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Response Surface Methodology (RSM) for Optimizing Ultrasound Assisted Extraction of Bioactive Phenolic from *Artemisia Annua L* (Asteraceae) and Kinetic Approach

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Artemisia annua, Central Composite Design, Kinetics model, Optimization, Response Surface Methodology

ABSTRACT

The objective of this work is to apply a sustainable extraction technique like ultrasound-assisted extraction (UAE) to optimize experimental conditions, with the aim of maximizing the recovery of total phenolic compounds from *Artemisia annua* leaves. To explain the phenology of the mass transfer in this extraction process, Hervas and Peleg kinetic models were investigated. Single, interaction, and quadratic effects of three independent variables on extraction yields were evaluated using a central composite design through a response surface methodology. The extraction process was carried out successfully under optimal conditions of 15.97 min for extraction times; 68.99 °C for the temperature and a liquid-to-solid ratio of 40:1 mLg-1. Among the several extraction solvents and techniques investigated during extraction process, UAE and ethanol produced the best results. The UAE provides a promising method to obtain the maximum extraction yield of 20.02 % and the best recovery of total phenolics content (TPC) of (76.28 ± 0.07) mg EAG/g of extract). Regarding the optimization results, liquid-to-solid ratio was the most significant factor influencing the extraction yield. Moreover, the extraction time and liquid-to-solid ratio were the two important extraction parameters influencing the extraction kinetics. The suitable kinetics models was the Peleg's mathematical with the maximum extraction quantities of secondary metabolites Co (0.013 mg EAG/g DW) and the extraction rate coefficients k (0.012 s-1). The goodness of the fit was controlled by the magnitude of the coefficient of determination (R^2), and the square root of the mean square error (RMSE).

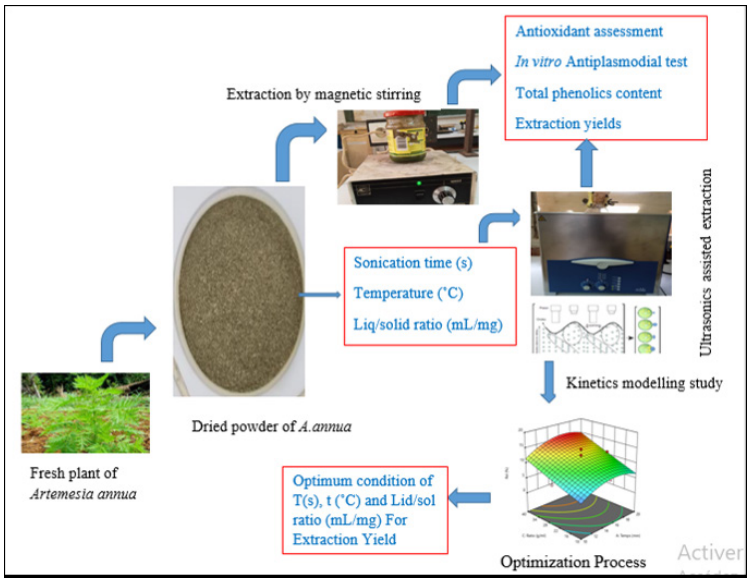

Figure 1: Graphical Abstract

INTRODUCTION

Artemisinin is a sesquiterpene lactone from *Artemisia annua* L. for Asteraceae family. Moreover, it is a current preferred antimalarial therapeutic molecule. *A. annua* belongs to the genus Artemisia, an aromatic plant renowned on the one side for its bioactive substances; and on the other side for its major constituent namely artemisinin used to fight against all the malaria causing protozoal organisms in the genus plasmodium. (Liu *et al.,* 2023). This medicinal plants, widely used in medicine is pharmacologically endowed with components active against several parasitic and other diseases. *A. annua*

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leaves have shown antifungal, antioxidant, and anti-inflammatory activities, stimulating digestion. Referring to cardiovascular diseases, the polyphenolic profile of the extract from A.annua L was assessed as a source of natural antioxidants. (Monika *et al.,* 2014). According to a specific botanical approach, *A. annua* is an annual herbaceous plant native to China, where it is known as Qinghao. It is a 4 species that can reach up to 2 m in height. Its glandular trichomes, present in the leave and flowers are the seat of artemisinin one of the main molecules endowed with active principles of the plant, others active constituents of *A. annua* L. are located in the glandular hairs, on the stems, the leaves, and the flowers (Sanogo, 2021). Several reviews have shown that the constitution of A.annua L varies from one geographical place to another and from one year to another because of the abiotic factors. Owing to the new attraction for natural products like polyphenolic compounds, flavonoids, alkaloids, they are all useful for a good health. Hence the need to subject this plant to extractive methods with different solvents in order to optimally benefit from its bioactive secondary metabolites. Developing, improving and optimizing a suitable extraction process of medicinal plants would ensure the quality of secondary metabolites with respective active ingredients. Moreover, an increased interest in this underscores the importance of choosing after a good investigations an appropriate extraction methods using different solvents to optimally recover these bioactive secondary metabolites. Investigating, improving, and optimizing extraction processes for medicinal plants is essential to ensure the quality and potency of bioactive compounds. Then Response Surface Methodology (RSM) is suitable for investigating responses influenced by independent variables, with their linear, interactions, and quadratic effects as originally described by Montgomery (2001).

