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Thème

Optimisation d'extraction par solvent des poly phénols assistée aux ultrasons et activité antioxydante de fruits de *Ziziphus lotus* L.

Soutenu le : 19 /09/ 2018 **Devant le jury composé de :**

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RAZIKA, RIMA

DEDICATION

For those who gave me everything without anything in return, there are no words to describe how much my parent has meant to me throughout all my life.

To my parent, my mother and father, who supported me with love and understanding. Without you, I could never have reached this current level of study. Thank you for giving me life and the joy of living.

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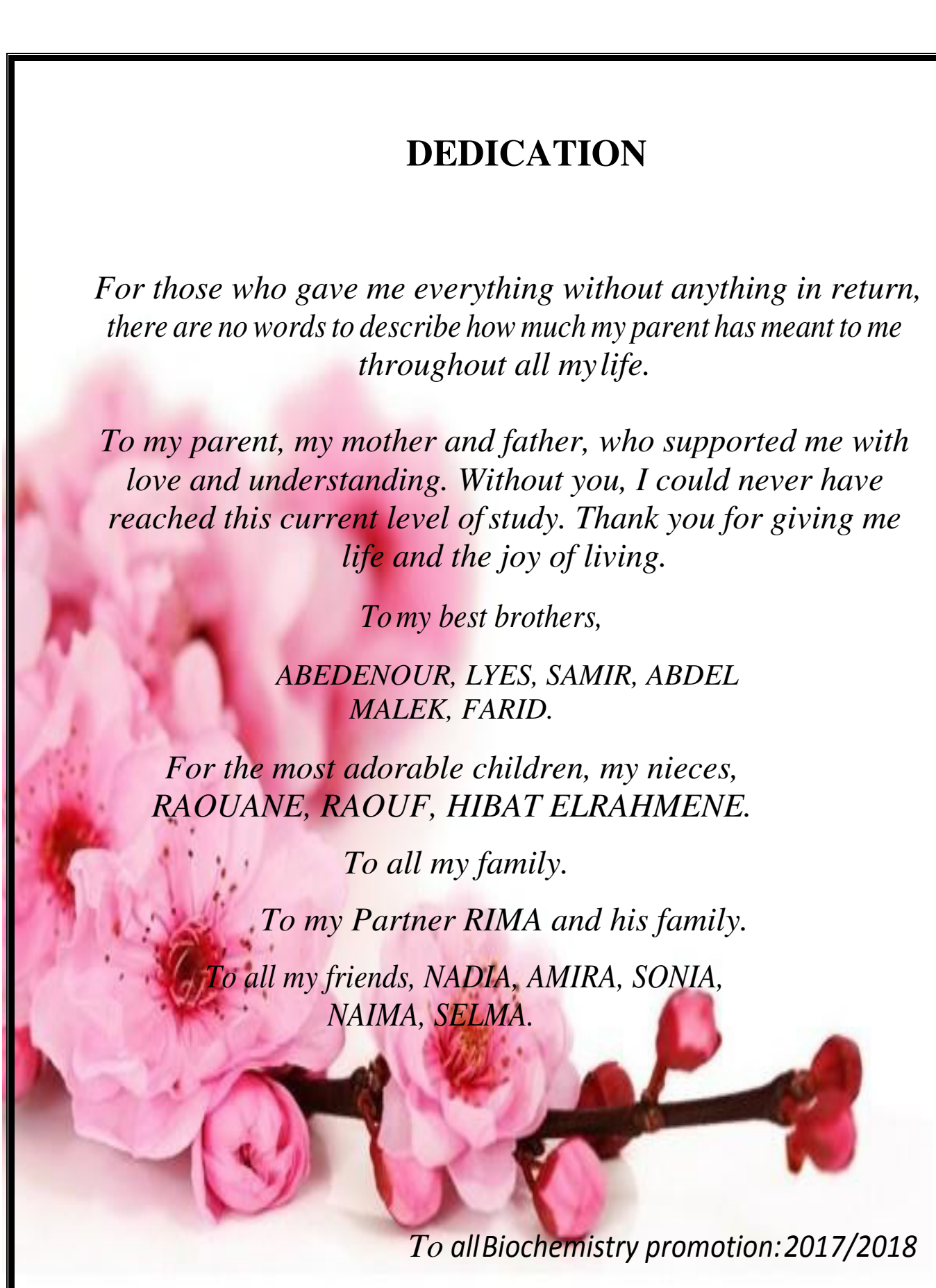

To all Biochemistry promotion: 2017/2018

RAZIKA

DEDICATION

First of all, I dedicate: this persents

My parents: my mother and father, the light of my life; thank you for all that you have done for me concerning my education which leads today to the realization of this study. Thank you for his giving me life and the joy of living.

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To my partner: RAZIKA and his family.

To all Biochemistry promotion 2017/2018

RIMA

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ANOVA: Analysis Of Variance

$\mathbf{A_w}$: Water activity

CE: Conventional Extraction

DPPH: 2,2-Diphenyl-picrylhydrazyl

DW: Dry weight

FFD: Fractional Factorial Design

PI: Phenols Index

GAE: Gallic Acid Equivalent

MAE: Microwave Assisted Extraction

MC: Moisture Content

PBD: Plackett - Burman Design

RMSE: Root Mean Square Error

RSA: Radical Scavenging Activity

RSM: Response Surface Methodology

TPC: Total Phenolic Compounds

TFC: Total Flavonoid Compounds

UAE: Ultrasound Assisted Extraction

Introduction

Introduction

Algeria is considered among the countries known for their taxonomic diversity given its privileged biogeographical position and its extent between the Mediterranean and sub-Saharan Africa **(Cheurfa et al., 2017).** The Algerian flora counts nearly 3,000 species belonging to several botanical families **(Arab et al., 2014).** In recent years the medicinals plants have attracted increasing attention because of their various potential health benefits and pharmacological activities **(Chen et al., 2018).**

Zizyphus lotus L, wild jujube, is one of the medicinals plants famous in the world, and its production has steadily increased in China in the last ten years. It is very common in North of Algeria. Although, its fruits are very appreciated by the Algerian population for the treatment of several pathologies like gastrointestinal disturbance, liver troubles, urinary infections, skin infections, insomnia, diarrhea, and diabetes **(Abdeddaim et al., 2014).** *Jujube* is characterized by its particular flavors and abundant nutrients, such as proteins, dietary fibres, minerals, and vitamins **(Yu et al., 2012).**

Extraction process is considering the first basic and important stage for separating bioactive compounds from raw materials **(Liu et al., 2017).** However, several methods are available for extraction of phenolics compounds from *Z. lotus,* including solvent extraction; maceration, mechanical agitation, ultrasound-assisted extraction, microwave-assisted extraction **(Chouaibi et al., 2012, Mkadmini Hammi et al., 2016, Ourzeddine et al., 2017).** Among of these methods, ultrasound-assisted extraction (UAE) technology has gained increasing popularity due to its advantageous properties, including high extraction efficiency, good reproducibility, low solvent consumption, speed, low cost,**.** environmental friendliness and easy scale up for industrial applications **(Li et al., 2016).** Furthermore, Due to the many factors that influence UAE, optimization of the extraction process parameters is an important step prior to eventual purification and application of the extracts **(Carciochi et al., 2015).**

Therefore, the main goals of this study were to optimize the ultrasonic-assisted extraction of phenolics compounds from *Z. lotus* fruits using Plackett and Burman Design (PBD) in order to select the suitable parameters extraction which may maximize the yield of total phenolics and flavonoids to be considered during the optimization process. In other hand, Fractional Factorial Design (FFD) and response surface methodology (RSM) are using to optimize the UAE to obtain maximum yield of TPC, TFC and determined antioxidant activity of UAE extracts.

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ChapterI

Literature Review

I.1.Overview of Jujube plant

Ziziphus lotus known as jujube, is a xerophytic shrub, belongs to the angiosperm Rhamnaceae family **(Gorai et al., 2010).**This family includes about 135–170 species of Zizyphus. It's commonly known by the Algerian population as "sedra" and the edible fruit called "nbeg" and it's dormant from October through March and mature plant flowers in May and June and produces fruits in August **(Maraghni et al., 2010).**

Z. lotus, is widespread in tropical and sub-tropical regions: Asia, Africa, North America, South America, Oceania and Europe with the center of diversity in Asia **(Ghalem et al., 2014) (Fig.1).** Different parts of the plant have found various uses in the food industry, such as, in the cosmetics in several forms; honey, tea, jam, juice, oil, loaf, and cake and pharmaceutical industries .It is used in Algerian traditional medicine for treatment of several pathologies such as digestive disorders, obesity, urinary troubles and skin infections **(Benammar et al., 2014, Chouaibi et al., 2012).**

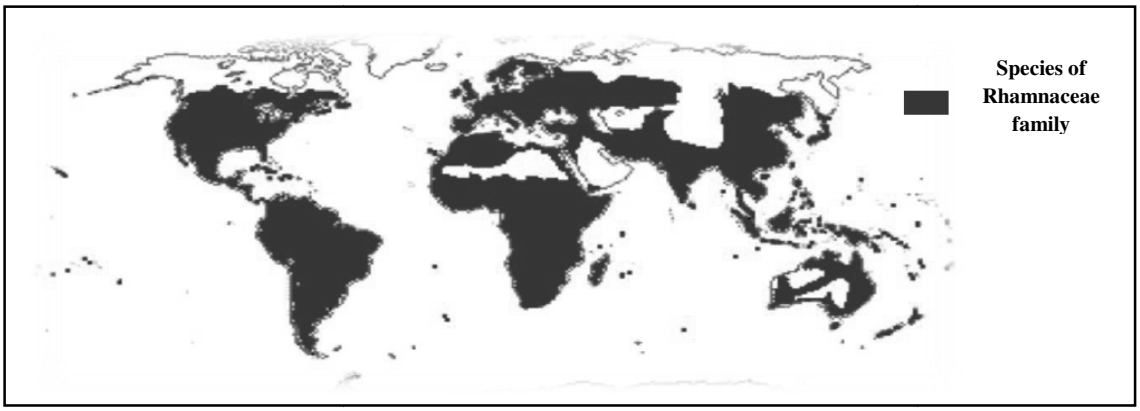

Figure 1: Distribution area of Rhamnaceae family in the world **(Dupont et al., 2012).**

I.2. Description and botanical classification

Z. lotus (jujube tree) is a deciduous, fruitful thorny shrub with intricately branched stems and smaller flowers and fruits. It forms tufts of a few meters in diameter, up to 2 to 3 meters in high with many ramifications and is found in depressions with deep sandy soil **(Maraghni et al., 2010).**The leaves are deciduous, glabrous, coriaceous, shortly petiolate and with entire margins. They are alternately oval in shape and small in size (15 x 10 mm, 1.5 to 2 times longer than wide), accompanied at its base by two stipules transformed into uneven and vulnerable spines **(Amara and Benabdeli, 2017).** Its fruits are round from 1 to 2cm in diameter. At maturity, it has a brown skin, a very sweetened and farinaceous ochre flesh, wrapping a small seed with a diameter of 4 to 5 mm **(Saadoudi et al., 2012), (Fig.2).**

