the compounds contained in this extract mentioned for the first time from pulp and peel. This will open up to other more innovative studies requiring the purification of this extract and the isolation of this majority compound in order to benefit more from their use as a nutraceutical agent.

Anticholinesterase activity

The AChE inhibition assay of *Zlp* for both MAE and UAE is represented in Table 3. MAE exhibited a significant effect on AChE inhibitory activity ($25.00 \pm 3.81\%$) when compared to UAE ($19.66 \pm 0.16\%$), this was in accordance with the antioxidant activities showing the higher positive effect by MAE than other techniques. These results are in agreement with our previous works on jujube seeds demonstrating a lowest IC_{50} value by MAE than by UAE (0.88 ± 0.02 and 0.93 ± 0.01 mg/mL, respectively) where we have seen an AChE inhibition of 50% in seeds which is twice lower than that of pulps, while a same tendency has been observed on the method of choice (MAE > UAE) [3, 4]. In comparison to data in literature, these results are mainly due to the concentrations of phenolic compounds present in jujube extracts which gave a proportional AChE inhibition of many plant matrix [67–69]. These results suggested that *Zlp* is a good AChE inhibitor and could be exploited against treatment of several desease mainly Alzheimer's disease and alleviation of severe constipation [4].

Antioproliferative activity

The antiproliferative activity of *Zlp* extracts was evaluated by measuring the cytotoxic effect on $HepG_2$ (human hepatocellular liver carcinoma cell) and MCF-7 (breast cell) lines as represented in Table 3. The recovery of jujube phenolic extracts that killed 50% of the cells (IC_{50}) was determined from dose–response curves. Similar observations than antioxidant assay were observed for this test. The antiproliferative effect on both $HepG_2$ and MCF-7 cell lines as observed to be maximum in extract obtained by MAE with lower IC_{50} value ($< 0.02 \pm 0.77$ and $< 1.36 \pm 5.48$ mg/mL, respectively) in comparison to that noticed by UAE extracts ($< 2.04 \pm 1.81$ and $< 3.99 \pm 3.36$ mg/mL, respectively), while demonstrating that our extracts exhibited minimum toxicity. A value of 0.1 mg/mL is the limit of toxicity to human cell lines [70]. Thus, we can notice that the jujube extracts excerce a significant higher effect against both MCF-7 and $HepG_2$ cells. Our results are in accordance to that obtained in our previous work on jujube seeds [3], which are mainly due to the nature of bioactive compounds present in jujube extracts including mainly flavan-3-ols such as monomer [as (−)-epicatechin, gallocatechin gallate, (+)-catechin] and other procyanidins, the extraction methods [3, 15]. Such finding demonstrated that *Zlp* is one of the most active antitumor agents that caused the severe cytotoxicity in the studied lines.

Conclusion

In this study, the microwave extraction process was applied according to a RSM modeling optimization to predict and estimate the behavior of some bioactive compounds namely total polyphenols, flavonoids and condensed tannins extracted from *Zlp,* as well as, the parameters influencing the biological activities (including $DPPH^{\cdot}$, FRAP, PAOT, AChE, MCF-7 and HPG_2 testes).

- The mathematical models generated by the RSM design proved to be appropriate for predicting the responses chosen in each experimental study. The optimal conditions obtained from optimization study were microwave power at 600 W, irradiation time of 180 s and 47 mL/g solvent-to-solid ratio and 51% ethanol (v/v in water). These are very important factors which afect both the yield and biological activities of jujube extracts.
- The comparison between the three extraction methods revealed the efficiency of MAE technique in terms of TPC, TFC and TTC than UAE and CSE ones. Under these conditions, MAE extract presented high content for TPC (7473.38 ± 740.55 mg GAE/100 g) with an extraction of 10 times lower approximately than that of UAE and CSE.
- Indeed, MAE showed the highest correlation values between antioxidant capacity and phenolic compounds, as demonstrated by $DPPH^{\cdot}$, FRAP and PAOT tests. This method presented a highest AChE effect and antiproliferative effects against MCF-7 and HPG_2 on jujube extracts.

- LC–MS/MS analysis identified 34 phytochemicals including primary and secondary metabolites. To the best of our knowledge, 10 phenolic compounds have not been reported before in *Zizyphus* species. Moreover, the *Zlp* are rich in phytochemicals that are responsible for high antioxidant activity.
- From an industrial point of view, the phenolic extracts obtained by MAE can be used by exploiting their biological properties (food additives, nutraceuticals, etc.) in order to replace the conventional extraction methods as CSE.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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