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(RESEARCH ARTICLE)

Green ultrasound-assisted extraction and optimization of phenolic compounds from *Savia officinalis* and *Olea europaea* leaves for antidiabetic nutraceutical applications

Arar K [1, 2, *], Ghouini A [3], Imoudache H [4] and Gaci F [5]

[1] *Pharmacognosy Laboratory, Department of Pharmacy, Faculty of Medicine, University Blida1, Algeria.*
[2] *Central Pharmacy Department, Blida University Hospital Center, Algeria.*
[3] *Physiology Laboratory, Department of Medicine, Faculty of Medicine, University Blida1, Algeria.*
[4] *Research and Development Center, Department of Analytical Chemistry, SAIDAL group, Algiers, Algeria.*
[5] *Laboratory of Phytochemistry and Organic Synthesis, Department of Chemistry, Faculty of Sciences, University of Batna, Algeria.*

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Abstract

This decade, type 2 diabetes and its complications present a major global public health crisis and a significant economic burden, even though it is chronic and often asymptomatic. The global prevalence of diabetes continues to increase at an alarming rate. The International Diabetes Federation estimated that 588.7 million adults aged 20-79 years were living with diabetes worldwide in 2024, with projections reaching 852.5 million by 2050 [1]. The consequences of this issue can lead to serious complications such as amputations, blindness, cardiovascular diseases, heart attacks, cognitive decline, and Alzheimer's disease, all of which place a significant strain on health and social security systems. Therefore, prevention is the key to avoiding this substantial health and economic pressure. In the prevention and overall management of type 2 diabetes, phytotherapy can be highly effective. The current challenge is to identify practical and safe products that contain plant extracts. Our goal is to develop a dietary supplement aimed at preventing Type 2 Diabetes (T2D), particularly during the prediabetes phase, which currently lacks effective therapies. Therefore, the creation of nutraceutical products to support patient care is essential. In this context, we conducted a study presenting original research on the green optimization of polyphenol extraction from *Salvia officinalis* and Olea europaea for an adjuvant dietary supplement, with a focus on the treatment of type 2 diabetes.

The present study aimed to optimize the green ultrasound-assisted extraction (UAE) process to maximize the recovery of bioactive polyphenols from *Salvia officinalis* and Olea europaea leaves, two Mediterranean plants used in traditional care for diabetic patients in northern Algeria, with well-documented antioxidant and antidiabetic properties. A Box-Behnken response surface methodology (RSM) was employed to evaluate the effects of glycerol concentration (10-90% v/v), temperature (20-80°C), and sonication time (5-15min) on total polyphenol content (TPC, mg GAE g^{-1}).

The polynomial model was statistically significant, with a p-value of 0.0029 and a coefficient of determination (R^2) of 0.9292, indicating high predictive accuracy. Among the variables analyzed, glycerol concentration had the most significant effect ($p < 0.001$), whereas sonication temperature had a moderate effect. The optimal extraction conditions were determined to be 50% glycerol, 50°C, and 10 minutes of sonication. Under these conditions, the maximum total phenolic content (TPC) achieved was 185 mg GAE per gram. This result is comparable to that obtained with ethanol-based extraction but was achieved under milder and safer conditions.

A comparative analysis showed that glycerol-based ultrasound-assisted extraction yielded polyphenol levels and antioxidant activity similar to those achieved with ethanol extraction. However, glycerol offers additional benefits such as biodegradability, energy efficiency, and safety suitable for drug use. The extract demonstrated strong DPPH

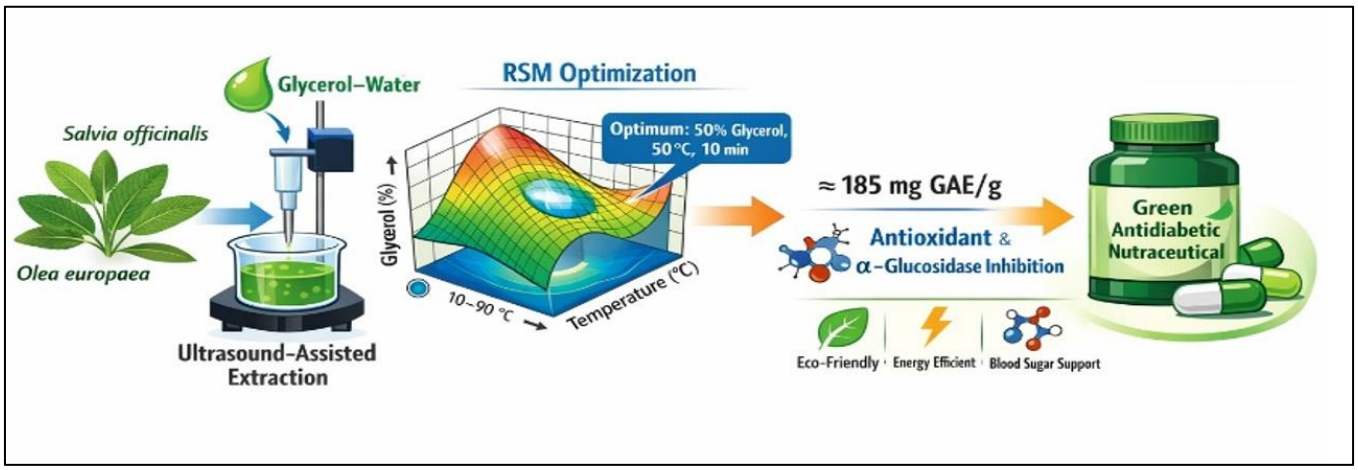
* Corresponding author: Arar K

scavenging and α-glucosidase inhibitory activities, highlighting its potential as a functional ingredient for the management of type 2 diabetes mellitus (T2DM).

This study presents a validated framework for green extraction to produce high-value phytochemical extracts. By integrating sustainable solvents with optimized processes, we establish a strong foundation for developing nutraceutical formulations that balance environmental responsibility with therapeutic effectiveness. Specifically, the use of *Salvia officinalis* and Olea europaea offers the opportunity to supply high-quality raw materials in sufficient quantities to support future enhancements in the pharmaceutical and agri-food sectors.

Keywords: Green extraction; Ultrasound; Optimization RSM; Polyphenols; Antidiabetic; Nutraceuticals

Graphical Abstract



1. Introduction

Natural products, especially plant-based products, are a substantial reservoir for the manufacture of future medicines [2]. Depending on the therapeutic effect sought, traditional use and subsequent research have developed processes for isolating plant molecules of interest, for local, oral, or injectable use.