Kingdom: Plantae

Division (Phylum): Tracheophyta

Class: Dichotyledoneae

Order: Rhamnoles

Family: Rhamnaceae

Genus: *Ziziphus*

Species: *Ziziphus lotus*

Figure 2: Photography of *Z. lotus* **(Adelia and Samavatib, 2015).**

I.3.Biochemicalcomposition of jujube

Z. lotus has been the subject of considerable studies because it is a source of several bioactive compounds which hold therapeutic potentialities for human nutrition, health promoting, and disease preventing such as, polyphenols, fatty acids, vitamins, and flavonoids, alkaloids, saponins, and other bimolecular **(Rsaissi et al., 2013, Abdoul-Azize et al., 2013).** Its medicinal properties depend on the part of the plant concerned and the extract used. For example, the fruits contain sugar (20 to 32%). protein (0, 8 to 2,1%). lipid (0,1 to 0,3%) and high levels of vitamins: A.C and E. In addition it is rich in minerals; Ca. Mg. Na. K and phosphorus. pollysaccharids and amino acids **(Abdoul-Azize, 2016, Ourzeddine et al., 2017) .**

The fatty acid composition of *Z. lotus* fruits revealed that their oil contained 13 compounds with the predominance of linoleic acid (18:2n-6) a precursor of n-6 fatty acids and the leaves were the richest source of vitamin E and linolenic acid (18:3n−3), a precursor of n−3 fatty acids **(Benammar et al., 2010, Ghazghazia et al., 2014).**

I.4.Phenolic composition

Polyphenols or phenolic compounds are secondary metabolites, ubiquitous widely exist in nature and food-industry by-products. They are differentiated from one another by their structure and molecular weight, and the resulting physicochemical and biological properties. Due to this enormous variety, there are reports of more than 10000 phenolic molecules and the list continues expanding**(Vazquez et al., 2015).** These compounds are characterised by the presence of multiple hydroxyl groups on aromatic rings and are divided into two main categories, the flavonoids and non flavonoids, based on the number of phenolrings and the way in which these rings interact. The main classes include phenolic acids and flavonoids **(Vauzour, 2017).**

- These phenolic compounds are present in different *jujube* tissues at various concentrations, as detailed in Table 1

Table 1: Contents of majors phenolics compounds of *Z. lotus* (Pulp, Seeds, and leaves).

Plant tissue	**Main component identified**	Content in mg/ 100g FM	**References**
Pulp	- Total phenols - Flavonoids - Tannins - Vitamin C	325 173 922 190,65	**(Abdeddaim et al., 2014, Abdoul-Azize et al., 2013, Benammar et al., 2010).**
Seeds	-Total phenolic acid -Total phenol -Vitamin C	25.24 14.68 170,84	**(Abdoul-Azize et al., 2013, Benammar et al., 2010, Chouaibi et al., 2012).**
Leaves	- Total phenols - Flavonoids - Vitamin C - Tannins	23 – 340 52.101 63,40 39	**(Bouaziz et al., 2009, Benammar et al., 2010, Lupea et al., 2008, Ghazghazia et al., 2014).**

I.5.Pharmacological activities

Many authors annunciated that the different parts(roots, leaves ,fruits, seeds) of *Z. lotus* have a great potential like, plants medicinal, with antimicrobial activities **(Bouaziz et al., 2009, Cheurfa et al., 2017, Ghazghazia et al., 2014, Naili et al., 2010, Rsaissi et al., 2013, Saiaha et al., 2016)** litholytic Activities **(Khouchlaa et al., 2017)** gastro-protective properties **(Bakhtaoui et al., 2014)** activation of immune system **(Abdoul-Azize et al., 2013, Benammar et al., 2010)** antidiabetic and antioxidant activities **(Benammar et al., 2014, Borgi et al., 2007, Chouaibi et al., 2012, Ghalem et al., 2014, Ghazghazia et al., 2014, Mkadmini hammi et al., 2015, Ourzeddine et al., 2017).**

II. Extraction methods of phenolic compounds

Extraction of phenolic compounds from plants is a crucial step for the valorization of these bioactive compounds. They depend on the method and the appropriate solvent that preserves their biological properties **(Mahmoudi et al., 2013).** Among of these methods:

II.1. Conventional extraction techniques (CE)

II.1.1. Maceration extraction (ME)

Maceration extraction (ME) is a conventional method frequently used in the extraction of phenolic compounds. The procedure consists in putting the sample in a solvent for a certain period of time and at a specific temperature. It is a simple technique, but very often requires long time periods (about 4 to 10 days) **(Albuquerque et al., 2017).**

II.1.2. Extraction by mechanical agitation

Extraction by mechanical agitation is a simple and low-cost method uses agitation or mixing action to extract the phenolic compounds. It consists of directly contacting a solid matrix in a suitable solvent (water, ethanol, methanol, acetone) under mechanical agitation at a given temperature for a period of time ranging from a few minutes to a week **(Lau et al., 2010).**

II.2. Novel extraction techniques

Modern extraction techniques are a major factor inflencing the qualité of extract and extraction yield. Among these methods :

II.2.1. Ultrasound assisted extraction (UAE)

Ultrasounds can be considered as the air vibrations of a frequency from 20 kHz to 100 MHz, and also caused by the mechanical waves propagated in solids, liquids and gases other than air. It is defined as sound waves chaving frequency that exceeds the hearing limit of the human ear (20 kHz) **(Azmir et al., 2013).**UAE is based on the principle of acoustic cavitation where, the propagation of the ultrasonic waves will generate a series of compressions and rarefactions in the molecules of the present solvent causing the formation of bubbles as a result of changes in temperature and pressure **(Fig.3) (Vilkhu et al., 2008).**

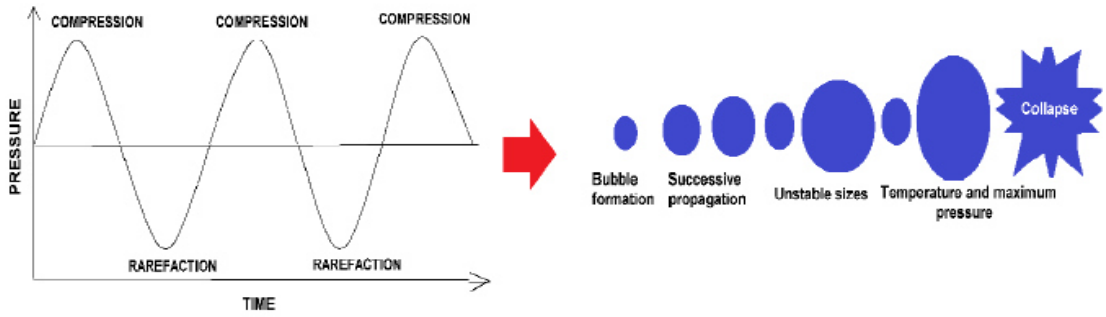

Figure 3: The principle of acoustic cavitation **(Medina-Torres et al., 2017).**

In general, several mechanisms involved in UAE have been identified. One of the positions of our mechanism is the fragmentation attributed to the collisions between particles and ultrasonic waves, which cause a reduction in the particle size, thereby facilitating mass transfer. Another is erosion which helps to improve the accessibility of the solvent by imploding the bubbles on the surface of the plant matrix. Sono-capillarity and Sonoporation, are able to improve the penetration of liquid through the channels produced by the bubble implosion and the alteration of the permeability of the cell membranes, respectively. Finally, the sheer stress mechanism produces the collapse of the cavitation bubble into the fluid, due to the oscillation phenomenon **(Fig.4) (Chemat et al., 2017).**

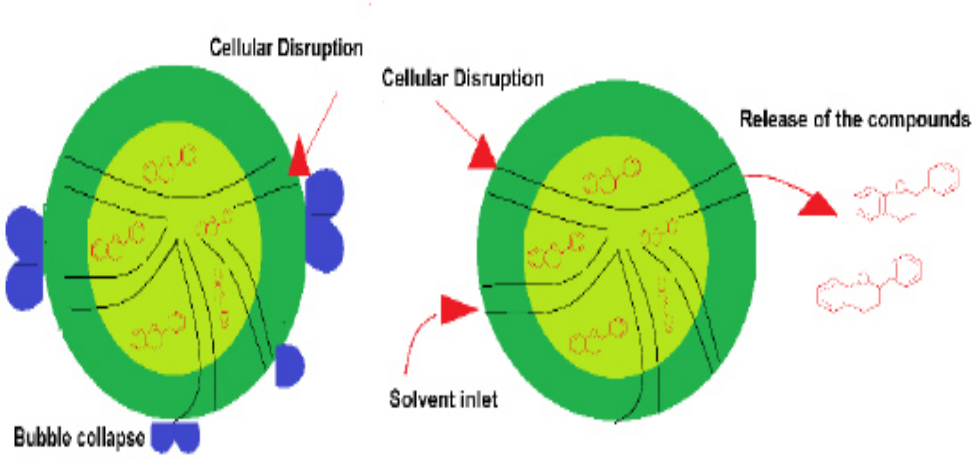

Figure 4: The mechanism of cell wall disruption (a) breaking of cell wall due to cavitation, (b) diffusion of solvent into the cell structure **(Medina-Torres et al., 2017).**

II.3. Microwave Assisted Extraction (MAE)

Microwaves are non-ionizing electromagnetic waves lie between infrared radiation and radio waves with a frequency range of 300 MHz–300 GHz, belonging to high frequency

electromagnetic wave. In order to avoid interferences with official communications domestic and industrial or scientific microwave devices generally operate at 2.45 GHz **(Heng et al., 2013).**

Microwave-assisted extraction (MAE) is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample and to increase the mass transfer rate of the solutes from the sample matrix into the solvent **(Shukla et al., 2013).**

The extraction of bioactive compounds from plants using microwaves has been the subject of considerable works published in scientific journals. Currently, the most widely are solvent-assisted microwave extraction "MAE", Microwave Assisted Processing "MAP" and microwave hydrodistillation under vacuum or vacuum microwaves. hydrodistillation "VMHD" and air training **(Bouaoudia-Madi et al., 2017, Chan et al., 2011, Dahmoune et al., 2015, Felkai-Haddache et al., 2016).**Furthermore, the principle of heating by microwave is based on direct impact between polar materials and solvents. It is governed by two phenomenon's: ionic conduction and dipole rotation **(Mandal et al., 2007).**

Compared to conventional heating method, the microwave heating results of the conversion of the energy of into materials by dipolar polarization, ionic conduction. However, in conventional heating, heat transferred to the sample volume by conduction or convection heating **(Gude et al., 2013).(Fig.5)**

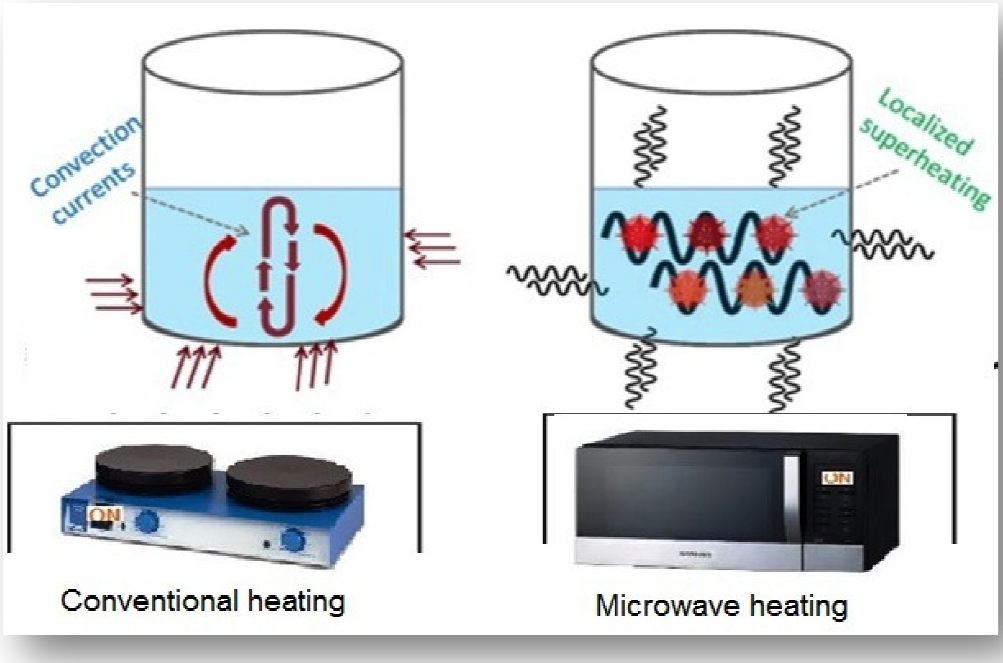

Figure 5: Conventional and microwave heating mechanisms **(Gude et al., 2013).**

II.4. Advantages and disadvantages of different extraction techniques

All extraction techniques have advantages and disadvantages (Table 2).