In the perspective of extraction, liquid–solid extraction involves transferring one or more solutes from a solid matrix into an extraction solvent. Designing an efficient extraction process requires solving complex challenges, such as modeling, to better understand and explained the kinetics and phenomenology of extraction process (Herzi *et al.,* 2013). Due to the numerous biological activities of Phenolic compounds, they are widely used for their antioxidant, antibacterial, antiviral, antidiabetics and anticancer activities. There are several extraction methods such as maceration, reflux extraction, Soxhlet extraction and supercritical fluid extraction called conventional method. However, these methods are either time-, energy- and solvent-intensive or too expensive for small-scale implementation (Chumnanpaisonnt *et al.,* 2014). To address this gap, the alternative extraction methods as microwave assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction (UAE) has been proposed. Specifically, ultrasonic assisted extraction have been developed to facilitate the recovery of active constituents from biomass resources. This special advantage is mainly attributed to the formation of cavitation bubbles during the progression of ultrasonic waves, followed by implosions generating "micro-jets". So the bioactive secondary metabolites obtained through extraction are widely utilized due to their natural properties, which include antioxidant, antimalarial antibacterial, antiviral, antidiabetic, and anticancer activities. Conventional extraction methods, such as maceration, reflux extraction, Soxhlet extraction, and supercritical fluid extraction are well established but, time, energy, and solvent, are prohibitively expensive for small-scale applications (Chumnanpaison *et al.,* 2014). To address these limitations, alternative methods have been developed, including microwave-assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction (UAE) and their derivatives. UAE in particular, has gained attention for facilitating the recovery of active plant constituents. The efficacy is due to acoustic cavitation, during which ultrasonic waves produce bubbles that collapse, producing micro-jets that disrupt cell walls matrix and increase mass transfer. Ultrasound assisted extraction has proven particularly efficient in improving the extraction yield of phenolic compounds (Nana *et al.,* 2021). When the objective is to maximize extraction yield from plant material, understanding extraction kinetics is essential. Sonication time emerges as a fundamental parameter, as rapid extraction is a key advantage of ultrasound-assisted methods (Prasad *et al.,* 2011; Liu *et al.,* 2010; Farhat, 2009). The efficiency of metabolite extraction depends on the rate at which secondary metabolites dissolve and reach equilibrium in the solvent. When solute diffusivity within the plant matrix is considered, the rate-limiting step is typically the diffusion of dissolved compounds from the matrix into the bulk solvent (Gertenbach, 2002). Thus, a steeper concentration gradient accelerates extraction. Ultimately, extraction performance depends on how quickly secondary metabolites dissolve and achieve equilibrium in the solvent phase (Herzi *et al.,* 2013).

LITERATURE REVIEW

Countless studies have been conducted on *Artemisia annua* with aim of identifying the active ingredients, without truly considering the extraction method. The kinetic approach to extraction remains less investigated, to the extent of our current knowledge. In general, extraction kinetics refers to the transfer of solutes from one phase to another. In the specific case of the extraction kinetics of secondary plant metabolites, it explains the speed at which solutes in a plant matrix in the presence of a solvent are transferred from the solid phase to the liquid phase. The solid phase is the plant cell and the liquid phase is the extraction solvent. Thus, the process of metabolite dissolution and diffusion is fundamental to the success of the kinetics. Extraction kinetics are influenced by factors such as temperature, concentration, the nature and polarity of the solvent, pH, particle size, etc. Extraction kinetics allow for yield optimization because, by monitoring the evolution of the extracted

mass over time, the optimal time to stop extraction and maximize yield can be determined. Another advantage of extraction kinetics is the reduction in energy and solvents, as a good understanding of kinetics will enable the best compromise to be found between extraction time, solvent polarity, and yield. Kinetics can also guide the choice of the most suitable alternative or conventional extraction method depending on the secondary metabolites to be extracted. Numerous kinetic models have been proposed by numerous authors to monitor the extraction of the phenolic compounds from plants; typical kinetic models of liquid-solid extractions include the empirical Hervas steady-state diffusion equation and the Peleg model (Peleg, 1988). Kinetic modeling is of great approach for understanding the diffusion phenomenon, mass transfer, and independent variables affecting extraction. Many material kinetic models for extracting secondary metabolites from plant have been reported before, such as first order models, two-parameter empirical models, and chemical kinetics and diffusion models based on Fick's law. Precisely, the two-stage rate model best describe the dynamics of alternative and traditional extraction techniques The two-site kinetic equation proposed by So and Macdonald is broadly used to model the extraction process (Zhizhe *et al.,* 2014). The extraction model stipulates that the extraction process relies on two stages: the washing stage first and the diffusion stage for the second stage. In the first stage, the secondary metabolites quickly extracted via external elusion. In the second one or equilibrium stage, all extraction processes are limited and the solute transfers slowly from the inside of the matrix to the solvent via internal diffusion. In addition, Mathematical models are useful engineering tools that facilitate the simulation, optimization, design and control of extraction processes. Furthermore, mathematical modeling can simplify process design and control to obtain optimization conditions and provide suitable information for large-scale extraction and preparation (Yonggang *et al.,* 2021). In fact, the kinetics of the extraction process is yet to be exploited. Thus, UAE is used as the main method for the extraction of phenolic of *Artemesia* annua L, and the working conditions including the extraction time, the temperature and liquid-to-solid ratio are optimized by RSM to obtain the highest phenolic yield and TPC. The kinetics of the extraction process will also be investigated. This kinetic modeling of ultrasonic extraction of phenolic compounds using Hervas and Peleg kinetic models was undertaken to provide comprehensive mass transfer phenology.

MATERIALS AND METHODS

Plant Material

Fresh *Artemisia Annua* L. plants were collected in the month of April, 2023 from a village in a suborn of Adamawa region of Cameroon. The harvested *A. annua* L. plants were taxonomically authenticated by a botanist, Doctor FAWA from the Department of Sciences and Technology of Organic Agriculture (STOA), the Faculty of Science of the University of Ngaoundere. A specimen was deposited and assigned a Voucher number 65647/HNC at the National Herbarium of Cameroon. After harvesting, the Leaves were cut and air dried at room temperature for ten days, to avoid denaturation of heat-sensitive molecules. The dried leaves were then pounded using a mortar and pestle set, and sifted using a sieve to obtain a finer powder. The powder was stored in a glass flask to protect them from humidity and kept for further investigations.

Methods

Ultrasound-Assisted Extraction procedure

During ultrasonic assisted extraction process, ultrasonic waves act on solid–solvent extraction method, by heating from the fast and continuous compression and expansion of the secondary metabolites (Mulet *et al.,* 2011). At the same time, these repeating and alternating expansion and compression with high and low pressure cycles generate an effect makes the solvent move easily and efficiently through microscopic channels in the vegetal matrix hence facilitates the release of secondary metabolites from plant. Moreover, ultrasonic method is taking as safe and clean because it relies on the use of non-ionizing radiation. However, ultrasound can induce reactions through the occurrence of acoustic cavitation, formation and collapse of small gas bubbles during extraction process (Rehman *et al.,* 2016). Precisely, 10 g of *Artemisia Annua* L. was introduced into a flat bottom flask, then 100 mL of different extracted solvent was added; the mixture is carried on gallows immersed in an ultrasonic bath (Figure 1A) for 20 minutes. The operation is carried out with an ethanolic solvent at 45°C. After thirty minutes of rest, the mixture is subjected to decantation. With Wattman filter paper the ethanolic mixture, is filtered and with the coffee filter paper, the mixture containing

