JJBS

Volume 18, Number 3,September 2025
ISSN 1995-6673
Pages 539 – 547

Jordan Journal of Biological Sciences

Optimizing the Extraction of Total Phenolic, Flavonoid Content, and Antiradical activity from *Salvia tingitana* Using Mixture Design and Triangular Surfaces

Amine Azzane[1], HouriaNekhla[2], and Mohamed Eddouks[1,*]

[1]*Team of Ethnopharmacology and Pharmacognosy, Faculty of Sciences and Techniques Errachidia, Moulay Ismail University of Meknes, BP 509, Boutalamine, Errachidia, 52000, Morocco;* [2] *Higher Institute of Nursing Professions and Health Techniques (ISPITS), Errachidia,Morocco;*

Received: December 6, 2024; Revised: March 29, 2025; Accepted: April 17, 2025

Abstract

Background: Salvia tingitana is a medicinal and aromatic plant with several medicinal properties. Pharmacological investigation revealed the antihyperglycemic and antihyperlipidemic proprieties of this herb. In addition, phytochemical screening of Salvia tingitana demonstrated the presence of polyphenols, flavonoids, and tannins.
Aims: The study aimed to evaluate the effect of different solvents on the yield of polyphenols and flavonoids, and molecules endowed with antiradical activity derived from Salvia tingitana.
Material and Methods: Ten solvents were used to determine their efficacy in extracting total flavonoid and total phenolic content, as well as other antioxidant molecules. 2,2- diphenyl-1-picrylhydrazyl (DPPH) scavenging was used to evaluate the antiradical activity. We used a simplex axial mixture design to optimize the extraction of polyphenols, flavonoids, and molecules with antiradical activity by using response surface methodology.
Results: The results indicated that extraction was influenced by the solvent nature. The most effective solvents for achieving good polyphenol and flavonoid yields were water, ethanol, and methanol concerning the part of solvent screening. The second part of the solvent mixture extraction revealed that pure water was the most appropriate solvent for extracting the highest amounts of total polyphenolic content (3.344±0.03 GAE/g). Concerning total flavonoid content, the equi-proportional solvent mixture "water-methanol" revealed the highest quantity (3.213±0.06 QE/g). In addition to the extraction solvent's nature, the mixture's nature had a significant impact on the anti-radical activity of Salvia tingitana. The percentage of inhibition of DPPH was higher in the extract obtained with the ternary mixture (103.15±4.20 %) the following proportions: ethanol 66.66%; methanol 16.66%; water 16.66%. Generally, the percentage of inhibition of DPPH was also influenced significantly and positively by the methanol proportion.
Conclusion: In conclusion, the study demonstrates that water, ethanol, and methanol act synergistically to improve the bioactive recovery. Thus, solvent combination methods may result in better yields of flavonoids and antiradical molecules compared to single extraction methods. Moreover, the use of solvents can help to reduce the amount of time and energy required to extract the desired compounds.

Keywords: *Salvia tingitana*; solvent extraction; polyphenols; flavonoids; antioxidant activity; surface design methodology.

1. Introduction

Plants belonging to the *Salvia* genus are increasingly recognized as a rich reservoir of biologically active phytochemicals, like polyphenolic, flavonoid, and phenolic acids compounds, well-known for their biological properties (Lu and Foo, 2022). The chemical composition of essential oils obtained from the aerial parts of Salvia plants has been determined and dominant components are 1,8-cineole, camphor, limonene, α-pinene, β-pinene, 3-carene, campene, p-cymene, sabinene, β-caryophyllene, and α-gurjunene (Lu and Foo, 2022; Kelen and Tepe, 2008). The bioactive compounds present in *Salvia* species exhibit a considerable antioxidant potential, rendering them well-suited for maintaining various physiological functions (Martino *et al.,* 2010; Asadollahi *et al.,* 2019; Khiyari *et al.,* 2014; Bisio *et al.,* 1998). Several studies have demonstrated that many Salvia species exhibit hypoglycemic, hypolipidemic, anticancer, antioxidant, antihypertensive, antimicrobial, and anti-inflammatory effects (Sharifi-Rad *et al.,* 2018; Li *et al.,* 2013; Azzane *et al.,* 2022). For this reason, some plants of this genus have been cultivated worldwide for their applications in traditional medicine and culinary practices (Askun *et al.,* 2010).

Salvia tingitana (ST) is known to contain a diverse range of phytochemical compounds, such as polyphenols, flavonoids, tannins, saponins, sterols, terpenoids, alkaloids, and carbohydrates (Azzane and Eddouks, 2022). These Compounds have garnered substantial attention due to their antioxidant and antibacterial properties (Bouaziz *et al*., 2009). In addition, Pharmacological investigation revealed the antihyperglycemic and antihyperlipidemic

* Corresponding author. e-mail: mohamededdouks2017@gmail.com; mohamed.eddouks@laposte.net.

proprieties of *Salvia tingitana* (Azzane and Eddouks, 2022).

The extraction yield is linked to various factors, including variety, fruit maturity, pedoclimatic conditions, extraction process, and polarity of the solvent used for extraction (Oliveira *et al*., 2001). The composition of the obtained extract, which influences its biological properties, is affected by the efficacy of the extraction method and the appropriateness of the extractor solvents. In addition, a successful extraction technique involves combining solvents in appropriate proportions. Choosing an extraction method requires evaluating factors such as accuracy, stability of extracted substances, resource availability, and processing costs, all contributing to the eventual biological application of the extract (Oliveira *et al.*, 2001). Bioactive compounds are generally extracted from plant material with alcohols, acetone, and water, but the selection depends on the compound of interest and the plant material (Zhang *et al.*, 2018). In the objective to determine bioactive compounds and antioxidant ability, optimization of the extraction proves to be an effective method for examining the impact of a single variable, particularly the solvent (Aazza, 2021). Utilizing response surface methodology (RSM) stands as a precise approach to enhance the extraction process (Saravana *et al.*, 2023).