Table 2: Advantages and disadvantages of novel and conventional extraction technologies.

Methods	Advantages	Disadvantages	References
Ultrasound assited extraction	Easy to handle; Safe(atmospheric pressure and ambient temperature); Moderate use of solvent; Reproducible; Reduction of the extraction time; Extraction yield important.	Required filtration step. Possible degradation of compounds at high frequencies	**(Medina-Torres et al., 2017, Samaram et al., 2013).**
Microwave assisted extraction	Fast; easy to Handle. Moderate use of Solvent; High extraction efficiency; Less solvent consumption; Consume me energy; Respect the environnement	Risk of Explosion, Expensive, required filtration step	**(Zhang et al., 2011).**
Conventional assisted extraction	Not use of sophisticated equipment; Good extraction efficiency; Simple to make	Risk to degradation of thermos-labiles Compounds; Consumes a lot of solvent; Long extraction time; Evaporation of the solvent; Limited choice of solvent, and risk of pollution.	**(Lau et al., 2010, Medina-Torres et al., 2017).**

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Chapter II Experimental Work

The natural phenolic compounds have received increasing interest in the last years, since a great amount of them can be found in plants and consumption of vegetables and that are known to have several health-benefitting properties, including reducing the risks of certain types of cancer, cardiovascular, heart and neurodegenerative diseases due to their antioxidant power **(Song et al., 2009).** In the present study, the total phenolic compounds, flavonoids concentrations and antioxidant activity of *Z. lotus* fruits were determined after the ultrasonic-assisted extraction (UAE), but what are the optimal extraction conditions of these substances?

II.1. Chemical reagents and equipment

All chemicals used were of analytical grades. The reagents used in the experiments are collected in Table 3.

Table 3: The reagents used in the present work.

	Chemicals	Purity%	Provider
Extraction	Methanol	99.7 %	SIGMA-ALDRICH
	Ethanol	96 %	Honeywell
	Acetone	99.5 %	BIOCHEM Chemopharma
	Distilled water	-	-
	Sodium hydroxide	98 %	CHEM-LAB NV
	chlorhydricacid	37 %	SIGMA-ALDRICH
Quantification of phenolic compounds	Folin-Ciocalteu	-	SIGMA
	Sodium carbonate anhydrous	96.9 %	BIOCHEM Chemopharma
	Aluminium chloride hexahydrate	97-102%	BIOCHEM Chemopharma
	Gallic acid	-	BIOCHEM Chemopharma
	Quercitin	-	BIOCHEM Chemopharma
Antioxydant Activity	DPPH	-	SIGMA-ALDRICH

The various apparatus used for analysis are listed in Table 4.

Table 4: Apparatus used for this present investigation.

Apparatus	brand
Precision balance	KERN 440-35N
Spectrophotometer UV-Vis	OPTIZEN 3220UV
Oven	Memmert
Ventilated oven	Venticell
Grinder	IKA A11 BASIC, Germany
Sieving	Retsch AS 400
Microwave	SAMSUNG, ME6124ST, Malaisie
Ultrasound apparatus	J.P.SELECTA, s.a
Magnetic stirrer	Stuart, Stir SB161. Magnetic motion MIX 15 eco
Water bath	Nuve bath
pH meter	METTLER TOLEDO

II.2. Plant material

- **Drying and Grinding**

Fresh *Z. Lotus* fruits were harvested from Djelfa city (Algeria) in august 2017 **(Fig.6).** The fruits have been well washed with tap water followed by distilled water to remove all impurities, drying was carried out in two stages, first a pre-drying in the open air to remove excess water, followed by drying in an oven. Representative samples of 10 g (triplicate) are brought to 103 ± 2°C until constant weight, to calculate the moisture content, while the other samples of the same weight and the rest of the harvest were dried in an oven at 40 °C equipped with ventilation in the dark about a week to protect the active compounds content from light oxidation **(Doymaz, 2004).** The samples are brewed every day, especially at the beginning of drying to facilitate it. After confirmation of the humidity test, dried samples were ground with an electrical grinder, the powder was passed through standard sieve and the fractions with particle sizes between 500µm and 50µm were used. The powder was stored in airtight bags until use.

The moisture content (MC) was calculated by expressing the weight loss upon drying as a fraction of the initial weight of sample used.

$$\boldsymbol{H\%} = \frac{(\boldsymbol{M_1} - \boldsymbol{M_2})}{\boldsymbol{P}} * \mathbf{100}$$

Where:

H%: Water content (%)

M_1: Mass capsule + fresh matter before drying in g,

M_2: Mass of the unit after drying g ,

P: Mass test specimen in g.

Figure 6: Photography of *Z. lotus* fruit.

II.3. Experiment design and extraction of phenol compounds

The experimental design was divided into two major parts. Firstly a screening design using Plackett- Burman Design (PBD) to determine the appropriate range of conditions for *Z. lotus* phenolic extraction and treating all the factors influencing extraction yields. After that, we use Fractional Factorial Design (FFD) to optimize the parameters of *Z. lotus* fruits extraction to obtain an extract with high total phenolic compound, flavonoids and antioxidant capacity.

II.3.1.Plackett-Burman Design (PBD)

The Plackett-Burman Design is screening designs based on Hadamard matrices. It completes the complete 2^k factorial designs and the 2^{k-p} fractional designs.

As with factorial designs, the PBD take only two factors -1 and +1 levels and there are usually no central points because we do not try to model the results.

II.3.1.1. Preparation of the experimental plan

II.3.1.1.1. Description of the study

PBD is one of statistical experimental designs used to identify the important factors among a large number of variables that influence extraction of TPC, TFC from *Z. lotus* fruits.

II.3.1.1.2. Response

PBD assay was used to select the deferent parameters that influence the extraction of TPC, TFC from *Z. lotus* fruits.

II.3.1.1.3. Goal of the study

The main purpose of PBD assay is screening all influencing factors on the extraction of phenolic compounds of Z. *lotus* fruits (Table 5).

Table 5 : The coded values and corresponding actual values of extraction parameters used in screening study.

Factors	Level -1	Level +1
Ethanol concentration (%)	10	90
Liquid to- solid ratio (mL/g)	20 : 1	50 : 1
pH	4	10
Time (min)	5	30
Ultrasonic pulsation (Con/Dis)	Continue	Discontinue
Temperature (°C)	20	60
Particle size (µm)	50	500

II.3.1.1.4. PBD matrix

There are seven factors influencing the quantities of total phenolic compound, flavonoids of *Z. lotus* fruits. So the screening design chosen is PBD assay which has 12 tests in triplicate (Table 6).

The factors that have been considered are reported in the table below with specific levels for each test:

Table 6: The actual values of extraction parameters used in screening study.

Test	Time (min)	Particle size (µm)	Solvent (%)	pH	Ratio liquid-solid (mL/g)	Ultrasonic pulsation (Con/Disc)	Temperature (°C)
1	30	500	10	4	20 : 1	Continue	20
2	30	50	10	4	50 : 1	Discontinue	20
3	30	50	90	10	50 : 1	Discontinue	20
4	30	500	90	4	20 : 1	Discontinue	60
5	5	50	90	4	20 : 1	Continue	20
6	5	500	90	10	20 : 1	Discontinue	20
7	5	500	10	4	50 : 1	Discontinue	60
8	30	50	10	10	20 : 1	Continue	60
9	5	500	10	10	50 : 1	Continue	20
10	30	500	90	10	50 : 1	Continue	60
11	5	50	90	4	50 : 1	Continue	60
12	5	50	10	10	20 : 1	Discontinue	60

II.3.2. Fractional Factorial Design (FFD)

On the basis of Plackett-Burman Design, major influence factors were selected. Then, a Fractional Factorial Design was conducted to optimize the UAE process.

- **Choice of the experimental design**

A 2^{6-2}plan is constructed from a base plan 2^4 that has **four** main factors and **tow** factors that are related to interactions. In our case we have six factors; we will use the first four columns to study four factors.

II.3.2.1. Preparation of the experimental design

II.3.2.1.1. Description of the study

In the present study, a Fractional Factorial Design (FFD) was examined for optimization of UAE process parameters (ethanol concentration, extraction time, and liquid-to-solid ratio, particle size, temperature, ultrasonic pulsation) to obtain maximum yield of total phenolics, flavonoids and antioxidant activity of UAE extracts from *Z. lotus* fruits.

II.3.2.1.2. Factors

The levels used in FFD assay as reported in Table 7.

Table 7: The levels values of the optimization parameters used in UAE.

Factors	Level -1	Level +1
Ethanol concentration (%)	10	90
Liquid to- solid ratio (mL/g)	20 : 1	50 : 1
Time (min)	5	30
Ultrasonic pulsation (Con/Dis)	Continue	Discontinue
Temperature (°C)	20	60
Particle size (µm)	50	500

II.3.2.1.3. FFD matrix

The factors that have been considered in the FFD are presented in Table 8 with specific levels for each test (16 tests in triplicate):

Table 8: The actual values of extraction parameters used in optimization process.

Test	Solvent (%)	Ratio liquid-solid (mL/g)	Time (min)	Ultrasonic pulsation(Con/Disc)	Temperature (°C)	Particle size (µm)
1	10	50 : 1	30	Disontinue	60	500
2	90	50 : 1	5	Discontinue	60	500
3	10	20 : 1	5	Continue	20	500
4	90	20 : 1	5	Discontinue	20	500
5	90	50 : 1	30	Continue	60	500
6	90	20 : 1	30	Continue	20	500
7	10	50 : 1	5	Continue	60	500
8	90	20 : 1	5	Continue	60	50
9	90	20 : 1	30	Discontinue	60	50
10	10	20 : 1	30	Discontinue	20	500
11	90	50 : 1	5	Continue	20	50
12	10	20 : 1	5	Discontinue	60	50
13	10	50 : 1	30	Continue	20	50
14	10	50 : 1	5	Discontinue	20	50
15	90	50 : 1	30	Discontinue	20	50
16	10	20 : 1	30	Continue	60	50

II.3.2.1.4. Choice of the degree of experiment

In the case of the six factors, the basic plan 2^4, has four columns for the four factors, six columns for the second order interaction, four columns for the third order interaction, and one column for the fourth order interaction.