The use of plants with anti-diabetic potential is no exception to this rule. Many investigations have focused on plants as possible sources of new glucose-lowering agents, and many plants have already been examined. Today, more than a thousand plants have been identified as potential anti-diabetic drugs. Still, only a few have been evaluated in accordance with pharmaceutical and scientific regulations to determine their effectiveness. It is therefore important to characterize these molecules, which are potential factors in the development of antidiabetic products, through phytochemical analysis of plant extracts and purification of bioactive compounds before clinical trials.

The availability, low cost, and fewer side effects make herbal preparations the main player in all available therapies, especially in rural areas [3]. In addition, many plants provide a rich source of bioactive chemicals, which are free of unwanted side effects and possess potent pharmacological [4] actions. Plants have also always been an exemplary source of medicines; many of the medicines currently available are obtained directly or indirectly from these plants.

Given the limitations observed with modern antidiabetic drugs, the search for new antidiabetic molecules derived from plant extracts would be a good alternative.

Nowadays, it is recommended to treat diseases such as diabetes with medicinal plants [5] because these plants contain various phytoconstituents, including phenolic compounds, flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, which can exhibit remarkable antidiabetic activities [6].

The recent rise in herbal medicine offers an opportunity to identify natural molecules that can regulate carbohydrate metabolism, avoiding the side effects of synthetic substances.

The anti-hyperglycemic effects of the herbs are usually attributed to their ability to improve pancreatic tissue function by increasing insulin secretion or reducing intestinal glucose absorption [7].

Despite the presence of anti-diabetic drugs on the pharmaceutical market, the treatment of diabetes with medicinal plants is becoming increasingly important. Herbal medicines and plant components with negligible toxicity and no adverse effects are notable therapeutic options for the treatment of diabetes worldwide [8].

A large number of plants are used in traditional medicine practices. The search for naturally occurring active ingredients from medicinal plants that can treat metabolic disorders, such as diabetes, is of great interest to the field of medicine. Many plants are traditionally considered antidiabetic, some of which are at the origin of the development of drugs such as metformin, thanks to Galega officinalis. Indeed, the history of biguanides dates back to the use of Galega officinalis as a treatment for diabetes in the Middle Ages. Guanidine, the plant's active component, was used to synthesize several antidiabetic compounds in the 1920s, including metformin [9].

In addition, experimental work has been carried out to verify the antidiabetic activity of some of these plants and to identify the active compounds responsible for this activity. The illustrious example is contained in the journal Bindu and Narendhirakannan [10], which classified and described 81 plants with antidiabetic, anti-hyperglycemic, anti-lipemic, and insulin-mimetic properties.

Medicinal plants are an important source of bioactive compounds used in pharmaceuticals, nutraceuticals, and traditional medicine systems. *Salvia officinalis* L. (sage) and Olea europaea L. (olive) are both well-known for their roles in traditional medicine, especially in northern Algeria, specifically in the Blida region. These plants are recognized for their high phenolic content, which is associated with various biological activities, especially their antioxidant and antidiabetic effects [11,12]. Phytotherapy continues to be a popular practice in central Algeria for treating diabetic patients, often used alongside conventional chemical treatments. This persists despite the region's socio-economic development and advancements in medical care, as highlighted in a study conducted by Professor K. Arar and colleagues. [13]. This study suggests that incorporating phytotherapy alongside medical treatment for patients with type 2 Diabetes is necessary, particularly due to significant blood glucose imbalances, as indicated by an HbA1c level of approximately 10. This level is well above the recommended glycemic targets for individuals with diabetes. Such findings underscore the inadequacy of current medical treatments prescribed for these patients, which include metformin, sulfonylureas, and the continued use of GLP-1 receptor agonists for managing diabetes. Therefore, there is an urgent need to develop nutraceutical products that can be integrated with effective antidiabetic medical treatments to improve the management of T2D. This study reveals that using phytotherapy alongside medical treatment for patients with Type 2 Diabetes is warranted, given the significant blood glucose imbalance, as indicated by an HbA1c of approximately 10. This level is well above the recommended glycemic targets for patients with diabetes. This situation highlights the inadequacy of current medical treatments prescribed for these patients, such as metformin and sulfonylureas, as well as the ongoing reliance on GLP-1 receptor agonists for diabetes management. Consequently, there is a pressing need to develop nutraceutical products that can be integrated with effective antidiabetic medical treatments for managing T2D [15,16].

Supplementing with herbs and nutrients is essential for managing diabetes, a conclusion unanimously agreed upon by scientists. Diabetologists have recently determined that a therapeutic supplement combining both nutrients and herbs is necessary to enhance diabetes treatment. [14].

Polyphenols play a vital role in reducing oxidative stress and regulating carbohydrate metabolism by inhibiting specific enzymes. Consequently, the efficient extraction of polyphenols is essential for maintaining the quality, effectiveness, and consistency of herbal medicinal products. This significance is underscored in the quality guidelines provided by the World Health Organization, the Food and Drug Administration, and the European Medicines Agency [17,18].

Traditional Soxhlet extraction is commonly used for thorough extraction; however, it is energy-intensive and time-consuming. In contrast, ultrasound-assisted extraction has emerged as a more sustainable and efficient alternative, offering reduced solvent consumption, shorter extraction times, and improved mass transfer [19]. The current study compares two extraction techniques through factorial design optimization, emphasizing phytochemical yield and in vitro biological activities related to metabolic disorders.

Our primary objective is to use national plant resources to manage type 2 diabetes. A key question in diabetes treatment is: What are the most effective methods for managing this condition? Unfortunately, no study has provided a definitive answer to this question. Diabetes specialists remain uncertain about advising patients on herbal supplements, such as fenugreek seeds or coffee, as well as other alternatives. Additionally, there is ambiguity regarding the role of

nutraceuticals, particularly in the prediabetic phase of the disease [13]. Our previous research focused on an ethnopharmacological study to identify the most commonly used plants for treating type 2 diabetes in Algeria. We also conducted a preliminary phytochemical analysis on selected plants. Following this, we optimized the extraction methods and isolated the extracts from natural bioactive products through fractionation. Our goal was to develop nutraceutical products for prediabetic patients, specifically targeting perception and care.

In light of the data and the context surrounding the search for molecules with optimal antidiabetic properties, we propose using green ultrasound-assisted extraction to obtain polyphenols from *Salvia officinalis* (sage) and Olea europaea (olive). This process will involve optimization and an assessment of the antioxidant potential of the extracts. The main objective of our work, arising from the issue of type 2 diabetes, is to enhance the value of these plant resources for effective disease management.