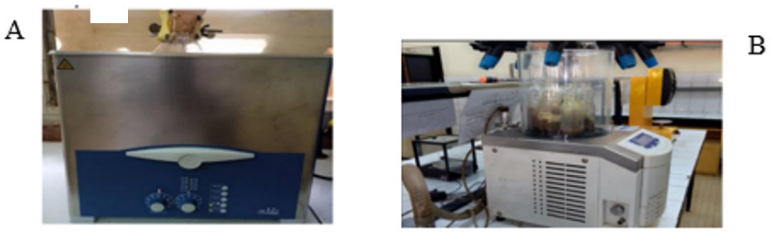

Figure 2: The Clifton Range Ultrasonic bath (A) Laboratory freeze dryer Alpha 1-2 LSCbasic (B);

water is filtered. Then, the ethanolic filtrate obtained is concentrated using a rotary evaporator, under partial pressure to obtain the solvent-free extract. The aqueous extracts were lyophilized using the Laboratory freeze dryer Alpha (Figure 1B)

Quantitative Estimation of Total Phenolic Content (TPC) and Biological Activities of *a. Annua* L Extract

TPC of the *Artemisia annua* L. extracts were obtained spectrophotometrically using the Folin-Ciocalteu colorimetric method with a slight modification (Singleton *et al.,* 1999). Gallic acid was used as reference and the results were expressed as gallic acid equivalents (GAE) (mg GAE/g extract)

Antioxidant Investigation Using DPPH Scavenging Assay Method

The *in vitro* antioxidant assessment using DPPH scavenging assay was evaluated by the method used by Mansouri with a little modification (Mansouri et al (2005). In this test, the antioxidants reduce the 2,2-diphenyl-1-picryl-hydrazyl radical having a purple color in to a yellow stable compound, the intensity of the color of which is inversely proportional to the capacity of the antioxidants present in the medium to donate protons. A.annua L. extract were mixed and left in the dark for 30 min. What is more, the decrease in absorbance of the mixtures was measured by a UV–Visible spectrophotometer (Germany Spectrophotometer UV/vis sp 8001) at 760 nm. Antioxidant potential of A.annua extracts leaves was quantified by the standard curve of butylated hydroxytoluene (BHT). Results were expressed in milligram (mg) BHT per g of A.annua extract. The IC50 values were determined graphically by linear regression. The antiradical activity is estimated according to the equation below

%antiradical activity = [(A control – A sample) / A control] x 100 eqn.1

where initial A is the absorbance of the control reaction and A sample is the absorbance of the test extract or purified organic compound.

***In Vitro* Antiplasmodial Investigation Using Chloroquine-Sensitive Malaria Parasite Strain nf54**

The SYBR Green and tetrazolium-based colorimetric assays were used to measure parasite growth inhibition during the antiplasmodial activity of the samples. (Smilkstein *et al.,* 2004).

Extraction Kinetics Modeling

Referring to kinetic study, two empirical models were used because of their relative ease of use and implementation (Tuhran *et al.,* 2002a), the empirical models of Hervas and the one of Peleg

Hervas Model

The Hervas kinetic model is applied to study and optimize the extraction of secondary metabolites from plants. In its mechanism, kinetic parameters such as the diffusion coefficient k and the equilibrium concentration Co allow respectively the evaluation of the diffusion capacity of the metabolites (Fick's law) and their concentration at the final equilibrium state.

$$C = C_0 (1 - e^{-kt}) \quad \text{eqn.2}$$

Where C and C_0 (µg E/gDW) are the concentration of metabolite in the medium (solvent) respectively during extraction time t (s) and equilibrium, and k (s-1) is the kinetic constant of extraction of the first order.

Peleg Model

Peleg's empirical model is used to describe kinetics model such as water absorption, solid-liquid extraction, and rehydration in food science. But within the framework of this work, Peleg empirical model is used to describe the extraction of various bioactive compounds from *A. annua* and mass transfer during the ultrasonic assisted extraction. The Peleg model estimates the theoretical (Ce) extraction yields as a function of time according to the equation as well as the extraction rate in the first minutes (C_0). The Peleg equation with slight modification is expressed as follows,

$$Y(t)=Y_0+t/(K_1+K_2\,t) \quad \text{eqn.3}$$

Where C(t) is the solutes yields (g/100 g) at t time, C_0 is the solutes yields (g/100 g) at t0, time 0, K_1 is linked to the constant rate of extraction of solutes expressed in min.g/100 g, K_2 provides information on the balanced solutes extraction yields ($C\infty = 1/K_2$, g/100 g). In the present work, C0 = 0 at time t = 0 then:

$$t/(Y(t)-Y_0)=K_1+K_2\,t \quad \text{eqn.4}$$

Taking into account the equilibrium condition, three kinetic parameters emerge from these equations, namely: The initial extraction rate B0 (µg E/ (s.gDW), the extraction capacity of the phenolic compounds of the Peleg model at l balance (C0) (µg E/gDW), and the extraction rate coefficient k (s- 1); defined in the equation: $B_0=1/K_1$; $C_0=1/K_2$; $k=K_2/K_1$

Experimental Design Using Response Surface Methodology (RSM)

Respond Surface Methodology (RSM) is an optimization techniques used for the modelling and analysis of a process in which an output variable of interest is influenced by the controlled factors. As RSM, Central Composite Design (CCD) involving three different factors: extraction time (X1), temperature (X2) and liquid-to-solid ratio (X3) is the experimental tool used in this work. The phenolic extraction from *A. annua* L was assessed based on the face-centered experimental plan, as shown in Table 1. Analysis of Variance (ANOVA) by Design Expert 6.0.6 software is the statistical method used for the validation parameters. Three-dimensional plots and their respective contour plots were represented taking in to account the various effect of the levels of the factors X1, X2, and X3. From these three-dimensional plots, the simultaneous interaction effects of the three factors on the response were studied. Furthermore, the

results of single-factor experiments were selected as the central points of the RSM experiments and the lower and upper levels of the optimal points were applied for the 17 runs. The optimum domain was also identified based on the main parameters in the overlay plot. To reduce the random error and to ensure accuracy the experiment was carried out in triplicate, and each result was compared with the predicted values to determine the suitability of the model.

Statistical Analysis

Table 1: Different input levels tested in the 2^3 central composite designs

Factors	Range levels				
	Codes	**Units**	**-1**	**0**	**+1**
Time	X1	min	10	15	20
Temperature	X2	°C	40	55	70
Liquid-to-solid ratio	X3	mL/g	1/10	1/25	1/40

ANOVA is the statistical tool used to evaluate the influence and the degree of importance of sonication time (X1), temperature (X2) and liquid-to-solid ratio (X3) their cumulative effects and their quadratic effects on the response. The suitability of the models was assessed using absolute mean deviation analysis (AMDA) and experimental and an adjusted correlation coefficient (R^2). A low AMDA and a high R2 value indicate a good fit between experimental values and predicted data and so a reliable and suitable model. Optimization and modeling of the extraction yield were carried out using the stat graphics plus XVI. Software and the 3-D plot were done on sigma plot 12.0 software

RESULTS AND DISCUSSION

Extraction Yield of Secondary Metabolites from *Artemisia annua* L.