One might be tempted to choose the fourth-order interaction for aliasing the first additional factor, namely:

$$\mathbf{5 = 1234}$$

The other additional factor would be aliases on the order 3 interactions, for example:

$$\mathbf{6 = 123}$$

These choices lead to three independent alias generators:

$$\mathbf{I = 12345 = 1236}$$

The dependent generators are reckoned from these independent generators by multiplying them 2 - 2, 3 in 3 and 4 in 4. We are not going to make all these calculations because the software can make them for us. But he is interesting to calculate one or two well chosen:

$$\mathbf{12345 * 1236 = 456}$$

We see that we get a three-digit generator. The chosen plan is therefore a resolution plan III, which means that main factors will be aliased to second order interactions. This is not very good. Is it possible to achieve a design where main effects are confounded with 3-factors interactions .For this, it is necessary to alias the additional factors on the interactions of order three or four

$$\mathbf{5 = 234}$$

$$\mathbf{6 = 134}$$

We obtain three independent alias generators:

$$\mathbf{I = 2345 = 1346}$$

When calculating the dependent generators from these independent generators, there are no generators containing less than four digits. The design is therefore of resolution IV, which means that no main effects are aliased with two-factor interactions, but two-factor interactions are aliased with each other. If the first hypothesis of interpretation is acceptable (negligible third-order interaction), we obtain directly the coefficients, therefore the effects of the main factors and not the contrasts.

II.4. Ultrasound assisted extraction

The dried peel powder was extracted in an ultrasonic apparatus with working frequency fixed at 20 kHz**.** For the extraction, 1 gram of the powder was placed in an amber glass bottle containing the extraction solvent **(Dahmoune et al., 2013).** The suspension was exposed to acoustic waves under variations of ethanol concentration, sonication time and liquid-to-solid ratio, temperature, pH, particle size, ultrasonic pulsation. The temperature was controlled continuously. After the extraction, the solution was filtered through filter paper and was centrifuged at 10000 x g for 10 min the supernatants were collected for the analytical determinations.

Figure 7: UAE equipment used during extraction of phenolic compounds (Photo from Lab. N 08).

II.5. Microwave assisted and Conventional extraction

Microwave assisted extaction (MAE) and Conventional extraction (CE), made for comparison, was carried out in the exactly same conditions without ultrasound and microwave parameters. Thus,1 gram of *Ziziphus lotus* L powder was extracted with solvent during 5 min and 24 h for MAE and CE respectively **(Dahmoune, Nayak et al. 2015).**

Figure 8: Equipment used in MAE and CE (Photo from Lab. N 08) .

II.6. Analytical determinations

II.6.1.Total phenolic content

The determination of TPC in the extracts were done according to the method of **(Georgé et al., 2005).**Oxidations of phenolic compounds with this reagent include reaction with the mixture of $H_3PW_{12}O_{40}$ and $H_3PMo12O_{40}$ acids in the alkaline medium. At this reaction a mix of blue oxides is formed **(Lapornik et al., 2005).**A volume of 500 µL of diluted fruits extract with distilled water was added to 2.5 mL of 10-fold diluted Folin–Ciocalteau reagent. The solution was mixed and incubated at room temperature for 2 min. After 2 min, 2 mL of 7.5% sodium carbonate (Na_2CO_3) (v/v) were added. After incubation at 50° C for 15 min, the absorbance of the sample was measured at 760 nm against a blank (made as reported for the sample) byusing a UV–VIS Spectrophotometer. The assay was performed in triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid, (R^2= 0.999). The TPC were expressed as mg of gallic acid equivalent (GAE) per gram of powder on dry weight (DW) basis (Appendix 1).

II.6.2. Total flavonoids content

The total flavonoid contents were estimated according to the aluminum chloride method of **(Adedapo et al., 2008)** based on the formation of a complex flavonoid-aluminum. Briefly, 1 mL of extracts was mixed with 1 mL of 2 % $AlCl_3$. After 15 min of incubation in the dark, the absorbance of the mixture was determined at 430 nm. Each analysis was carried out in triplicate. The total flavonoid content was calculated from a calibration curve was generated with the standard solution of quercitin, (R^2= 0.9996). The TFC were expressed an mg of quercitin equivalent (EQ) per gram of powder on dry weight (DW) basis (Appendix 2).

II.6.3.Phenols index

The phenols index is a method based on the direct measurement of the absorbance at 280 nm of the various diluted extracts **(Spigno et al., 2015).** The results are expressed in absorbance unit.

II.7. Antioxidant activity

II.7.1. Scavenging activity against the DPPH* radical

The radical-scavenging activity of samples was evaluated by the DPPH• assay. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidant (Ar OH) with concomitant formation of a colorless hydrazine (DPPH-H) **(Molyneux, 2004).** The free radical-scavenging activity (RSA) of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract. The free radical-scavenging activity (RSA) was measured, following **(Achat et al., 2012)** method modified briefly, 25ul of extract was added to 1ml of DPPH solution (60 µmol/L in methanol) and the mixture was left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol instead of sample solution was used as a control.

The total RSA of each extract was expressed as the percentage of DPPH reduced and was calculated by the following equation:

$$\% \textbf{ Inhibition} = \textbf{RSA} = \frac{\textbf{A0} - \textbf{A}}{\textbf{A0}} \times \textbf{100}$$

Where:

$\mathbf{A_0}$: absorbance of DPPH solution without any antioxidant;

A: absorbance of DPPH solution after reaction with the extract.

All experiments were performed in triplicate.

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Chapter III
Results
And discussion

III.1. Evaluation of moisture content

The results of the moisture test show that the powder tested has a humidity of 10.67%.This indicate that our powder is poor in water. **Kim et al., (2013)** have reported in their studies a water content value of 36.56% which is more than that of our sample. Furthermore, Similar results was found by **(Kumar and Suneetha, 2014),**who obtained a water content value of 12.4%. Thus, According to **Ruiz-Rodriguez et al., (2011)**, the water content can vary from one fruit to another by the influence of several conditions such as: the geographical distribution of the fruits, the different conditions imposed by the environment, as well as their exposures to different conditions soil and climate.

III.2. Screening study: Plackett and Burman Design (PBD)

In this part, screening study was performed in order to determination of suitable parameters extraction to obtain the maximum TPC and TFC yield from *Z. lotus* fruits to be considered during the optimization process.

The effects of several influential extraction parameters; ethanol concentration, extraction time, and liquid-to-solid ratio, particle size, pH, temperature, ultrasonic pulsation were systematically studied for set-up of the optimal extraction conditions for TPC and TFC yield from *Z. lotus* fruits. The results are shown in Table 9.

Table 9: Results of PBD assay for UAE, expressed as mg $_{(GAE/100g\ DW)}$ and mg $_{(EQ/100g\ DW)}$ for TPC, TFC and PI respectively.

Extract	TPC	TFC	PI
1	1318.74 ± 107.50	366.90 ± 10.23	0.42 ± 0.02
2	1752.64 ± 333.90	513.73 ± 30.72	0.18 ± 0.02
3	499.62 ± 139.81	294.38 ± 6.00	0.03 ± 0.02
4	759.65 ± 253.04	366.37 ± 2.40	0.17 ± 0.03
5	967.19 ± 127.61	198 ± 10.11	0.29 ± 0.17
6	57.95 ± 47.37	154.32 ± 5.05	0.01 ± 0.007
7	2213.26 ± 291.98	780.88 ± 21.90	0.28 ± 0.01
8	1584.88 ± 81.37	574.61 ± 52.95	0.74 ± 0.04
9	3778.66 ± 469.42	1056.25 ± 181.47	0.32 ± 0.007
10	976.12 ± 355.05	436.00 ± 8.18	0.01 ± 0.007
11	1666.17 ± 307.76	369.13 ± 19.40	0.10 ± 0.06
12	1575.70 ± 47.65	552.05 ± 265.50	0.81 ± 0.01

Results are reported as means ± S.D.

The results illustrated in Table 9 show that the phenolics compounds of Z. *lotus* fruits varies between 57.95 ± 47.37 -3778.66 ± 469.42 $mg_{(GAE/100g\ DW)}$for TPC and 154.32 ± 5.05 - 1056.25 ± 181.47 $mg_{(EQ/100g\ DW)}$ for TFC. The data of this study were near than with the study of **Ghazghazi et al.,(2014), Hammi et al.,(2015)**, who found that the TPC and TFC of Z. *lotus* fruits varies between 297- 4078.2 mg $_{(GAE/100g\ DW)}$ and122 $mg_{(EQ/100g\ DW)}$ .These results can be explained by internal factors, the degree of maturation of the fruits and storage time and extrinsic factors such as geographical and climatic factors, but also the genetic factors **(Khouchlaa et al., 2018).**

The effects of various extraction parameters on the TPC, TFC and PI yield were studied using a scatter plot matrix. It can be see that each scatter plot shows the relationship between a pair of variables as TPC-TFC, TPC-PI and TFC-PI **(Fig.9).**According to this figure, the all six pairs of variables are positively correlated. As one variable increases, the other variable increases too. The strongest relationship appears to be between TPC and TFC. The data points in the scatter plot for TPC and TFC are the most tightly clustered long an imaginary line.

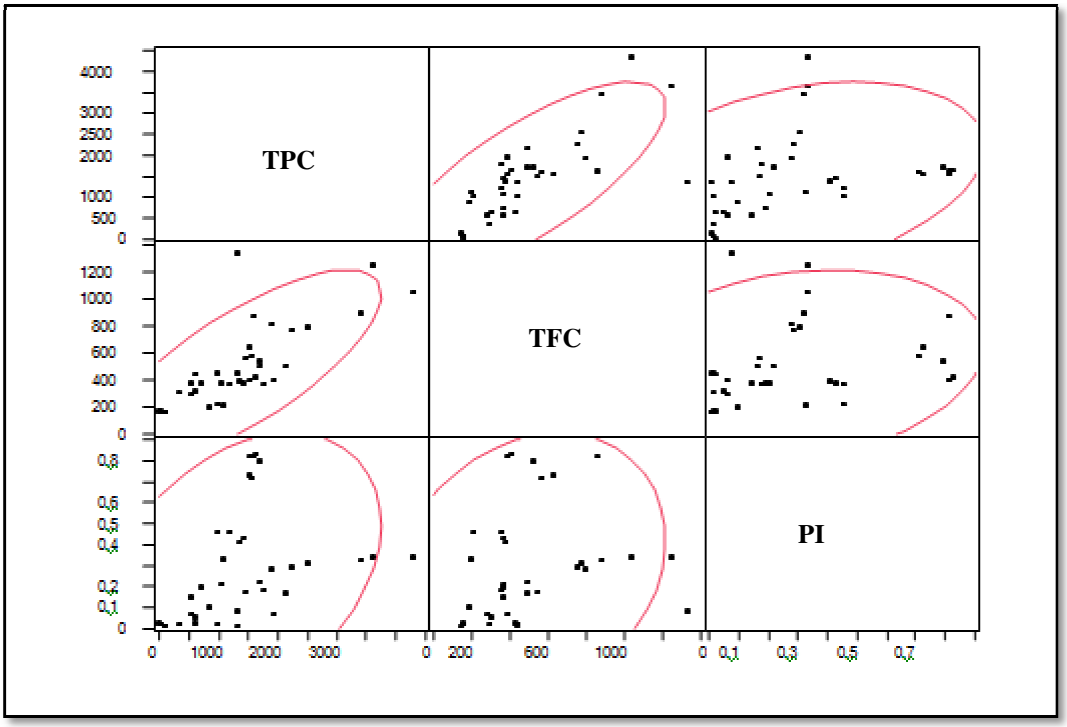

Figure 9: Scatter plot for TPC, TFC and PI from *Z. lotus* fruits.