2. Materials, methods, and study design

The extraction of bioactive compounds from *Salvia officinalis* and *Olea europaea* was carried out using two methods: Soxhlet extraction and ultrasound-assisted extraction. We optimized the experimental conditions through full factorial designs (2n2 and 2n3) and response surface methodology. This process adhered to the guidelines established by the European Pharmacopoeia and the recommendations of the European Medicines Agency for method optimization and robustness assessment. Ethanol and glycerol were chosen as solvents for the extraction due to their suitable polarity and regulatory acceptability for herbal preparations, making them preferred choices in pharmaceutical applications.

2.1. Plant Material and Preparation

Fresh leaves of *Salvia officinalis* L. and Olea europaea L. were collected from the Blida region in northern Algeria, North Africa. These medicinal plants are traditionally used to care for diabetic patients. The aerial parts of the leaves of Olea europaea and *Salvia officinalis* were authenticated by Professor Bouhouhou, a taxonomist at the ENSA Herbarium in Algiers, where specimens of each plant have been deposited.

After collection, the samples were dried in the shade. The leaves and aerial parts were then ground into a fine powder using a Ciatronic® grinder. The resulting powder was stored at room temperature, protected from light and humidity, for further analysis.

2.2. Solvents and Chemicals

Analytical-grade glycerol (≥ 99.5%) and ethanol (96%) were procured from Sigma-Aldrich. Gallic acid 1-hydrate was obtained from Panreac Quimica, Barcelona, Spain. Folin-Ciocalteu reagent was purchased from Biochem, Chemopharma, France, along with sodium carbonate (Na2CO3), DPPH (2,2-diphenyl-1-picrylhydrazyl), α-glucosidase, and p-nitrophenyl-α-D-glucopyranoside from Sigma-Aldrich, Germany.

2.3. Phytochemical study

In this initial phase, a chemical screening was conducted to characterize the primary classes of bioactive compounds. The antidiabetic activity was evaluated in vitro by measuring inhibition of intestinal alpha-glucosidase using a spectrophotometric assay.

2.4. Crude extract of bioactive molecules

The extraction of bioactive molecules from the studied plants was conducted using distilled water as the extraction solvent, reflecting its common use by local populations. To obtain the aqueous extract, crushed plant material was mixed with distilled water in a 1:10 ratio, then refluxed at 60 °C for 30 minutes. Afterward, the system was allowed to cool, and the extract was recovered through filtration and freeze-drying.

2.5. Phytochemical screening

The procedure was carried out using the techniques described by Trease and Evans [20] and Bruneton [21].

2.6. Extraction Procedures

2.6.1. Soxhlet extraction

Conventional Soxhlet Method: The extraction was conducted using a glass Soxhlet-type apparatus. Twenty grams of plant powder were placed into an extraction cartridge, and 300 ml of organic solvent was added to a 500 ml flask. A heater was used to provide the necessary heating.

Extraction was performed with either 60% v/v ethanol or dichloromethane under reflux conditions. The extraction time was set for 120 minutes, with a heating power of 200 W and a temperature of 80 °C.

For each solvent, the extraction parameters for the bioactive molecules were optimized by varying the extraction time and temperature. Data analysis was performed using StatEase DOE software (Minneapolis, MN, USA), Serial No. 3925-8505-3422-4528. The power of the test was determined to be 85.7%.

Table 1 Design of Experiment Factors Used

Factor	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean
Extraction time	min	Numeric	Continuous	100,00	120,00	-1 → 100,00	+1 → 120,00	110,00
Heating power	watt	Numeric	Continuous	100,00	200,00	-1 → 140,00	+1 → 200,00	170,00

2.6.2. Ultrasound-assisted extraction

Extractions were performed in an ultrasonic bath (EMAG Emmi-D280, 40 kHz, 200 W) equipped with temperature control. A sample of 2.00 g of powdered plant material was combined with 40 mL of solvent, using either ethanol-water or glycerol-water mixtures at specified concentrations. The suspension was sonicated under the conditions specified by the factorial design.

2.6.3. Experimental design and optimization strategy

Full factorial designs (2^2 and 2^3) and response surface methodology were utilized to optimize extraction parameters and evaluate their effects on extraction yield and polyphenol content.

Ethanolic extraction

The experimental model employed is a complete factorial design with three factors (2^3) and three responses. The factors examined are: Solvent concentration, Ethanol, Temperature, and Sonication time.

The responses measured include Total polyphenol levels, free radical-scavenging activity (DPPH levels), and in vitro alpha-glucosidase inhibition rates. Each test is repeated three times. The structure of the experimental design is presented in Tables 2 and 3.

Table 2 Summary of Ultrasonically Assisted Extraction Method Factors

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Solvent concentration	%	Numeric	20,00	60,00	-1 ↔ 20,00	+1 ↔ 60,00	40,00	19,22
B	Temperature	°	Numeric	40,00	80,00	-1 ↔ 40,00	+1 ↔ 80,00	60,74	19,60
C	Temps Sonication	min	Numeric	5,00	15,00	-1 ↔ 5,00	+1 ↔ 15,00	10,00	4,80

The experimental plan was developed using Design Expert (DOE), which calculated the parameter values, specifically the average temperature and standard deviation.

Table 3 Summary of the construction data of the experimental design by the ultrasonic extraction method

Parameter	Description
Software	Design-Expert®
Version	13.1.2.0
Study type	Factorial experimental design
Design type	Two-level factorial
Model	3FI (three-factor interaction)
Randomization	Randomized
Total experimental runs	27
Center points	2
Blocks	None

Optimization of extraction using glycerol through a Box–Behnken response surface design

The Box-Behnken Design is a higher-order experimental design that allows the examination of as many main effects as possible with a minimal number of trials. This response surface design is employed to develop a quadratic mathematical model based on continuous variables. In our study, we focused on three factors: the concentration of the extraction solvent (glycerol), temperature, and sonication time (as shown in Tables 4 and 5). The response being measured is the total polyphenol content.

The mathematical model can be expressed as follows:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + e$$

Table 4 Summary of factors: ultrasound-assisted glycerol extraction method

Factor	Variable	Unit	Minimum (−1)	Maximum (+1)	Mean	Standard Deviation
A	Glycerol concentration	%	10	90	50	28.28
B	Temperature	°C	20	80	50	21.21
C	Sonication time	min	5	15	10	3.54

Independent variables and experimental levels used in the Box–Behnken response surface design for glycerol extraction optimization.

Coded values −1 and +1 correspond to the lower and upper levels of each experimental factor used in the response surface methodology.