The powder form of A.annua L plant was subjected to different extraction methods using different extraction solvents in order to compare the suitable method and the best extraction solvent. It appears from these investigations that ethanol extract obtained by UAE have the highest extraction yields 10.22%. These results are similar to those from the work of Rhianna in 2013, who found a better yield using ultrasound assisted extraction for the extraction of artemisinin from *A. annua.* (Briars *et al.,* 2013). This can justify the effectiveness of ultrasound in accelerating extraction which can be explained by the intensification of material transfer using the mechanical effect of ultrasound allowing rapid exudation into the solvent until reaching an equilibrium where the stage is governed by diffusion (Chemat *et al.,* 2016). More, similar results were obtained by Adjogble et al; during their work on the phytochemical study of *A. annua* L from Togo in 2019 which found a low extraction yield using distilled water for extraction (Adjogble *et al.,* 2019). This could be explained by a good solubility of secondary metabolites in ethanol and also better penetration of these into plant cell and which would cause the bursting of plant tissues (Toma *et al.,* 2001). Then the cavitation phenomenon, combined with the low viscosity of ethanol (1. 07 m Pa.s), promote the good solubility, the good diffusion and then the easy mass transfer from the plant matrix to the extraction solvent.

Total Phenolic Content from *Artemisia annua* L.

Table 2: Yields of different extraction methods and different solvent of *Artemisia annua* L.

Extraction Methods	Solvent used	Yields (%)
UAE	EtOH (45°C)	10.22
	EtOH/H2O (1:1)	06.00
	H_2O (45°C)	05.40
Infusion	H_2O (30 min)	04.89
Magnetic stirring	EtOH (3 H)	06.94
	EtOH/H2O (1:1)	07.40
	H2O (3 H)	04.20

The total phenolic content of these extracts were evaluated. It appears from these assay that ethanol extract obtained by UAE and by maceration have the highest content. Thus, from these investigations, UAE is the suitable extraction method and ethanol, the best solvent for the extracts with high extraction yields and high phenolic compounds. TPC of the extracts obtained different techniques vary from (46.58 ± 0.31 to 76.28 ±) 0.71 mg GAE/g of extract. The highest value (76.28 ± 0.71) mg GAE/g of extracts results from the ultrasound assisted extraction with ethanol solvent. The ethanolic extracts reveals a high content compared to the aqueous extracts regardless of the technique used. Such results were found by Touil Yacer in 2022 with contents of 21,21 mg Touil Yacer in 2022 noticed that ethanol extracts phenolics from plants *Artemesia* genus better than distilled

water GAE/g ethanolic extracts and 9,05 mg GAE/g of aqueous extracts (Touil., 2022). Likewise, the work of Diabi still in 2022 on another species and another family, *Haloxylon articulatum* (Amaranthaceae) also confirmed that the contents of total phenolics are higher in the extracts obtained by ultrasonics assisted extraction than those obtained by maceration (37, 82 ± 0, 42 µg GAE/mg of extract) (Diabi *et al.,* 2022) this is still due to the good solubility of phenolic compound in alcohol than water.

Biological Activities

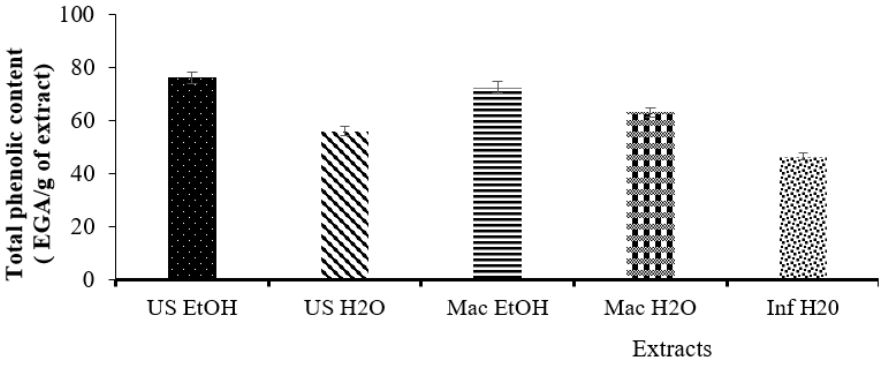

Figure 3: Total phenolic content from *Artemisia annua* L.

A low viscosity solvent can more easily seep into the plant matrix and access the target compounds. However, an approach linked to the solubility of secondary metabolites suggests the insolubility of artemisinin, the main antiplasmodial agent of *A. annua* in water hence the low extraction yields of aqueous and hydroethanolic extracts and the week biological activities

Quantification of DPPH Scavenging Assessment of Extracts from *A. annua* L.

Countless investigations of biological activities of *A. annua* L. have demonstrated their antioxidant capability which could be due to the presence of secondary metabolites in general and specifically to the phenolic compounds (Septembre-Malaterre *et al.,* 2020). It emerges from our results that all the extracts present strong antiradical activity. Once again, ultrasonic ethanolic extracts show better activities compared to aqueous extracts. Their IC50 values close to that of the reference (0, 45 ± 0, 21 et 0, 33 ± 0,07) mg/ml for ethanolic extracts obtained by ultrason and by magnetic stirring respectively and (mg/mL) value of BHT taken as standard. These results are similar to those obtained by Bubuean during his work on the comparative analysis of A annua extraction techniques in 2020 which presented a high antiradical activity of the microwave ethanolic extract compared to the extracts obtained by conventional method (Bubuean *et al.,* 2020).

Antiplasmodial Investigations of Different Extracts

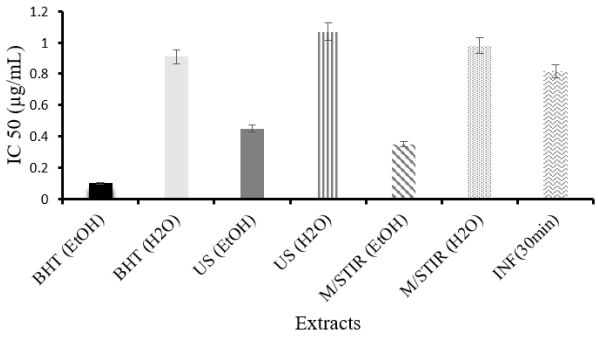

Figure 4: IC50 values of different extracts from antioxidant evaluation of *A. annua* L.

from *Artemisia annua* L.