The actual by predicted plot shows the actual TPC and TFC versus the predicted TPC and TFC. As the predicted values come closer to the actual values, the points on the scatter plot fall closer around the red line **(Fig.10).**

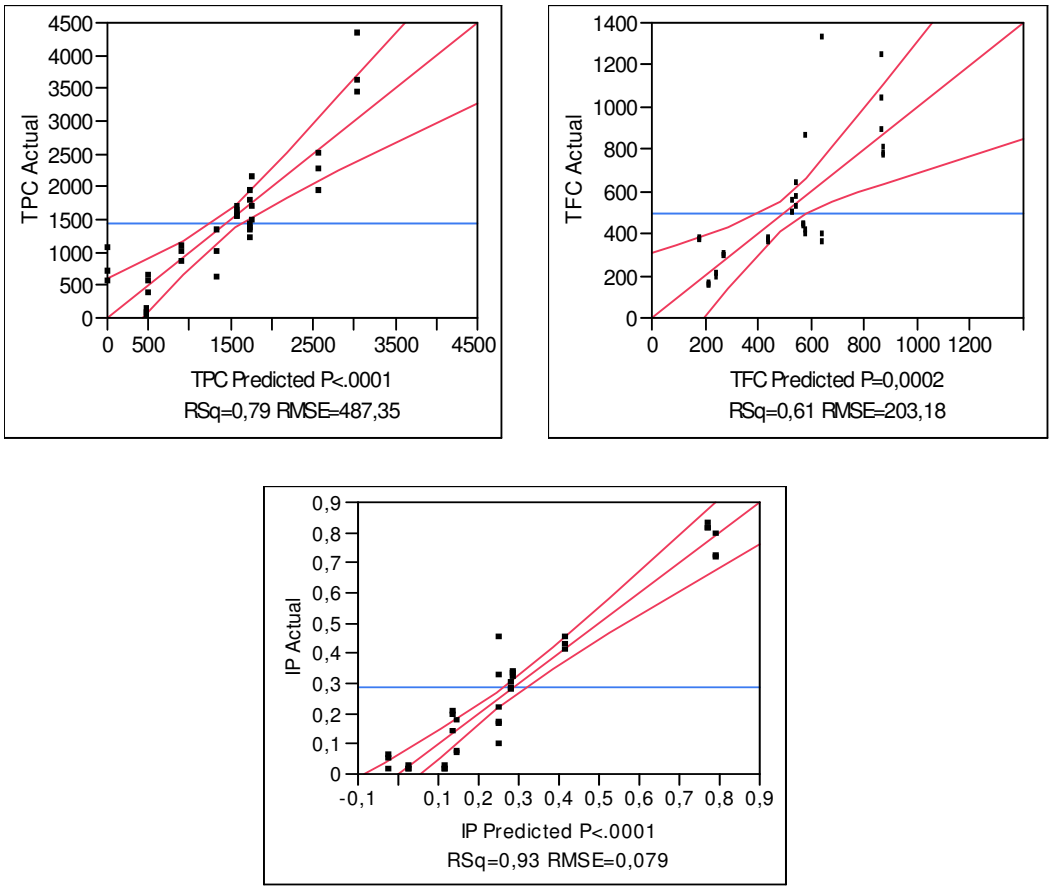

Figure 10: Comparison between experimental and predicted values of UAE content for TPC, TFC yields and PI.

According to the three graphs **(Fig.10),** it can be seen that that model terms have a high degree of correlation between the observed and predicted with $R^2$of 0.79, 0.61 and 0.93 for TPC, TFC and PI respectively.

III.2.1. Single factor analysis method for UAE

According to the results presented in the Table 10, the factors have an effect significant (p <0, 05) on the extraction of TPC, TFC from *Z. lotus* fruits are:

a) Ethanol concentration;

b) Liquid to- solid ratio;

c) Ultrasonic pulsation;

d) Extraction time.

Furthermore, the other factors such as temperature; particle size have no significant effect (p > 0.05) for TPC and TFC, but also can be an effect significant for PI (p <0,05) (Appendix 3), for this reason we considered that temperature and particle size have an effect during the optimization process. However, the pH has no significant effect (p > 0.05) on the extraction of TPC, TFC and PI.

Table 10: Screening study for selection of appropriate extraction conditions for TPC and TFC.

TPC			TFC		
Term	**Individual p-Value**	**Simultaneous p-Value**	**Term**	**Individual p-Value**	**Simultaneous p-Value**
Solvent (%)	**<.0001***	<.0001	**Solvent(%)**	**0.0096**	0.2088
Liquid to- solid ratio (mL/g)	**<.0001***	0.0005	**Liquid to- solid ratio (mL/g)**	**0.0155**	0.3010
Ultrasonic pulsation	**<,0001***	0,0033	**Time (min)**	0.1205	0.9676
Time (min)	**<.0001***	0.0040	**Temperature (°C)**	0.1473	0.9859
Size of particules (µm)	0.0783	0.8706	**Ultrasonic pulsation**	0.2387	0.9999
Temperature(°C)	0.5021	1.0000	**Size of particules (µm)**	0.5692	1.0000
pH	0.7410	1.0000	**pH**	0.7961	1.0000
Sol(%)* Liquid to- solid ratio (mL/g)	0.0017	0.0341	**Sol(%)* Liquid to- solid ratio(mL/g)**	0.4287	1.0000
Sol(%)* Ultrasonic pulsation	0.0043	0.1050	**Sol(%)*Time (min)**	0.4882	1.0000
*** Ultrasonic pulsation**	0.0042	0.1001	**Liquid to- solid ratio(mL/g) *Time(min)**	0.1345	0.9783
Sol(%)*Time (min)	0.0017	0.0343	**Sol(%)*Temperature(°C)**	0.3267	1.0000

a. Ethanol concentration

Extraction process was carried out at different ethanol concentrations of 10% and 90%. The phenolic compounds content of *jujube* mash is significantly affected by ethanol concentration ($p < 0.0001$). This result is related to the extraction solution polarity which the polarity of 10% ethanol solution may be similar to that of the phenolic compounds of *Z. lotus* , resulting in high extraction yield **(Liu et al., 2017)**. In addition, Water and low concentration of ethanol can easily get access to cells, but a high concentration of ethanol can cause protein denaturation, preventing the dissolution of polyphenols and then influencing the extraction rate **(Dahmoune et al., 2015) .** Similar results was found by **(Yang et al., 2009).**

b. Liquid to- solid ratio

As in other extraction techniques, liquid-to-solid ratio is an important parameter that influences the recovery of phenolic compounds **(Dahmoune et al., 2015).**The results illustrate that the phenolic compounds content of *jujube* mash is significantly affected by liquid to-solid ratio ($p < 0.0001$). This result can be explain by the phenomenon mass -transfer principle and the distribution of ultrasonic energy density in the extraction solutions**,** Which a high solvent to-solid ratio can facilitate the ultrasonic extraction process due to a high concentration gradient between the solid raw materials and the bulky solvent **(Zhao et al., 2014, Tao et al., 2014)**. Our results are in agreement with the works of **Hammi et al., (2015).**

c. Ultrasonic pulsation

The extraction process was carried out using ultrasonic pulsation continue and discontinue .The content of phenolic compounds content of *jujube* is mash significantly affected by ultrasonic pulsation ($p < 0.0001$). Generally, cavitation effect plays an important role in the process of ultrasonic extraction of phenolic compounds **(Zhang et al., 2017).** When, the extraction device is set to continuous mode the ultrasound is continuously delivered over time. This mode is used to obtain an essentially thermal effect. In other hand, when the ultrasonic device is set to discontinuous mode, the ultrasound is fractionally cut in time .This mode is used to suppress the thermal effect and retains only the mechanical effect **(Rezic et al., 2008).**

d. Extraction time

Extraction time is crucial in minimizing energy and cost of the extraction process **(Chew et al., 2011).**The results illustrate that the phenolic compounds content of *jujube* mash is significantly affected by extraction time ($p < 0.0001$). The data of this study are in agreement with the works of **Liu et al., (2013).**

e. Temperature

The results show that the phenolic compounds content of *jujube* mash is not significantly affected by temperature ($p > 0.05$). The temperature may increase the yield of extraction of phenolic compounds by different properties such as: viscosity, diffusivity, solubility and surface tension **(Dranca and Oroian, 2016).**In general, ultrasonic temperature has a impact on phenolic compounds but did not impact significantly **(Dailey and Vuong, 2015).** Our results are in agreement with the works of **Hayta and İşçimen., (2017).**

f. Particles size

The phenolic compounds content of *jujube* mash is not significantly affected by size particles ($p > 0.05$). In a general way, the size particles have an effect on the yields of phenolic compounds where the higher extraction efficiencies can be achieved by applying smaller particle sizes, which results in an increase in mass transfer surface and in quantity of soluble fraction **(Maksimovic et al., 2012).**

III.3.Optimization of UAE progress (FFD assay)

Fractional Factorial Design (FFD) is among the most important statistical contributions to the efficient exploration of the effects of several controllable factors on a response of interest. Two advantages of these designs have already been noted: a large number of factors may be studied in a relatively small number of runs. Additionally, each factor's effects are estimated with maximum precision **(Gunst and Mason, 2009, Voelkel, 2008).** In this study, FFD assay was performed in order to determination of suitable parameters extraction to obtain maximum yield of total phenolics, flavonoids and determined antioxidant activity of UAE extracts from *Z. lotus* fruits .The results are presented in Table11. Results are reported as means ± S.D.

Table 11: Results of optimization process.