Table 5 Summary of the data from the Box-Behnken experiment

Parameter	Description
Software	Design-Expert®
Version	13.1.2.0
Study type	Response Surface Methodology (RSM)
Design type	Box–Behnken design
Model	Quadratic model
Randomization	Randomized experimental runs

Total runs	17
Blocks	None
Build time	1.0000 ms

2.6.4. Determination of Extraction Yield, Total Polyphenol Content, Antioxidant Activity (DPPH Assay), and α-Glucosidase Inhibitory Activity

Extraction yield was determined as the percentage of the mass of dried extract in relation to the mass of the initial plant material. Total polyphenol content was measured using the Folin–Ciocalteu method and is expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g). Antioxidant capacity was assessed using the DPPH radical scavenging assay as described by Brand-Williams et al. [22]—evaluation of the inhibitory activity of intestinal α-glucosidase in vitro by spectrophotometric assay. The inhibitory activity of α-glucosidase was determined according to the method described by Kee et al. [23]. Absorbance readings were then taken at 405 nm using the spectrophotometer. The inhibitory activity of α-glucosidase was expressed as a percentage of inhibition.

2.7. Response surface modeling and statistical analysis

Experimental design and regression analyses were performed using Design-Expert 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). Model adequacy was evaluated through analysis of variance (ANOVA) at 95% confidence level.

The significance of the regression coefficient was assessed using the $p<0.05$ criterion. Model fitness was confirmed by the coefficient of determination (R^2), adjudged R2, the lack-of-fit test, and residual analysis.

Response surface and contour plots were generated to visualize interactions among variables and identify the optimal extraction region. Verification experiments were conducted under predicted optimal conditions to confirm model validity.

All analyses were performed in triplicate, and data were reported as the mean ± standard deviation.

2.8. Validation and comparative efficiency

To evaluate scalability and solvent efficiency, optimal glycerol-based UAE conditions were compared with ethanol-based UAE and conventional Soxhlet extraction (figure 1). Comparative parameters included yield (%), TPC (mg GAE g-1), DPPH scavenging activity (%), and α-glucosidase inhibition (%). Extraction efficiency and energy consumption were calculated to assess process sustainability and industrial feasibility.

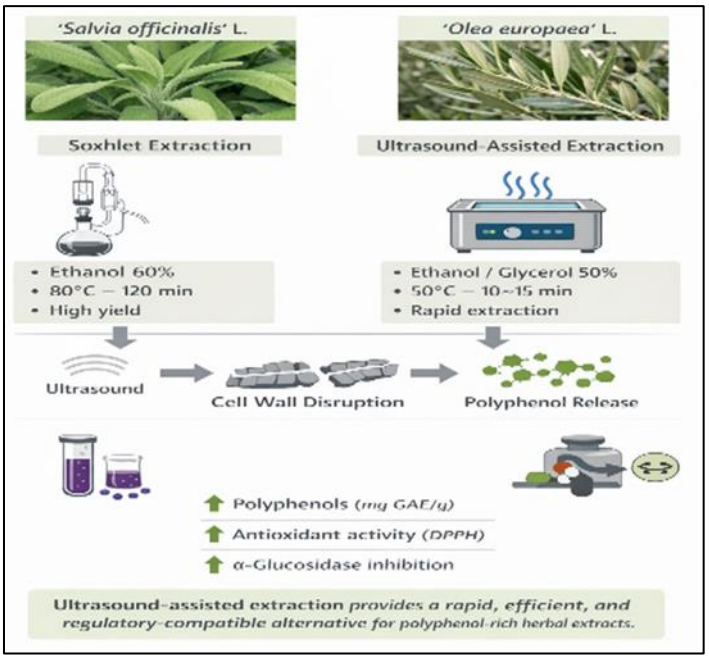


Figure 1 Soxhlet vs. Ultrasound-Assisted Extraction of Polyphenols

3. Results

In this research work, which is part of the valorization of the flora of therapeutic interest used in the treatment of diabetes mellitus, we optimized extraction using glycerol as a green solvent under assistant-extraction conditions. To assess a possible substitution of ethanol with glycerol (an eco-friendly, low-cost alternative), we optimized the extraction parameters using a Box-Behnken-type experimental design. Factor 1: Glycerol concentration (10 – 50 – 90% v/v); Factor 2: Temperature (20 – 50 – 80°). Factor 3: Sonication time 5 – 10 – 15 min. Response: Total polyphenols.

3.1. Experimental matrix and observed responses

3.1.1. Extraction at Soxhlet

- In the present study, the bioactive content of sage (S. officinalis) was affected by the extraction method.
- The bioactive content is represented by the responses associated with the extraction yields and polyphenol levels.
- The extraction was carried out successively by ethanol and dichloromethane.

Table 6 Ethanol extraction of *Salvia officinalis* with Soxhlet Mean and standard deviation of responses

Response	Number of Trials	Mean	Standard deviation
Low Extraction Time: 100 min Heating force Low: 140 W			
Yield (%)	3	19,55	0,57
Total Polyphenols (Eq mgAG/g E)	3	132,33	1,53
Extraction time High: 120 min Heating force Low: 140 W			
Yield (%)	3	21,75	0,96
Total Polyphenols (Eq mgAG/g E)	3	143,67	3,79
Low Extraction Time: 100min Heating Force Top: 140 W			
Yield (%)	3	24,25	0,85
Total Polyphenols (Eq mgAG / g E)	3	156,67	1,15
Extraction time High: 120min Heating Force High: 200 w			
Yield (%)	3	28,35	1,59
Total Polyphenols (Eq mgAG / g E)	3	181	1

3.1.2. Results of the optimization of the extraction by the surface method for the surface response: Box-Behnken

The results of the optimization of the ethanolic extraction of *Salvia officinalis*, assisted by ultrasound: Summary (Table 7,8)

Table 7 Optimisation de l'extraction éthanolique, assistée par ultrasons, de *Salvia officinalis*

<table>
<tr><td colspan="5">EXTRACTION - Salvia officinalis. Method: Ultrasonically Assisted Extraction
Solvent: Ethanol</td></tr>
<tr><td colspan="2">DESIGN OF EXPERIMENT</td><td colspan="2">FACTORS: 3</td><td>REPLIES: 27 tries</td></tr>
<tr><td colspan="2" rowspan="3">Complete 2n3 Factorial Design

Polynomial Postulated Model
First degree</td><td colspan="2">Ethanol concentration
(% v/v)
Level -1: 20
Level +1: 60</td><td rowspan="3">Determination of total polyphenols
(Eq mgAG / g E)

Test DPPH
(% Act. anti-radicalaire)
Inhibition In vitro
Alpha glucosidase
(% Inhibition)</td></tr>
<tr><td colspan="2">Temperature
(degrees Celsius)
Level -1: 40
Level +1: 80</td></tr>
<tr><td colspan="2">Sonication Time
(min)
Level -1: 5
Level +1: 15</td></tr>
<tr><td colspan="5">MODELING</td></tr>
<tr><td colspan="2">Respnses</td><td>Total Polyphenols</td><td>Test DPPH</td><td>Inhibition glucosidase</td></tr>
<tr><td colspan="2">Model</td><td>Significant à p = 0,0001</td><td>Significant à p = 0,0001</td><td>Significant à p = 0,0001</td></tr>
<tr><td rowspan="7">Coefficients