It appears from Table 3 below resulting from the in vitro antiplasmodial essay that the ethanolic macerate exhibits the best antiplasmodial activity with an IC50 of 2.69 µg/mL compared to all other extracts. Similarly, aqueous extracts do not show any antiplasmodial activity (IC50> 100) and the rest of the extracts present the active fractions against *plasmodium falciparum* according to the categorization of kumari in 2016 (Kumari *et al.,* 2016). We noted that the aqueous extracts are not active, suggesting the absence of the artemisinin molecule in the aqueous extracts. Those results could be easily explained by the fact that artemisinin, the main active antiplasmodial molecule of *A. annua* is not soluble in the pure water or have a very low solubility in water with a solubility of (0.07 ±0.01) mg/mL compared to ethanol with a solubility of (3.20 ± 0.03). (Sales *et al.,* 2021). More after quantitative analysis of different extracts, Thin layer chromatography (TLC)

(Figure 4) and Fourier Transform Infrared Spectroscopy (FTIR) used to scan test samples and observed chemical properties showed respectively the absence of artemisinin in the aqueous extracts and the absence of the vibration frequency of artemisinin (Tawatar 2023) confirming the insolubility of artemisinin in distilled water . Several results of work on *A. annua* corroborate these results (Sales *et al.,* 2021, Nassirou *et al.,* 2015, Gbaguidi *et al.,* 2015) who showed good antiplasmodial activity of the ethanolic extract (IC50= 0,7µg/mL) of *A. annua* and with those of Gbaguidi and Wang which confirmed antiplasmodial activity in hydroethanolic extracts (Wang *et al.,* 2007). However. A review carried out by Alesaeidi and Miraj en 2016 shows that some flavonoids (a class of polyphenols) of A annua have a synergistic effect for antimalarial activity which confirms the antiplasmodial activity observed in the aqueous extract infused for 30minutes (Alesaeidi *et al.,* 2016).

Based on the investigations results above, UAE is the suitable extraction method and ethanol, the best extraction

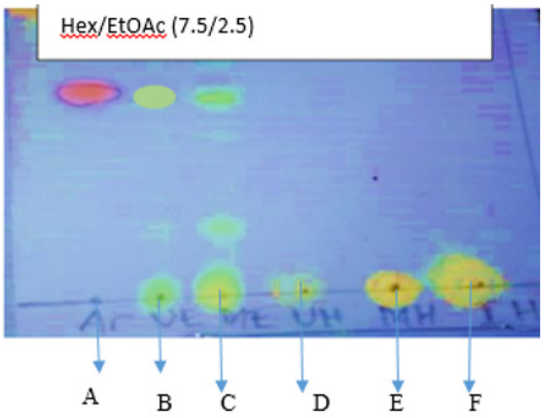

Figure 5: Thin Layer chromatography of different extract from *A. annua* L.
A: Standard (pur Artemisin); B: Ethanolic UAE extract; C: Ethanolic Magnetic stirring extract; D: Aqueous UAE extract; E: Aqueous magnetic stirring extract; F: Infusion extract

Table 3: Antiplasmodial results on *Plasmodium falciparum* Dd2

Extracts/compounds	IC501 (µg/mL)	IC502 (µg/mL)	Mean	SD
Ultrasonics/EtOH	09.840	06.680	8.260	1.570
Ultrasonics/H2O	>100	>100	>100	ND
Ultrasonics EtOH/H2O (1:1)	28,690	23,900	26,295	2,395
Mag.stirring/EtOH	3,464	1,918	2,691	0,773
Mag.stirring/H2O	>100	>100	>100	ND
Mag.stirring/EtOH/H2O (1:1)	16,760	11,910	14,335	2,425
Infusion (30 min)	48,450	45,400	46,925	1,525
Artemisinin	0,0333	0,0332	0,0094	0.0007
Chloroquine	0,2787	0,2783	0.0890	0.0064

solvent with the high extraction yields. Between all the seven extracts obtained, the ethanolic extract obtained by ultrasound assisted extraction revealed the presence of several classes of secondary metabolites including flavonoids, phenolics compounds tannins terpenoids… etc. We carried out all these investigations with the aim of comparing the extraction techniques of secondary metabolites, and extraction solvents. We can conclude that the green technique that is ultrasonic-assisted extraction used in this work presented a better performance in the extraction efficiency. Therefore, the need to study its kinetics to improve the extraction yield, maximize the recovery of total phenolic content and thus optimize the active ingredients through an experimental design using a response surface methodology (RSM).

Kinetic Modeling of Ultrasound-Assisted Extraction

The extraction kinetics for phenolic compounds were studied by evaluating the fit to experimental data of Hervas and Peleg models precisely the second-order diffusive models. The kinetic profile of the biomolecule's extraction from *Artemisia annua* L applying ultrasonic waves adjusted by the Peleg model and the Hervas model. The trend of the extraction curves indicates similarity with the kinetics of the sorption process of Peleg's model. The profiles of the extraction curves of biomolecules from the leaves of *Artemisia annua* L. indicate a high extraction rate in the initial extraction phase up to 20 minutes followed by a decrease of extraction rate. The extraction rate is controlled by the resistance to mass transfer in the liquid film and by intraparticle diffusion (Levenspiel, 2013). So,

exposure for 20 minutes increases the extraction rate of biomolecules from intraparticle diffusion. Moreover, the interpretation of the mechanism occurs during mass-transfer process and the standard solving methods for the adsorption kinetic models are very important for the implementations of the kinetic models (Wang *et al.,* 2020). The equations of the two kinetic models were generated by fitting experimental data from ultrasound-assisted extraction of secondary metabolites from *Artemisia annua* L. The diffusion constant (k) is 1.01. $10^{-4}s^{-1}$ for the Hervas kinetic model and 0.0012 s^{-1} for Peleg kinetic model. The solid-solvent extraction method can be considered as the reverse of an adsorption phenomenon; then, the basics of adsorption kinetic equations can be applied to solid-solvent extraction and Peleg's kinetic model can be applied to experimentally evaluate the extraction rate constant such as Peleg rate constant (K_1), Peleg capacitance constant (K_2) and others. The fitting curve and four kinetic parameters (K_1, K_2, B_0, and C_0) were obtained during the diffusion phase. This diffusivity value describes the rate at which these metabolites diffuse through the ultrasonic extraction solvent. From Peleg's kinetic model, the concentrations of secondary metabolites released at equilibrium (C_0=0.013) are obtained as well as the extraction speed coefficients k= 0.0012 s-1 (Table 4). In addition, the initial extraction rates Bo of biomolecules obtained are 11.11 µg E/ (s.gDW). The higher value of these secondary metabolites. The suitability of the model in relation to the experimental measurements was expressed using a determination coefficient R^2 (the model will be more valid as the value of R2 is close to 1). By applying Peleg and Hervas kinetic model described above to these experimental data, the values of K, K1, K2, C_0 and B_0 and AMDA were calculated. These values are presented in Table 4.