Extract	TPC $mg_{(GAE/100g\ DW)}$	TFC $mg_{(EQ/100g\ DW)}$	RSA (%)
1	4673.43 ± 375.23	767.76 ± 18.59	91.37 ± 1.84
2	824.35 ± 158.12	408.47 ± 20.18	76.86 ± 4.03
3	1668.89 ± 172.91	341.20 ± 2.40	86.72 ± 0.13
4	947.42 ± 43.72	222.13 ± 57.73	75.18 ± 2.55
5	1101.42 ± 84.93	557.95 ± 94.76	77.73 ± 0.07
6	1260.86 ± 74.16	175.97 ± 11.05	87.11 ± 1.72
7	4172.22 ± 432.28	816.28 ± 17.14	86.72 ± 0.13
8	488.57 ± 114.96	136.63 ± 36.88	54.28 ± 7.11
9	1244.62 ± 32.87	311.30 ± 21.36	88.47 ± 1.93
10	2259.75 ± 124.09	487.54 ± 25.43	82.20 ± 0.88
11	1556.75 ± 350.09	383.55 ± 65.70	41.30 ± 0.82
12	1788.19 ±167.84	378.96 ± 29.74	88.11 ± 4.03
13	3501.59 ± 87.05	666.79 ± 18.02	89.30 ± 0.48
14	3540.41 ± 281.20	733.67 ± 88.66	77.73 ± 1.93
15	1052.01 ± 302.24	447.81 ± 43.51	76.14 ± 2.67
16	2023.97 ± 114.67	441.90 ± 58.71	85.10 ± 1.84

Statistically Different according to ANOVA and Tukey's test. TPC, TFC and RSA (%).

The results presented in Table 11show that the highest amount was attributed to 4673.43 ± 375.23 $mg_{(GAE/100g\ DW)}$ and 767.76 ± 18.59 $mg_{(EQ/100g\ DW)}$ for TPC ,TFC respectively. The TPC obtained in this study was so much higher than those obtained by **Chougui et al., (2015)**.

the phenolics compounds and

III.3.1. Analysis of variance (ANOVA) for optimization process

Table 12: The results of analysis of variance for the experimental results on TPC, TFC and antioxidant activity.

TPC		TFC		RSA	
Term	**Pseudo p-Value**	**Term**	**Pseudo p-Value**	**Term**	**Pseudo p-Value**
Solvent (%)	**<.0001***	**Liquid to- solid ratio (mL/g)**	**<.0001***	**Solvent (%)*liquid-solid (ml/g)**	**0.0539**
Liquid to- solid ratio (mL/g)	**0.0002***	**Solvent (%)**	**<.0001***	**Solvent (%)**	**0.0572**
Solvent (%)*liquid to- solid ratio (mL/g)	**0.0010***	**Time (min)**	0.2548	**Liquid to- solid ratio (mL/g)**	**0.0644**
Solvent (%)*Temperature (°C)	0.1660	**Ultrasonic pulsation *Temperature (°C)**	0.2836	**Temperature (°C)**	0.2540
Time (min)	0.2947	**Solvent (%)*Liquid-solid (ml/g)**	**0.3154**	**Time (min)**	0.4243
Solvent (%)*Particle size (µm)	0.2953	**Liquid-solid (ml/g)* Ultrasonic pulsation**	0.3305	**Solvent (%)*Time (min)**	0.4426
Particle size (mic)	0.3976	**Temperature (°C)**	0.3441	**Ultrasonic pulsation *Temperature (°C)**	0.5089
Liquid to- solid ratio (mL/g)* Time (min)	0.4115	**Particle size (µm)**	0.4659	**Particle size (µm)**	0.5122
Time (min)*Temperature (°C)	0.4850	**Solvent (%)*Time (min)**	0.5122	**Solvent (%)*Temperature (°C)**	0.5263
Solvent (%)*ultrasonic pulsation	0.5403	**Time (min)*Temperature (°C)**	0.5247	**Ultrasonic pulsation**	0.5482
Liquid-solid (ml/g)* ultrasonic pulsation	0.6056	**Ratio liquid-solid (ml/g)*Time (min)**	0.5274	**Time (min)*Temperature (°C)**	0.6681
Ultrasonic pulsation *Temperature (°C)	0.6429	**Ultrasonic pulsation**	0.5309	**Liquid-solid (ml/g)* Ultrasonic pulsation**	0.6726
Ultrasonic pulsation	0.7819	**Solvent (%)*Particle size** (µm)	0.7784	**Solvent (%)*ultrasonic pulsation**	0.8400
Temperature (°C)	0.7922	**Solvent (%)*ultrasonic pulsation**	0.9285	**Solvent (%)*Particle size (µm)**	0.8813
Solvent (%)*Time (min)	0.8237	**Solvent (%)*Temperature (°C)**	0.9806	**Liquid to- solid ratio (mL/g)* Time (min)**	0.9478
Liquid-solid (ml/g)*Temperature (°C)	/	**Liquid-solid (ml/g)*Temperature(°C)**	/	**Liquid-solid (ml/g)*Temperature (°C)**	/

The ANOVA was used to analyze the model for the significance of each coefficient and the interaction strength between each independent variable. The pseudo *p- value* less than 0.05 indicate model terms are significant (Table 12). In this case ethanol fraction and liquid to- solid ratio were the major factors affecting the extraction of TPC, TFC and antioxidant activity. However it can be seen that the interaction between ethanol fraction and liquid to-solid ratio is significant on the extraction of TPC and antioxidant activity with a probability p <0. 05. In other hand ,the interaction of ethanol concentration with liquid to- solid ratio is insignificant with the probability p = 0.31 on the TFC yield.

The reponses obtained after having performed the 16 trials of the experiment design in a **y**ate order will be processed using software JMP (Version 13) **(Fig.11).**

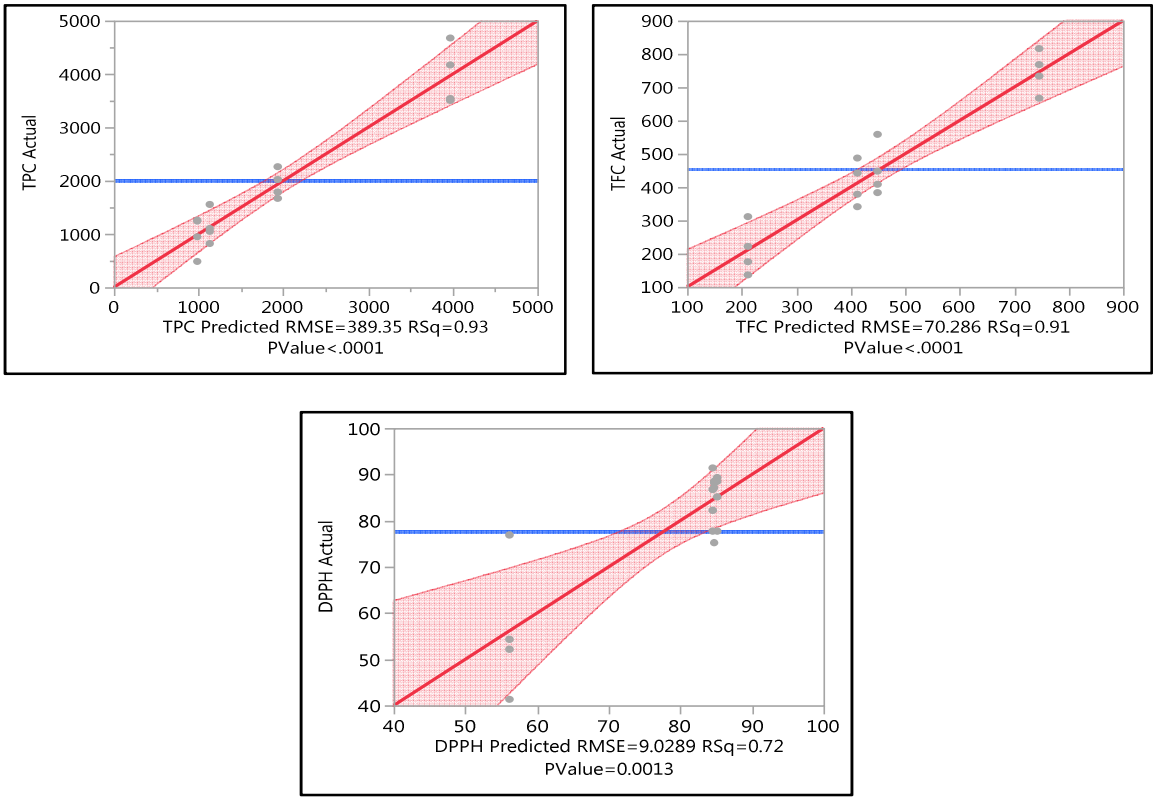

Figure 11: Comparison between experimental and predicted values of UAE content for TPC, TFC yields and DPPH scavenging.

As can be seen, the result of analysis of variance for the fitting model values of probability (P>F) less than 0.01 from TPC, TFC and DPPH scavenging indicate that model terms have a high degree of correlation between the observed and predicted data. Finally, we can conclude that the regression explains almost the total of the two measured responses since the significance of the risk is less than 10 %.

To evaluate the goodness of the models, different descriptive statistical analysis such as determination coefficient (R^2) were used. The R^2values for TPC, TFC and DPPH were 0.79 ,0.61 and 0.93 ,0.91 and 0.72 for PBD and FFD respectively, which implied that the sample variation was statistically significant at 79%, 93 % for TPC and 61%,91% for TFC and 72 for RSA(%) 21% ,39 % , and 7 % , 9 % , 28% respectively of the total variance could not be explained by the models.

III.3.2. Optimal extraction conditions

The aim of this study is to maximize the extraction yield of phenolic compounds of Z. *lotus* fruits. The desirability function makes it possible to determine in advance the optimal values of the factors studied in order to achieve this goal while maximizing the value of the function. The **Fig.12** show that the optimum conditions for extraction yield of TPC, TFC and antioxidant activity resulted in 10% ethanol and liquid to- solid ratio: 50:1 mL/g with a desirability value of 0.551. At this point, the investigated responses were theoretically calculated as TPC: 3971.918 mg GAE/100 g DW and TFC: 746.1317mg EQ/100 g DW, and antioxidant activity: 85.15%.

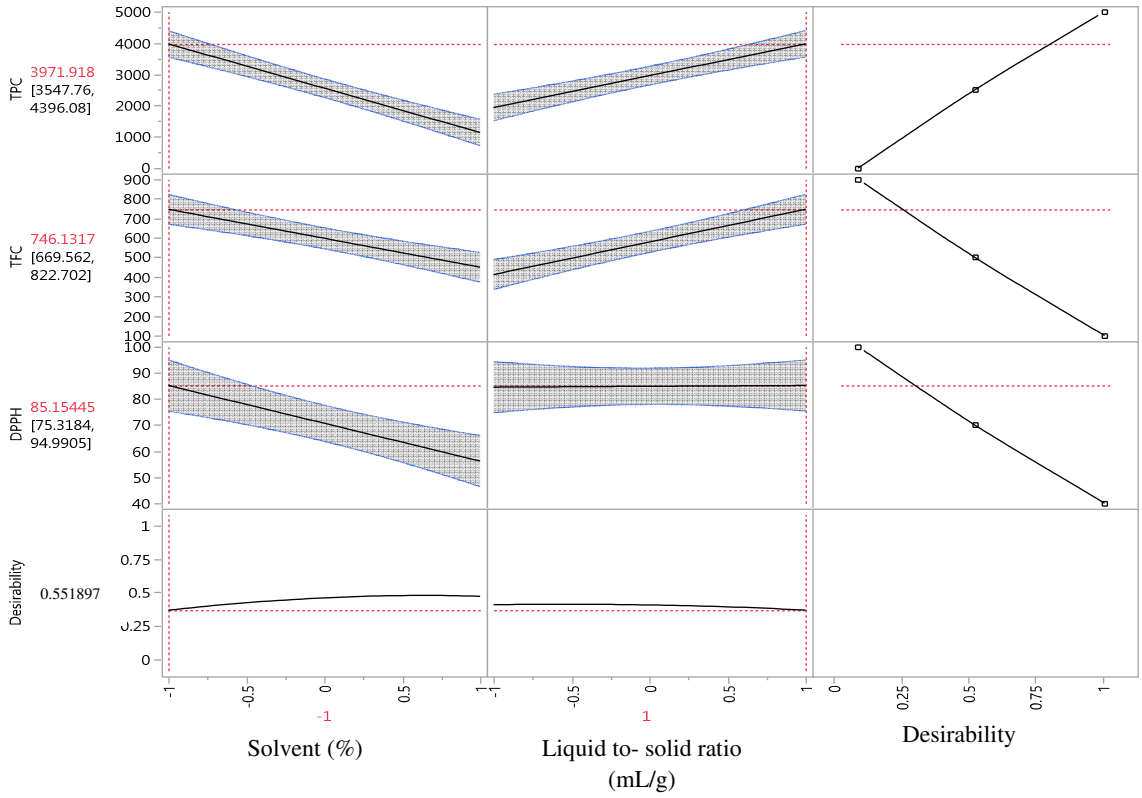

Figure 12: Profiles for predicted values and desirability function.