Value
Significance</td><td rowspan="2">Concentration Ethanol</td><td></td><td></td><td></td></tr>
<tr><td>p = 0,0001</td><td>p = 0,0001</td><td>p = 0,0001</td></tr>
<tr><td rowspan="2">Temperature</td><td></td><td></td><td></td></tr>
<tr><td>p = 0,0001</td><td>p = 0,0001</td><td>p = 0,0001</td></tr>
<tr><td rowspan="2">Sonication Time</td><td></td><td></td><td></td></tr>
<tr><td>p = 0,0001</td><td>p = 0,0001</td><td>p = 0,0001</td></tr>
<tr><td>Interaction</td><td></td><td></td><td></td></tr>
<tr><td colspan="2">Lack of adjustment</td><td>Significant (p = 0,0001)</td><td>Significant (p = 0,0001)</td><td>Significant (p = 0,0001)</td></tr>
<tr><td colspan="2">Coefficient of determination: R^2</td><td>0,9646
Acceptable</td><td>0,9582
Acceptable</td><td>0,9638
Acceptable</td></tr>
<tr><td colspan="5">INTERPRETATION</td></tr>
<tr><td colspan="5">Ultrasound-assisted extraction yielded a high extraction yield of 203 mg EAG/g at a solvent concentration of 60%, a temperature of 80 °C, and 15 min of sonication. The resulting models allow us to predict responses for the proposed factor values; the significance of the values obtained depends on the model's accuracy. The p-values from the ANOVA analysis ($p < 0.05$) indicate that all proposed models are statistically significant.</td></tr>
</table>

Correlation between different factors and responses (figs. 2, 4)

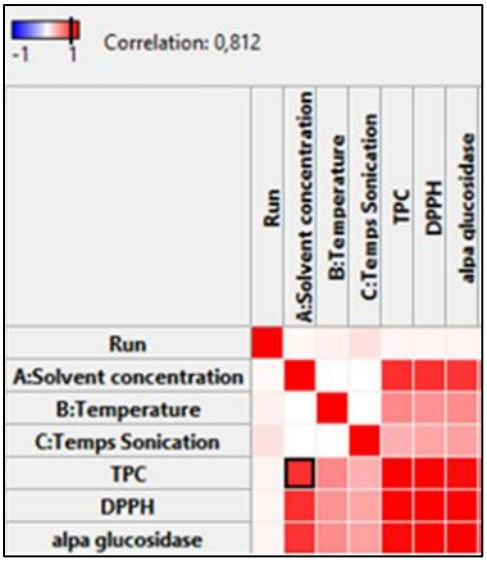


Figure 2 Correlation matrix

We observe a strong correlation between solvent concentration and the total polyphenol content, percent DPPH inhibition, and alpha-glucosidase inhibition. Conversely, all responses show a weaker relationship with extraction temperature and sonication time.

Analysis of how the three factors influence different responses.

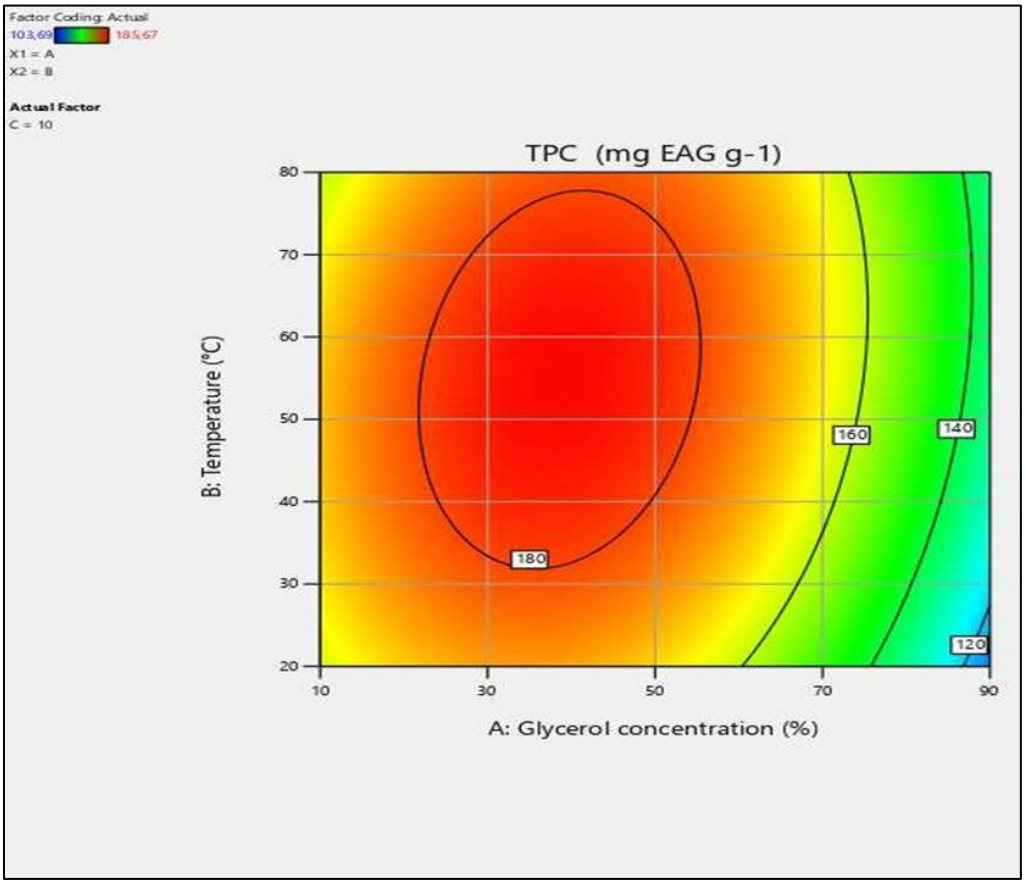


Figure 3 Numerical optimization of solvent glycerol extraction parameters. Boxenken