C_0 (µg E/g MS): the concentration of metabolites at equilibrium K (s-1): the kinetic extraction constant AMDA: Absolute Means Deviation Analysis K1 (s.gMS/µgE): the Peleg rate constant of solute; K2 (gMS/µgE): the Peleg capacity constant of the solute; B_0 (µgE/s.gMS): the initial solute extraction rate; C_0 (µgE/gMS): The equilibrium extraction yield; K(s^{-1}): the kinetic extraction constant. The values of the absolute analysis of the mean deviation (AADM) was 0.25, which implied good agreement between the experimental data and calculated with the Peleg model. K^1 is a linked constant the mass transfer rate, for example, the lower the K^1, the higher the initial solid-liquid extraction rate and K^2 is a constant linked to the maximum solid-liquid extraction capacity, i.e. say that the lower the K^2, the higher the solid-liquid extraction capacity. K^1 (73 s.gMs/µgE) and K^2 (0.090 mg/µgE) values are in close agreement with those of (Milićević (2021), in his work on the application of the Peleg model to study the kinetic modeling of ultrasonic-assisted extraction of phenolic compounds from cereal brands. It cannot describe well the behavior of the UAE process during the entire extraction period. It is necessary to explore more extraction models (Yonggang *et al.,* 2021). The suitability of Peleg's kinetic equation for adjusting the UAE of secondary metabolites is attributed to the ability of the equation to explain why the initial state of extraction is fast and very slow after the equilibrium state. The Figure 5 above depicts similar curve shapes between the experimental data curve and those of Peleg model for Ultrasonic assisted solid-liquid extraction of *A. annua* L. In addition to the advantages linked to ultrasound-assisted extraction mentioned above, knowledge of a good kinetic model allowing the transfer of material from

Table 4: Extraction parameters related to mass transfer according to Hervas and Peleg models

Hervas model				Peleg model						
Settings	**K (….)**	**R^2**	**AMDA**	**K_1**	**K_2**	**C_0**	**B_0**	**K**	**R^2**	**AMDA**
Values	1 . 0 1 . 10^{-4}	0.99	-0.40	73	0.090	0.013	11.11	0.0012	0.92	0.25

AMDA: Absolute Mean Deviation Analysis

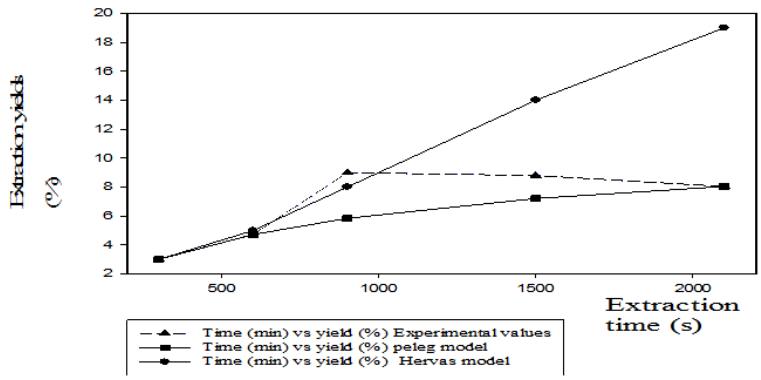

Figure 5: Suitability of experimental values with Peleg and Hervas values

the plant or animal matrix is necessary for optimize the extraction method and thus maximize biological activities through the active ingredient.

Optimization of the Extraction Process

The Influence of Extraction Conditions on The Extraction Yield

To obtain an extract with optimal biological properties, to develop a correlation between the three factors, a response surface methodology (RSM) implemented using a central composite design (CCD) of 17 experiments was used after that "one factor at time" design was applied to study the effects of independent variables. The effects of the variables sonication times (s) X1, temperature ($_oC$) X2, and liquid to solid ratio (g/mL) X3 evaluated shows that time and liquid to solid ratio have a significant effect at the 5% threshold on the response or output variable of interest here the extraction yield. In Table 5 below, are recorded the experimental plan with the predicted values and the experimental values of extraction yields. The definition of the minimum and the maximum level for each of the factors to delimit the experimental domain were obtained at the end of a rigorous experimental plan (Table 1)

It looks from the table above that the extraction yields are between 3.4 % and 15.8 %. To improve the extraction yields the adequateness of the model equation for predicting the optimum extraction yield values was investigate using the above selected optimal conditions. Table 5 also shows that the predicted optimum conditions

Table 5: Experimental and predicted values of ultrasound assisted extraction yields of phenolics from A.annua L. using central composite design

Run	Uncoded variable			Extraction yields (%)	
	X1 (min)	X2 (°C)	X3 (mL/mg)	Predicted values	Experimental values
1	15	55	25	11.2	11.94
2	10	40	10	4.9	4.39
3	20	40	10	7.2	8.10
4	10	70	10	3.4	3.33
5	20	70	10	4.6	3.99
6	10	40	40	7.7	7.95
7	20	40	40	13.3	13.01
8	10	70	40	15.2	13.94
9	15	55	25	12.7	11.94
10	20	70	40	15.8	15.95
11	10	55	25	6.3	7.86
12	20	55	25	10.9	10.72
13	15	40	25	11.3	10.92
14	15	70	25	10.1	11.86
15	15	55	10	7.9	8.16
16	15	55	40	14.8	15.92
17	15	55	25	14.7	11.94

Every result represents the average of at least three measurements.

for the secondary metabolites recovery from *A. annua* are X1=15.97 min, X2= 69.99°C and X3= 1/40 mL/mg. for the extraction yield of 15.95%. In order to determine the equation of the model, the regression coefficients of the linear, interaction and quadratic terms of the model were calculated using the least square technique and are given in equation below (Yang *et al.,* 2013). It was shown that two linear parameters, one quadratic and one interaction parameters were highly significant at the level of $P < 0.01$, the quadratic parameters $X2^2$, $X3^2$, and the interaction parameters X1X2, X1X3, were insignificant ($P > 0.05$). Wavering the non-significant parameters ($p>0.05$), the last final predictive equation obtained is given below:

Yield = 27,467 + 3,914X1 - 0,261*X3 - 0,106* X12+ 0,08*X2*X3 eqn.5

Were X1, X2, and X3 are extraction time, temperature and liquid-to-solid ratio respectively.