III.3.3. Validation of the models

The optimized conditions obtained by RSM were used to validate the predictive model of extraction for TPC, and antioxidant activity from *Z. lotus* fruits. Table 13 shows that experimental values are reasonably close to the predicted values confirming the validity and the adequacy of the predicted models. The experimental data were within 90% confidence interval of predicted values.

Table 13: Comparison between the predicted and experimental data of optimum conditions for TPC, TFC and antioxidant activity.

		TPC (mg GAE/100gDW)		**TFC (mg EQ/100 g DW)**		**RSA (%)**	
Solvent (%)	**Ratio (mL/g)**	**Prédited**	**Expérimental**	**Prédited**	**Expérimental**	**Prédited**	**Expérimental**
10	1/50	**3971.91**	**3640.42±281.20**	**746.13**	**753.7± 88.66**	**85.15**	**78.58 ± 0.71**

All values represent means± SD

III.3.3.Analysis of response surface

The 3D response surface is the graphical representation of regression equation. It provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables **(Zhong and Wang, 2010).** The effects of the independent variables and their mutual interaction on the extraction yield of TPC, TFC and antioxidant activity can be seen on three dimensional response surface curves shown in **Fig.13** and **Fig.14.**

III.3.3.1. Effect of interaction of ethanol concentration and liquid to- solid ratio on of TPC and TFC yields

The Fig.13 showed the 3D response surface, the combined effect of ethanol concentration and solid–liquid ratio on the extraction yield of TPC and TFC. The yields decreased significantly with an increase in ethanol concentration and liquid to- solid ratio. According to **Prasad et al., (2011)** the extraction of phenolic compounds depends largely on the polarity of solvents and the compounds, a single solvent might not be effective for the isolation of a bioactive compound. Hence, a combination of alcohol with water is more effective in extracting phenolic compounds than alcohol alone. This result was in accordance with the data obtained by **(Tao et al., 2014).** Similar results was found by **(Wang et al., 2014),**Who obtained a best extraction of Flavonoids from *Portulaca oleracea* by low concentration of ethanol

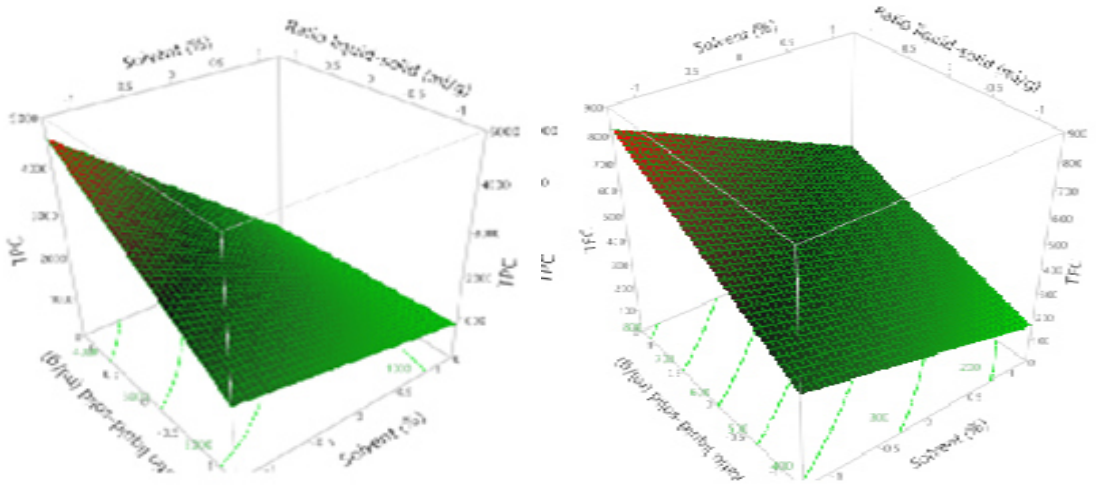

Figure 13: Surface plot of the TPC and TFC yields as a function of solvent and liquid to-solid ratio.

III.3.3.2. Effect of interaction of ethanol concentration and liquid to- solid ratio on antioxidant activity

Fig.14 is a response surface plot showing that the increase in ethanol concentration and liquid to- solid ratio enhanced the antioxidant activity of the extracts. The type of solvent and the polarity affect the transfer of electrons and atoms of hydrogen which is a key aspect in measuring antioxidant activity. Thus, the antiradical activity depends on the structure conformation of phenolic compounds to their electron transfer capacity / hydrogen donor **(Brand-Williams et al., 1995).** It's could be due to synergistic effects between various classes of antioxidants present in the extracts (phenolic compounds, flavonoids, tannins, vitamins…) **(López et al., 2010)**. This results obtained in this study was near than those obtained by **(Kim et al., 2013)** which was 89,42% from *jujube.*

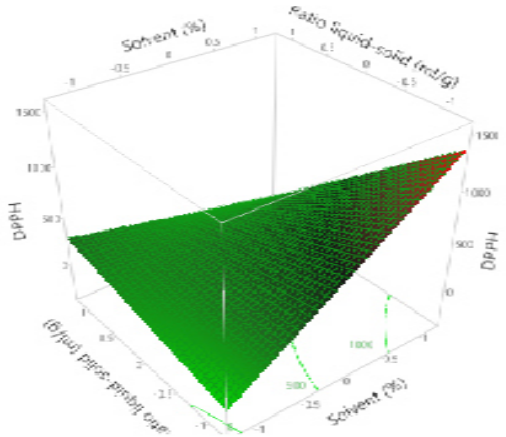

Figure 14: Response surface plot showing interaction between ethanol and liquid to- solid ratio for antioxidant activity**.**

The final mathematical models are expressed by Eq. 01, 02 and 03 represents TPC, TFC and RAS yields of *Z. lotus* (y_1, y_2 and y_3 respectively) as a function of concentration of solvent (X_1) and Liquid to- solid ratio (X_2).

$$Y_{TPC} = 2006.53 - 947.03X_1 + 546.23X_2 - 472.11X_1X_2$$

$$Y_{TFC} = 454.87 - 124.39X_1 + 142.91X_2 - 23.957X_1X_2$$

$$Y_{\%DPPH} = 77.63 - 7.2X_1 - 6.98X_2 - 7.30X_1X_2$$

III.3.5.Comparison between UAE and MAE and conventional extraction methods (CE)

The efficiency of TPC and TFC extraction and antioxidant activity using UAE was compared with MAE and maceration extraction by mechanical agitation. The conditions of different techniques were obtained with ethanol 50%, liquid to- solid ratio: 35:1 mL/g, for UAE and ethanol 50%, 30:1mL/g for MAE, extraction by mechanical agitation respectively and their results are summarized in Table14.

Table 14: Comparison of extraction yield of TPC, TFC and antioxidant activity from *Z. lotus* fruits by Ultrasound assisted extraction (UAE), Microwave assisted extraction (MAE), and conventional extraction (CE).

Extraction methods	Time	TPC (mg GAE/100g DW)	TFC (mg EQ/100 g DW)	RSA (%)
UAE	15 min	4730.38 ± 163.75	587.09 ± 30,36	84.84 ± 0.61
MAE	5 min	4553.51 ± 709.72	576.84 ± 324.44	85.78 ± 8.71
CE	24h	4434.02 ± 277.74	587.09 ± 30.36	83.39 ± 3.24

Results are reported as means ± S.D

The results of the two alternative extraction technologies, MAE and CE gave comparable TPC and TFC yields and antioxidant activity for fruits of *jujube* .However, these experiments indicated that ultrasonic assistance and microwave enhanced the efficiency of TPC and TFC extraction by shortening the extract time; it normally takes one day for conventional extraction and only few minutes for MAE and UAE. The extraction yield using MAE, reached a maximum in 5 min, revealed MAE was reported to be more efficient compared to other extraction methods such maceration ME and UAE (Table14).This higher efficiency was due to the heating mechanism of microwave. It offers a rapid transfer of energy to the extraction solvent and raw plant materials **(Bouaoudia-Madi et al., 2017).**

Moreover, Sonication also improved the extraction efficiency by and favoring the solubilization of the targeted compounds. This result can be explain by the ultrasonic cavitation phenomenon which is responsible of modifications on the plant material inducing a disruption of plant cell walls when the cavitation bubbles collapse at the surface of the solid matrix. Thereby, mass transfer is enhanced, and the contact surface area between solvent and plant material increases **(Meullemiestre et al., 2016, Dranca and Oroian, 2016).**

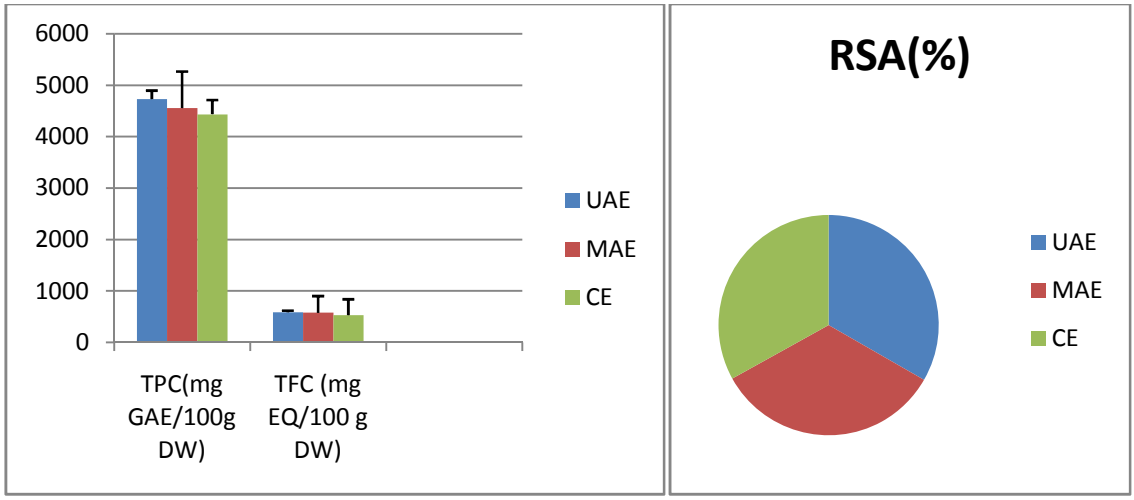

Figure 15: Comparison of bioactive components (TPC, TFC) yields and antioxidant activity between UAE and MAE and CE from *jujube*.