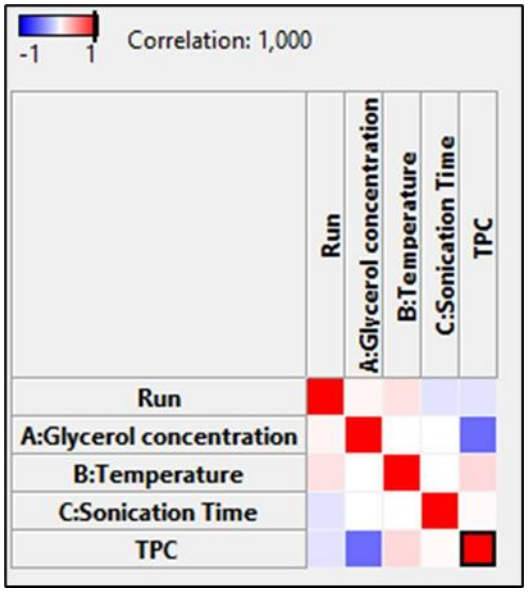


Figure 4 Correlation matrix

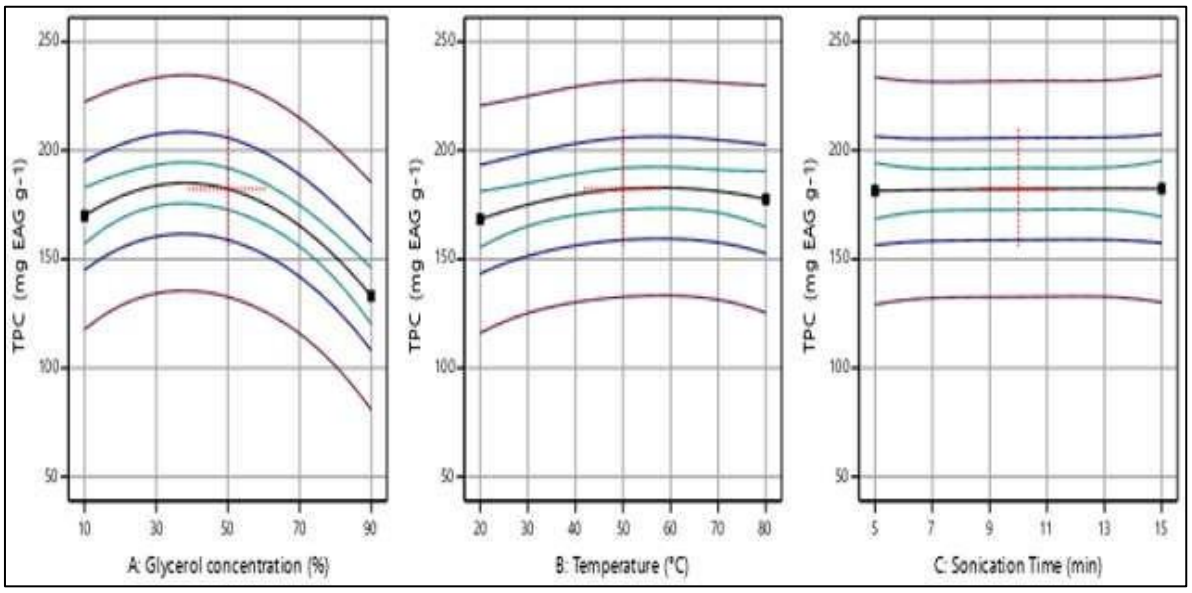


Figure 5 Regression curves of the TPC response as a function of the three factors

Table 8 Optimization of Ultrasound-Assisted Extraction of *Salvia officinalis* with Glycerol Summary

EXTRACTION - *Salvia officinalis* **Method: Ultrasonic Assisted Extraction Solvent: Glycerol**		
EXPERIENCE PLAN	**FACTORS: 3**	**RESPONSE: 17 attempts**
	Glycerol concentration **(% v/v)** Level -1: 10 Level +1: 90 Intermediate level 0: 50	**Determination of total polyphenols**

<table>
<tr><td rowspan="2">Response surface

Applied Polynomial Model Quadratic</td><td>Temperature (degrees Celsius)
Level -1: 20
Level +1: 80
Intermediate level 0: 50</td><td rowspan="2">(Eq mgAG / g E)</td></tr>
<tr><td>Sonication Time (min)
Level -1: 5
Level +1: 15
Intermediate level 0: 10</td></tr>
<tr><td colspan="3">MODELISATION</td></tr>
<tr><td colspan="2">Response</td><td>Total Polyphenols</td></tr>
<tr><td colspan="2">Model</td><td>Significant at p = 0.0029</td></tr>
<tr><td rowspan="8">Coefficients

Value
Significance</td><td rowspan="2">Glycerol concentration: A</td><td></td></tr>
<tr><td>p = 0,0007</td></tr>
<tr><td rowspan="2">Temperature: B</td><td></td></tr>
<tr><td>Not significant</td></tr>
<tr><td rowspan="2">Sonication Time: C</td><td></td></tr>
<tr><td>Not significant</td></tr>
<tr><td>Interactions : AB AC BC</td><td>Not significant</td></tr>
<tr><td>A^2</td><td>Significant at p = 0.0002</td></tr>
<tr><td>B^2 C^2</td><td>Not significant</td></tr>
<tr><td colspan="2">Lack of adjustment</td><td>Significant (p = 0.0048)</td></tr>
<tr><td colspan="2">Coefficient of determination: R^2</td><td>0,9292</td></tr>
<tr><td colspan="3">INTERPRETATION</td></tr>
<tr><td colspan="3">There was an optimal content of organic solvent (glycerol) at which phenolic compounds were best extracted: 50% glycerol for a temperature of 50°C and a sonication time of 10 minutes. In addition, the organic solvent content influenced the extraction linearly. The influence of the linear term was negative.</td></tr>
</table>

The sonication time shows a positive effect up to the threshold (≈10-15 min), Beyond Which the gain becomes marginal. So the matrix (Table 8) highlights the robust optimal zone centered on 50% (v/v) glycerol, 50°C, and 10 min sonication, Conditions offering the best compromise between extraction efficiency and polyphenol preservation. These results justify the use of response surface methodology to refine the overall optimum and to model the A×B, A×C, and B×C interactions.

The total polyphenol content values obtained across the 17 experimental conditions ranged from 103 to 185 mg GAE g-1. The highest TPC was observed at 50% glycerol, 50 °C, and 10 min sonication; extreme conditions, notably 90% glycerol and 80°C, resulted in a second significant decrease in polyphenol yield.

This trend highlights a nonlinear effect of solvent concentration, suggesting that the balance between solvent polarity and viscosity is crucial for maximizing extraction efficiency. The observed variability across experimental runs validated the application of response surface methodology to identify optimal process conditions.

3.2. Model fitting and statistical validation

The second-order polynomial model was applied to describe the relationship between TPC and the three independent variables (A: glycerol Concentration, B: temperature, and C: sonication time).