The analysis of variance (ANOVA) for the experimental results given in Table 6 shows that the strong correlation indicated the suitability of the model to predict the extraction yield at optimal conditions. This study suggests these optimal conditions could be significantly useful for further scale-up of the UAE of phenolics from *A. annua* L.

This analysis reveals that the effect of four factors significantly influences at the threshold of 5% the extraction yield of secondary metabolites from *A. annua*. The linear effect of time, the solid-to-liquid ratio, the interaction effect of temperature and the solid-to-liquid

Table 6: Analysis of variance (ANOVA) for the experimental results of extraction yields for UAE of *A. annua* L.

Parameters	Sum of square	DF	RMS	F value	P-value
X1: time(s)	5.11225	1	5.11225	7.55	0.0007
X2: temperature ($_oC$)	0.55225	1	0.55225	0.82	0.3964
X3: Liq-solid ratio (mL/mg)	37.636	1	37.636	55.59	0.0001
X1X2	1.16281	1	1.16281	1.72	0.2314
X1X3	0.227813	1	0.227813	0.34	0.5800
X2X3	6.21281	1	6.21281	9.18	0.0003
$X1^2$	4.70875	1	4.70875	6.96	0.0008
$X2^2$	0.203657	1	0.203657	0.30	0.6004
$X3^2$	0.00651076	1	0.00651076	0.01	0.9246
TE	4.73884	7	0.676978		
TC	64.4162	16			

The models with ($p < 0.05$) and ($F > 1$) were considered significant.
X1: Sonication time (s), X2: temperature (^oC), X3: Liquid-to-solid ratio
TE: Total Error; TC: Total correlation; RMS: root mean square; DF: Degree of freedom

ratio, contribute to increasing the extraction yield and the quadratic effect of time tends to reduce it. The results are in agreement with those obtained by Nana in 2016 in the process of optimizing the microwave assisted extraction of active substances from *T. roka.* (Nana, 2016). Moreover, the obtained total error and total correlation indicates the goodness of fit of the extraction method of the secondary metabolites from *A. annua.*

The analysis of variance of the regression coefficients consists of analyzing the impact of the independent variables (X1. X2 and X3) and $p<0.05$. In order to determine the degree of influence of the factors (times, temperature and solid-to-liquid ratio) on the extraction of bioactive substances, the p-values are used. The

Table 7: Regression coefficients and ANOVA results with validation conditions.

Validation indicators	Extraction yields	Standard Values
R^2 (%)	98.27	100
R^2adjusted (%)	96.06	100
SEE	0.82	1
AADM	0.07	[0 -0.3]
Bf	0.87	1
Af_1	1.08	1
Af2	1.008	1

R^2 (%): Determination coefficient; AADM: Absolute Mean Deviation Analysis
SEE: Standard Error of Estimate; Bf: Biais factor; Af_1, AF_2: Accuracy factor

significance of each coefficient. Which also indicates the intensity of interaction of each parameter. The smallest p-values present the greatest significance of the corresponding factor (Liu *et al.,* 2021). The results obtained are represented in table 6. Furthermore, still thanks to the validation indicators above, the desirability of the predicted factor of the predicted model must be closer to 1. Thus the model obtained is valid (Joglekar *et al.,* 1987). In this research work, just 0.018 of the variations are not explained by the model. In addition, in statistical science, a model is valid if the accuracy and biais factor are between 0.75 and 1.2 (Dalgaard and Jorgensen (1998); Baş and Boyac (2007). And the present work gave values of 1.08; 1.008 and 0.87 respectively; as for the AADM the obtained value is 0.07 confirming once again the validation of the kinetic model (Table7)

Graphical Analysis of Results

The experimental domain having been defined from the variation of three factors, given the difficulty in reproducing in a simple way the variation of the response, we therefore resort to cuts and projections which consist of fixing a given level to certain factors. The representation spaces of the response surfaces being defined from the variation of two factors (Figure 6). Design Expert software was used to construct the graphics below. At the end of the graphical analysis, the model equation was restored in two forms: Response surfaces 3D plot and

iso-response curves. The horizontal plane of the figure materializes the range of variation of two factors; the vertical axis materializes the variation of the extraction yields from the model. Beyond two factors, it is necessary to maintain at a constant level the factor whose variations are not described in the horizontal plane.

It emerges as main results from the response surface illustrations above that: the liquid-solid ratio and the temperature taken together contribute to increasing the extraction yield and this very significantly with the p-value of 0.0003 (Table 6). The corresponding response surface curve figure 6A shows that these effects tend to increase