The statistical analysis of TPC, TFC yields and antioxidant activity revealed no significant differences ($p < 0.05$) between the extraction methods (UAE, MAE, CE) **(Fig.15).**The results obtained are: 4730.38 ± 163.75 (mg GAE/100gDW), 587.09 ± 30.36 (mg EQ/100 g DW), 84.84 ± 0.61 (%) and 4553.51 ± 709.72 (mg GAE/100g DW), 576.84 ± 324.44 (mg EQ/100 g DW), 85.78 ± 8.71(%) and 4434.02 ± 277.74(mg GAE/100g DW), 587.09 ± 30.36 (mg EQ/100 g DW), 83.39 ± 3.24 (%) for UAE , MAE and CE respectively. The same results were reported by **(Chan et al., 2011).**

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Conclusion And Perspectives

Conclusion

In this study, an efficient UAE technique was developed to extract phenolic compounds from *Z. lotus* fruits. The operating parameters were optimized using Plackett-Burman Design (PBD), Fractional Factorial Design (FFD) and response surface methodology (RSM).

PBD was successfully used to screening study where the appropriate experimental factors influencing extraction of TPC, TFC are ethanol concentration; liquid to- solid ratio; ultrasonic pulsation; extraction time. The FFD assay was performed to optimize the parameters extraction of *Z. lotus* fruits to obtain an extract with high phenolic compound and antioxidant capacity.

The optimum extraction conditions were as follows: Ethanol 10% and Liquid to- solid ratio of 50:1 mL/g).This conditions were attributed to 3640.41 ± 281, 20 mg (GAE/100g), 753, 67 ± 88.66 mg (EQ/100g DM) and an anti-free radical activity DPPH of 78, 58%. This result indicates that these conditions are the best condition of extraction yields of TPC, TFC antioxidant capacity of *Z. lotus* fruits.

MAE is more efficient than UAE and conventional extraction method to obtain maximum yields of TPC, TFC and antioxidant activity from *Z. lotus* fruits.

Concerning the antioxidant activity, a good correlation has been found between content of bioactive compounds and antioxidant activity. These results confirm the interesting potential of this plant as a valuable source of natural bioactive molecules in food and medical industry.

However, it would be desirable to complement this work with:

- Study the other biological activities (*In vivo* and *In viro*) of the optimal extract;
- Characterization of phenolic compounds present in extracts by HPLC MS;
- Characterization the other substances present in *jujube* fruits.

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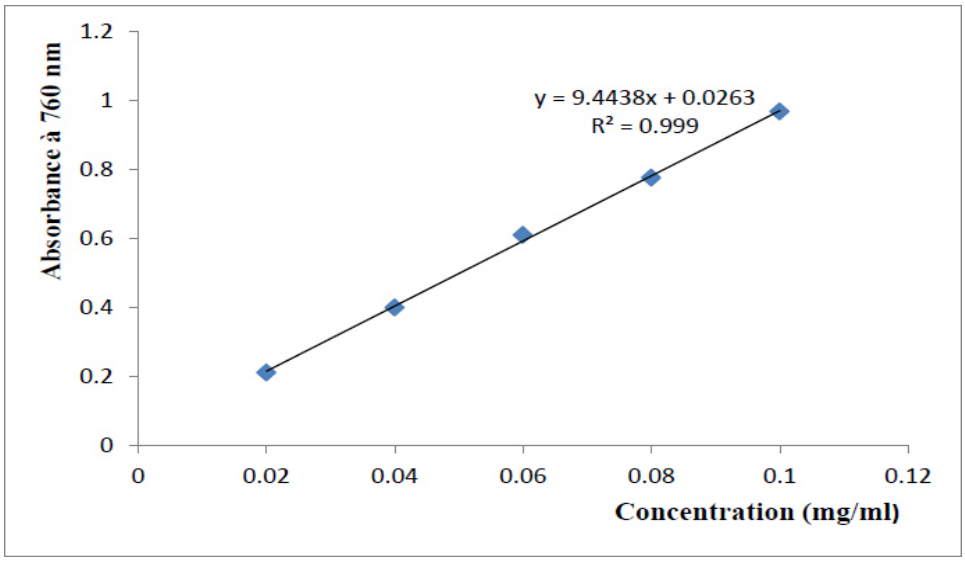
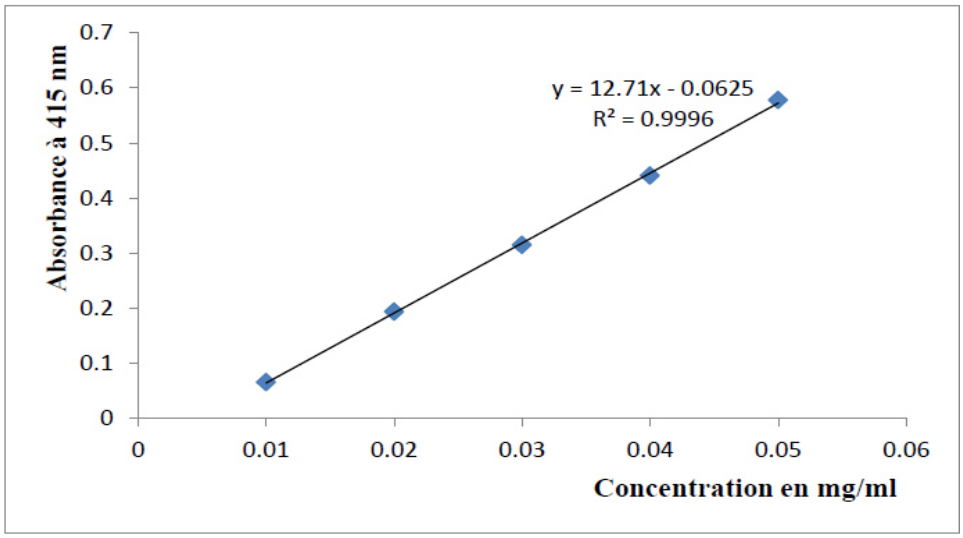
Appendix



Appendix 1: Calibration courbe of gallic acid.



Appendix 2: Calibration courbe of quercitin.

Appendix 3: Screening study for selection of appropriate extraction conditions for PI.

Term	Individual p-Value	Simultaneous p-Value
Solvent (%)	<,0001	<,0001
Liquid to- solid ratio (mL/g)	<,0001	<,0001
Size of particules (µm)	<,0001	0,0009
Temperature(°C)	<,0001	0,0015
pH	0,0032	0,0815
Ultrasonic pulsation (Con/Dis)	0,0109	0,2427
Time (min)	0,0644	0,7950
Sol(%)* Liquid to- solid ratio (mL/g)	0,4938	1,0000
Liquid to- solid ratio (mL/g)* Size of particules (µm)	0,0071	0,1935
Sol(%)*Size of particules (µm)	0,1413	0,9768
Sol(%)*Temperature(°C)	0,0090	0,2289

Abstract

Ziziphus lotus fruit is very rich in phenolic contents and present a high antioxydant activity. The aim of this study is optimization of extraction conditions of total phenolic compounds and total flanovoids content from Z. *lotus* fruit, using ultrasound assisted extraction (UAE). The content of phenolic compounds is given according to the method of Folin-Ciocalteu and the content of total flanovoids content were estimated according to the aluminum chloride method ,the antioxydant activity is determined by the measurement of free radical-scavenging (DPPH). The effect of two independent variables, ethanol concentration and liquid-to-solid ratio on the phenolic compounds yield and the antioxidant activity was estimated by using response surface methodology (RSM). The best condition was obtained with ethanol 10%, liquid to- solid ratio of 50:1 mL/g. These conditions resulted in total phenolic content concentration of 3640.40 ± 281.20mg (**GAE/100gDw**) and 753.67 ± 88.66mg (**EQ/100g Dw**), free radical-scavenging activity 78, 58%. The experimental values were reasonably close to the predicted values confirming the validity of the predicted models.

Keywords: *Ziziphus lotus*, optimization, phenolic compounds, ultrasonic, antioxydant activity, response surface methodology.

Résumé

Le fruit de *Ziziphus lotus* est très riche en composés phénoliques et présente une activité antioxydante élevé. L'objectif de ce travail est l'optimisation des conditions d'extraction des polyphénols et des flavonoïdes, en utilisant le plan Factoriel Fractionnaire. La teneur en composés phénoliques est déterminée selon la méthode de Folin-Ciocalteu et les flanovoids est estimé par la méthode chlorure d'aluminium, aussi leur activité antioxydante est déterminée par la mesure de l'activité anti-radical DPPH. L'effet de deux variables indépendants nommés concentration d'éthanol, ratio solide-liquide sur le rendement des composés phénoliques et l'activité antioxydante ont été estimée en utilisant la méthodologie de réponse de surface (RSM). Les meilleures conditions obtenues pour l'extraction des polyphénols et flavonoides totaux sont : le solvant éthanol 10%, ratio solide-liquide : 1 : 50 g/ml .Ces conditions permettent d'avoir une teneur en polyphénols de 3640.40 ± 281.20 mg (EAG/100g Ms) et 753.67 ± 88.66 mg (QE/100g Ms) et une activité anti-radical DPPH de 78,58%. Les valeurs expérimentaux sont proche des valeurs prédites ce qui confirme la validité du model mathématique.

Mots clés : *Ziziphus lotus*, optimisation, composés phénoliques, ultrason, activité antioxydante, méthodologie de réponse de surface.

ملخص

فاكهة النبق غنية بالمركبات الفينولية و لها نشاط نشاط بيولوجي كبير والهدف من هذا العمل هو تحسين شروط استخلاص البوليفينول و الفلانوفويد باستخدام الموجات فوق الصوتية. تحدد محتوى المركبات الفينولية وفقا لطريقة فلان سيوكالتو و الفلانوفويدات تم تقديرها بواسطة طريقة كلوريد الألومنيوم كما يتم تحديد نشاطها المضاد للأكسدة من خلال قياس النشاط المضاد للأكسدة بقياس الاستطاعة الارجاعية. تم تقدير تأثير اثنين من المتغيرات المستقلة ،تركيز الإيثانول ونسبة السائل إلى الصلب على إنتاجية المركبات الفينولية والنشاط المضاد للأكسدة باستخدام منهجية سطح الاستجابة.الظروف الجيدة لاستخراج المركبات الفيلونية هي ايثانول 10%, نسبة الصلبة-السائل1/50 غ/ل. هذه الظروف سمحت لنا باستخراج كمية البوليفينول تقدر 3640.40 مغ معادل حمض قاليك 100 /غ و و كمية الفلانوفويد تقدر ب 753.67.40مغ كرستين100/غ و نشاط مضاد للأكسدة يقدر ب 78.58%.القيم التجريبية قريبة من القيم المتوقعة والتي تؤكد صحة النموذج الرياضي.

كلمات المفاتيح: النبق, التحسين ,المركبات الفينولية ,الموجات فوق الصوتية ,النشاط المضاد للأكسدة, منهجية سطح الاستجابة.