The fitted regression equation (in coded variables) was as follows:

$$TPC+ 180,45+10,32A-1,45B+2,87C-23,14A^2-1,28B^2-0,92C^2.$$

3.2.1. Model significance

Amended this variance (ANOVA) revealed that the overall model was statistically significant (p=0.0029), with a determination coefficient R2=0.9292 and Adjusted R2=0.9014, indicating that approximately 93% of the total variance in TPC could be explained by the model.

Among the independent factors, glycerol concentration (A) exhibited the strongest and most significant linear and quadratic effects (p<0.001), whereas temperature (B) and sanitation time (C) contributed marginally within the studied range (p >0.05). Interaction terms (AB, AC, BC) were statistically insignificant, confirming that the solvent effects primarily governed the TPC response.

The lack-of-fit test was significant (P=0.0048), reflecting inherent experimental variability typical of ultrasound-assisted systems. However, the right R2 and acceptable residual distribution support the adequacy of the model for predictive purposes.

3.2.2. Effect of extraction parameters

3.2.2.1. Influence of glycerol concentration

As illustrated in the response surfaces (Fig.3), TPC increased sharply from 10% to approximately 50% glycerol, then declined sharply beyond 70%. This optimum corresponds to a solvent polarity window that enhances solubility and diffusion of moderately polar phenolics such as rosmarinic acid, caffeic acid, and oleuropein. While excessive glycerol (>70%) likely increases viscosity, reducing mass transfer and gravitation efficiency. Similar nonlinear patterns have been reported for green solvents in phenolic extraction from aromatic plants. [24,25,26].

3.2.2.2. Effects of temperature and sonication time

Moderate hitting (40-50°C). Improved extraction yields due to enhanced solute and decreased solvent viscosity.

However, temperatures above 60°C reduced TPC, likely due to thermal degradation of polyphenols or oxidative polymerization.

Sonication time had a minor but positive effect up to 10-15min; beyond this, additional gravitation produced negligible gains and may promote oxidation, consistent with previous UAE optimization studies [27,28].

3.2.2.3. 3D response surface interpretation

The 3D response surface and contour plot depict the dependence of TPC on glycerol concentration and temperature at a constant sonication time of 10 min. The surface reveals a distinct optimum plateau centered at 50% glycerol and 50°C. Corresponding to the maximum predicted TPC (185mg GAEg $^{-1}$)

Below 30% glycerol, solvent polarity decreases phenolic solubility, whereas above 70%, the system becomes too viscous for efficient cavitation.

The well-defined curvature of the response surface supports the statistical significance of the quadratic term (A^2) and confirms the existence of a local, rather than linear, optimum.

3.2.2.4. Comparative Evaluation of Extraction Methods

To benchmark the performance of green ultrasound-assisted extraction against conventional techniques, optimized glycerol and ethanol systems were compared to Soxhlet extraction under standard conditions (Table 6,7,8).

Both ultrasound-assisted systems produced significantly higher TPCs than Soxhlet extraction, demonstrating the efficiency of acoustic cavitation in enhancing polyphenol recovery.

Although ethanol yielded slightly higher TPC (≈ 203 mg GAE g^{-1}), the glycerol-based process operated under milder conditions (50 °C) and used a non-toxic, biodegradable solvent, making it more sustainable and safer for food-grade applications.

Furthermore, the bioactivity profiles (DPPH and α-glucosidase inhibition) of glycerol extracts were comparable to those obtained with ethanol, confirming that green extraction can achieve both ecological and functional equivalence.

3.2.2.5. Model validation and predictive reliability

Experimental validation under the predicted optimal conditions (50 % glycerol, 50 °C, 10 min) produced a mean TPC of 182 ± 3 mg GAE g^{-1}, in close agreement with the model's predicted value of 185 mg GAE g^{-1}.

The relative deviation (1.6 %) confirms the predictive accuracy and reproducibility of the RSM model.

Residual analysis revealed a random distribution around zero, indicating homoscedasticity and the absence of systematic bias.

3.2.2.6. Comparative Insights: Salvia officinalis vs. Olea europaea

While both S. officinalis and O. europaea demonstrated similar extraction kinetics under UAE, compositional differences influenced their overall polyphenolic yield and antioxidant profiles.

Salvia officinalis extracts were dominated by rosmarinic and caffeic acids, contributing to strong radical-scavenging activity, whereas O. europaea extracts were enriched in oleuropein and hydroxytyrosol, key contributors to α-glucosidase inhibition and anti-inflammatory activity [29,30,31,32].

The convergence of these complementary bioactivities positions a combined formulation as a promising nutraceutical candidate for glycemic regulation and oxidative stress mitigation in patients with T2DM.

3.2.2.7. Sustainability and Industrial Implications

From a process engineering standpoint, the glycerol-based UAE system offers several advantages: Reduced energy input (operating at ≤ 50 °C), shorter extraction times (≤ 10 min), elimination of volatile organic solvents, high reproducibility, and scalability using standard sonication reactors.

These characteristics align with the green extraction of natural products principles proposed by Chemat et al. (2022) (24) and reinforce the suitability of glycerol as a food-grade, sustainable solvent for industrial extraction of phenolic compounds.

4. Discussion

The integration of response surface modeling and green ultrasound-assisted extraction enabled the identification of an optimal solvent composition and process regime maximizing total polyphenol recovery from S. officinalis and O. europaea.

The findings demonstrate that solvent effects dominate extraction efficiency. Optimal TPC (~185 mg GAE g^{-1}) occurs at 50 % glycerol and 50 °C. Glycerol-based UAE achieves comparable efficacy to ethanol extraction while improving sustainability.

Both species exhibit complementary antioxidant and antidiabetic bioactivities, validating their inclusion in phytotherapeutic formulations for the management of T2DM.

Epidemiological data on polyphenol properties indicate a relationship between consuming a diet rich in polyphenols and the prevention of certain diseases, such as cardiovascular disease, atherosclerosis, allergies, diabetes mellitus, and cancer [33].

Similarly, many studies have evaluated the antidiabetic potential of phenolic compounds and extracts. Polyphenols reduce fasting and postprandial hyperglycemia by inhibiting the intestinal flora's disaccharidases (α-amylase and α-glucosidase) [34].

Finding new health products that are safe, effective, and affordable, especially those derived from plant extracts using plentiful, low-cost raw materials, poses a significant challenge today, particularly in the realm of sustainable development. Additionally, Oleaster and Common Sage have a long history of use in traditional medicine across Algeria, the Maghreb region, and beyond, notably as antidiabetic agents. This widespread use, especially among people with diabetes in Algeria and globally, has spurred research to better understand the true effects of these plants on glycemic control and overall well-being, both in the short and long term. However, the specific impacts on human health remain largely unconfirmed due to a lack of clinical studies. In fact, there remains a shortage of clinical research on homeostasis and glycemic regulation.