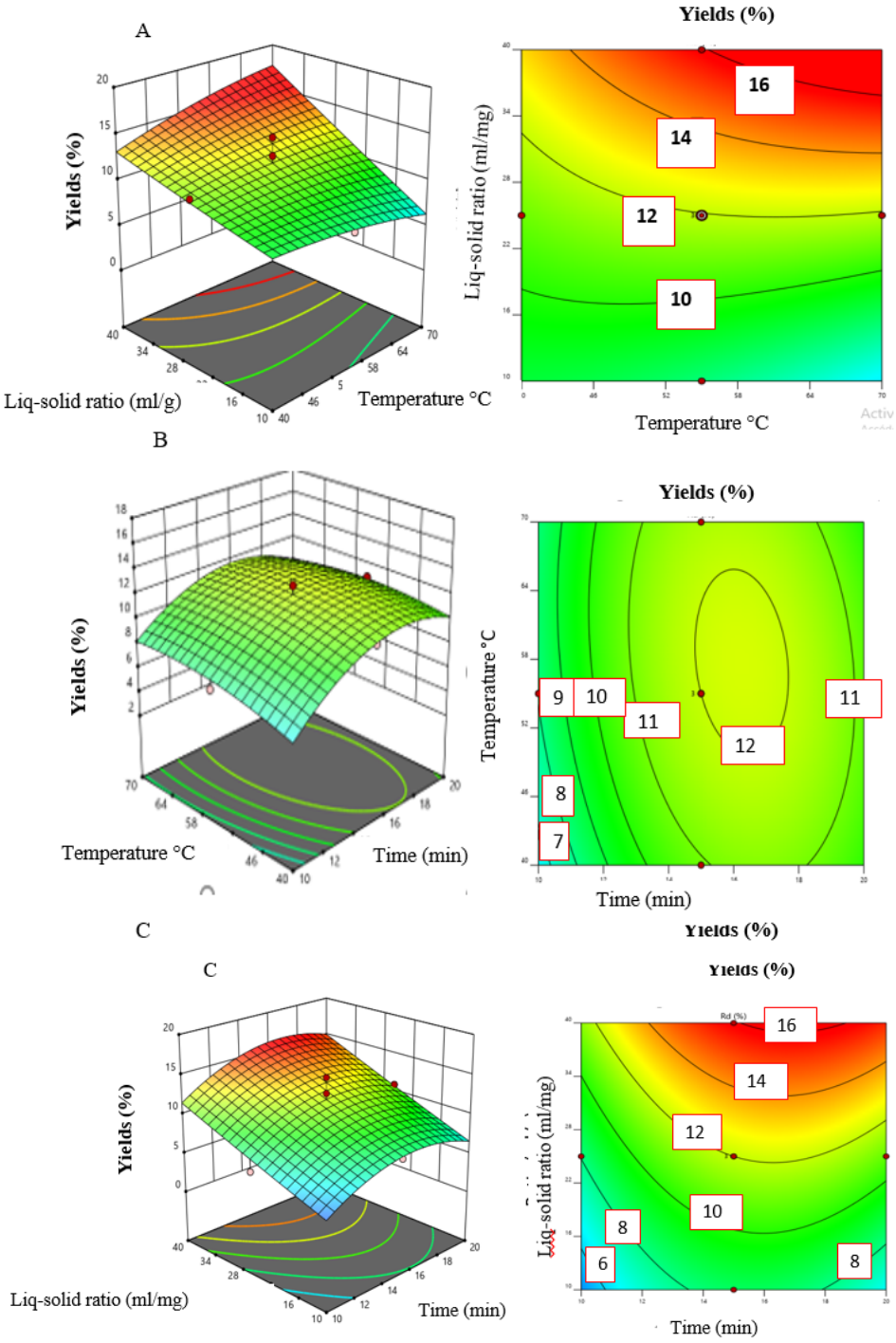

Figure 6: Response surface plots (3-D) and contour plots (2-D) for extraction yield of *A. annua* leaves extract in function of extraction time (min), extraction temperature (°C) and liquid-to-solid ratio (g/mL)

the extraction yield of biomolecules. According to (Gan and Latiff, (2011) a high temperature could cause, in addition to a softening of the plant tissue, the breakdown of interactions between phenolic compounds, thus

increasing their solubility and improving their diffusion speed and hence a better extraction yield. An increase in the volume of solvent makes it possible to penetrate the cells of the plant matrix, promoting the diffusion of secondary metabolites towards the solvent

Figure 6B presents the cumulative effect of the temperature and the time of the secondary metabolites of A annua which increase with time up to 17 min a yield of 10.80% then decreases from 10.80 to 7.80% at 20 min. As for the correlated effects of the liquid-to-solid ratio and the temperature, they also influence the extraction yield but not significantly just like the effect of the temperature and the extraction time with respectively the p-values of 0.5800 and 0.2314 (Table 6). In order for active compounds to be released into the environment, a minimum amount of time is required for the solvent to penetrate into the powder of the leaves, dissolve these active substances which then diffuse into the environment. It emerges from the Figure 6C that the cumulative effect of the extraction time and liquid-to-solid ratio tend to increase the extraction yield of biomolecules of *A. annua* L. This increase is reflected by the direct effect which has a positive and highly significant influence on the extraction. This could be explained by the fact that when a solvent material ratio increases, the yield of biomolecules also increases and perhaps probably due to the fact that an increase in the volume of solvent allows the solvent to penetrate the cell matrix plant promoting the diffusion of biomolecules towards the solvent. Thus, prolonged exposure leads to destruction of the structures of the compounds by heating; corresponding to the negative influence of the quadratic effect of time observed, on the extraction of bioactive molecules. Hence the observation of the reduction in extraction yield from 18 min. These results are in agreement with those of Nana in 2016 who observed that the quadratic effect of time has a negative influence on the extraction of bioactive molecules (Nana, 2016).

Verification of Predictive Model and Optimum Range of The Factors for Extraction Yields

For each optimal condition for the unique response, two experiment tests were carried out and the results obtained using the design-expert software. The optimal conditions to obtain the maximal extraction yield 17.69 % were a 15.97 min extraction times; a 68.99 °C of temperature and a liquid-to-solid ratio of 40:1 mL/g. To validate the adequacy of the model equation for predicting the optimum conditions, the model was evaluated by testing an experiment using the optimal conditions determined above, in Table 8 below are the results. A mean value of (18.89 ± 0.24) % was obtained from the actual experiments, which is in good agreement with the value of 20.02 % predicted by the model equation. These results validate the response surface methodology through a CCD used in this work thus the adequacy of the model describe the extraction process.

CONCLUSION

Table 8: Experimental and predicted extraction yield

Optimum condition			Extraction yields (%)	
X1 (min)	**X2(°C)**	**X3 (mLg-1)**	**Experimental**	**Predicted**
15.97	68.99	40:1	18.89 ± 0.24	20.02

Conclusively, ultrasonic assisted extraction with ethanol as solvent gave the best extraction yield (10.22%) and higher content in total polyphenols (76.28 ± 0.07 mg EAG/g of extract). The shorter sonication time required in UAE made this extraction method highly time, energy and solvent- consumption economic, then aligning closely with the principles of green chemistry. In fact, use of UAE could improve sustainability and then enhance eco-friendly secondary metabolites recovery. Kinetic investigation results showed that the model of Peleg adequately matched the UAE of phenolic compounds from *Artemisia annua* extracts. The experimental results, different from those used in the two kinetic models were compared to predicted values in others to confirm the accuracy of the kinetic model. For the entire UAE process, effective diffusivity values were determined for each response. The Absolute Mean Deviation Analysis (AMDA) of 0.07 values of the different models are between 0 and 0. 3, then the Peleg kinetic model best fits the ultrasound-assisted extraction of these secondary metabolites recovery. The optimum conditions of UAE of secondary metabolites from *A. annua* were 15.97 min of sonication times; a temperature of 68.99 °C and a liquid-to-solid ratio of 40:1 mL/g are necessary for the recovery of maximum secondary metabolites thus a high extraction yield.

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