The enhanced extraction of phenolic compounds observed in this study through ultrasound-assisted techniques aligns with other reports highlighting the advantages of UAE over conventional methods. Earlier work comparing conventional extraction and ultrasound-assisted extraction for *Salvia officinalis* demonstrated that UAE significantly shortened extraction times and increased phenolic yield due to enhanced cavitation and mass transfer, particularly when using ethanol–water mixtures as solvents [35]. Using a probe system, UAE under optimized conditions yielded up to ~60% higher phenolic content than conventional thermal extraction, supporting the efficiency gains also observed in our design for S. officinalis phenolics.

Similar comparative analyses have been reported for Olea europaea leaf extractions, where ultrasound and other green technologies have been systematically assessed across solvent systems. In a recent study, Huamán-Castilla et al. (2025) [36] compared several green solvents in the UAE for the sustainable recovery of antioxidant compounds from olive leaves, highlighting that green solvents (including aqueous systems) could rival or outperform conventional ethanol systems in terms of antioxidant yield and antiradical activity. This resonates with our observation that glycerol–water systems achieve TPC values comparable to ethanol UAE, while offering the added sustainability and safety advantages of a non-flammable, biodegradable solvent.

Comprehensive reviews on olive leaf valorization underscore that extraction method, solvent polarity, and processing conditions critically influence polyphenol yield and biological activity. Debs et al. (2023) [37]. provided an updated overview of olive leaf extraction techniques, noting that optimized solid–liquid and advanced extraction approaches substantially enhance recovery of oleuropein and related antioxidants, which are central to the antidiabetic and cardioprotective properties attributed to olive leaf extracts. Our results extend these findings by demonstrating that glycerol-based UAE can be incorporated into this suite of sustainable technologies without compromising the recovery of key bioactives.

In line with literature on ethanol concentration effects, studies on phenolic extraction from aromatic plants (e.g., Rosmarinus officinalis) and sage by-products have shown that increasing ethanol percentage improves the extraction of phenolics up to a point, after which the quadratic effect becomes limiting due to solvent polarity mismatch or compound degradation [38]. The quadratic response observed in our model, with a clear optimum around 50 % glycerol, echoes this behavior, reinforcing that intermediate solvent polarity often yields the highest phenolic content.

Comparative work on solvent systems further shows that ethanol extracts often exhibit higher total phenolic content than NADES extracts for certain matrices, highlighting that solvent physical properties (e.g., diffusivity, viscosity) can outweigh purely “green” designations if not optimized. For example, Siamandoura et al. (2023) [39] reported that ethanol extracts of olive leaf polyphenols had greater TPC than NADES extracts in many cases. However, NADES offered benefits in specific compound recoveries and stability profiles. Our glycerol-based approach parallels this nuance: while ECS systems such as ethanol might extract marginally higher TPC, strategically balanced glycerol concentrations yield competitive yields with superior sustainability profiles.

Additionally, research on the sustainable extraction of specific phenolics, such as hydroxytyrosol, using deep eutectic solvents (DES) highlights that advanced green solvent systems can achieve high yields with significantly reduced time and energy inputs, illustrating ongoing innovation in solvent engineering for phenolic recovery. This emerging field supports the broader trend that optimizing both solvent chemistry and processing parameters (e.g., temperature, sonication) is pivotal for scaling plant extract production for nutraceutical applications.

Together, these comparative findings underscore that Ultrasound-assisted methods consistently demonstrate higher extraction efficiency than conventional techniques. Solvent optimization — whether ethanol, glycerol, critically shaped yield and bioactivity, with intermediate polarity conditions frequently optimal. Advanced green solvent systems align with sustainable production goals and can support scalable nutraceutical development without sacrificing performance.

Comparing our optimized conditions to the broader literature confirms that glycerol-based UAE extraction of *Salvia officinalis* and Olea europaea phenolics achieves bioactive yields on par with established methods while advancing the sustainability profile required for modern industrial and clinical phytotherapeutic deployment.

Overall, this study bridges green extraction engineering and metabolic health applications, advancing the development of scalable, eco-efficient processes for nutraceuticals targeting metabolic syndrome and type 2 diabetes.

5. Conclusion

The present study successfully optimized a green ultrasound-assisted extraction process for the recovery of polyphenols from *Salvia officinalis* and *Olea europaea* leaves, integrating principles of sustainability, efficiency, and biological efficacy. Using a Box–Behnken response surface design, the effects of glycerol concentration, temperature, and sonication time were systematically analyzed. The resulting model ($R^2 = 0.9292$, $p < 0.01$) demonstrated excellent predictive accuracy, identifying 50 % glycerol, 50 °C, and 10 min sonication as optimal extraction conditions. Under these parameters, the total polyphenol content (≈ 182–185 mg GAE g^{-1}) approached that of ethanol-based extractions while maintaining eco-compatibility and full food-grade safety.

Comparative analyses confirmed that the glycerol-based UAE process yields and antioxidant activities equivalent to those of conventional ethanol extraction, but at lower energy costs, reduced solvent volatility, and improved environmental performance. The extraction profile revealed a local optimum for solvent polarity, consistent with previous studies on ethanol–water systems and deep eutectic solvents. These findings reinforce glycerol's value as a sustainable extraction medium that bridges the gap between laboratory efficiency and industrial scalability.

From a pharmacological standpoint, the bioactive profiles of the optimized extracts—dominated by rosmarinic acid, caffeic acid, luteolin, oleuropein, and hydroxytyrosol—underscore their potential as multifunctional phytotherapeutic agents for the treatment of type 2 diabetes mellitus. The complementary antioxidant and α-glucosidase inhibitory activities of *S. officinalis* and *O. europaea* extracts correspond to mechanisms previously demonstrated in vivo for glycemic control and oxidative stress modulation. Thus, beyond process optimization, this study establishes a scientifically validated foundation for formulating combined extracts as potential adjuvants in metabolic disease management.

Future studies should validate the optimized extraction parameters at pilot scale to assess solvent recycling efficiency, energy use, and product stability in industrial settings. The next stage of formulation development should investigate encapsulation or emulsification methods to improve the bioavailability and shelf life of polyphenols. Concurrent preclinical trials in models of diabetes and metabolic syndrome are essential to determine dose-response relationships, safety, and the potential for synergy with current antidiabetic treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest

Statement of ethical approval

All experimental procedures complied with institutional laboratory safety guidelines. No human or animal testing was conducted in this methodological phase. The plant materials used are non-endangered and were collected in accordance with local biodiversity regulations.

Statement of informed consent

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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