According to our knowledge, no reported investigation has employed solvents or solvent mixtures to extract active compounds from ST. In addition, some studies have been conducted on ST, but its full potential has not been fully explored. For this reason, the present study was undertaken to determine the optimal appropriate solvent mixture for extracting a higher quantity of total flavonoid content (TFC), total phenolic content (TPC), and molecules with antioxidant activity. The objective was to establish an efficient and reproducible model with the desired properties. It is important to develop a model with desirable properties that is efficient and reproducible.

2. Materiel and Methods

2.1. Plant Material

Fresh plant material of ST was purchased in March 2022 from a local market in the Tafilalet region (semi-arid area) of Morocco. This herb was air-dried in the shade at 40°C for one week in the laboratory. Taxonomic identification and authentication of the plant were conducted, and a voucher specimen (ST 25/35) was deposited in the herbarium of the Faculty of Sciences and Techniques, Errachidia.

2.2. Extraction Methodology

The extraction procedure was prepared as previously described (Tawaha *et al.*, 2007; El Baakili *et al.,* 2021). Briefly, 50 mg of the arterial part of ST was subjected to a 20-minute sonication with 1 ml of the selected solvents (water, acetonitrile, methanol, acetone, ethyl acetate, ethanol, chloroform, dichloromethane, toluene, and hexane). Following this, the mixture underwent centrifugation at 6000 rpm for 15 minutes, and the recovered supernatant was stored at 4 °C.

2.3. Estimation of total Polyphenol Content

TPC using Folin Ciocalteu method, polyphenol contents were measured according to the method described previously (Chansriniyom *et al.*, 2021; Azzane *et al*., 2023).

2.4. Estimation of Flavonoid Content.

As described by Kim et *al.* total flavonoid content (TFC) was measured (Kim *et al.*, 2003; Azzane *et al.*, 2022a).

2.5. Determination of DPPH Radical Scavenging Activity

In accordance with previous studies, anti-DPPH radical activity was measured (Louli *et al.*, 2024).

2.6. Optimizing Solvent Mixtures with Simplex Axial Design (SAD)

To illustrate the behavior of solvent mixtures used for extracting active ingredients from herbs, mixing models has proven effective (Garcia *et al.*, 2010). Due to its advantageous properties in exploring the experimental space, the simplex axial plane (SAP) was selected for optimizing the extraction process, particularly its vertices at (2/3, 1/6, 1/6), (1/6, 2/3, 1/6) and (1/6, 1/6, 2). It is interesting to note that the top point represents pure solvent (1 : 0 : 0;0 : 1 : 0;0 : 0 : 1); each intermediate point represents a permutation of binary mixtures (1/2: 1/2 : 0;1/2:0:1/2; 0:1/2:1/2), while the center point represents a ternary mixture (1 : 1 : 1).

To determine the TPC, TFC, and antiradical activity of ST this design of extraction, (DOE) allows greater precision in determining the proportion of solvents, such as water (X1), ethanol (X2), and methanol (X3), in the solvent mixture shown in Figure 1. Experimental data were fitted using a polynomial equation:

$$Y = \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X2X3 + \beta 123X1X2X3,$$

where $\beta 1$, $\beta 2$, $\beta 3$, $\beta 12$, $\beta 13$, and $\beta 23$ are interaction coefficients of linear, and nonlinear terms and Y is the response or predicted value.

2.7. Confirmation of the Model

With the surface response model (SRM), the extraction conditions for ST were optimized for maximum TPC concentration, TFC concentration, and antioxidant activity. To validate the model, the predicted values based on standard errors were compared with the experimental data obtained.

2.8. Statistical Analysis

Three independent experiments were conducted to determine the solvent proportions and each of TPC content, TFC content, and antiradical activity, and the results were summarized as mean + standard deviation. Using the analysis of variance (ANOVA), in objective to validated the multiple regression model ($p < 0.05$) and evaluated the significant effects of the variables and their interactions. Concerning the regression coefficients, the contour graphs and the model's response surface were produced. Tukey test was used to define the significant differences, with $p < 0.05$ as the significance criterion. STATISTICA (data analysis software system), version 10 (StatSoft, 2011) was used for the design and analysis of mixture design experiments.

3. Results and Discussion

3.1. Solvent Screening

Ultrasound assisted extraction (sonication) improves extraction efficiency because it accelerates dissolution, diffusion, and heat transfer. In addition to reducing extraction temperature and extraction time, this extraction method requires little solvent and energy consumption (Saravana *et al.*, 2023). As a result, sonication is used in this study to extract different compounds.

Ten solvents with various polarities ranging between 0.1 and 10.2 were systematically evaluated for their efficacy in extracting a great quantity of TPC and TFC. The data are detailed in Table **1**. Indeed, the results showed that water, ethanol, and methanol proved their ability for extracting highest amounts of TPC and TFC. In contrast, toluene and hexane showed the lowest TPC extraction, and hexane and acetonitrile presented the lowest TFC extraction. Interestingly, the findings demonstrate a substantial impact of solvent extraction capabilities on the yield of TPC and TFC. Polyphenolic compounds generally possess polar proprieties, and the pronounced polarity of water as a solvent enhances its efficiency in extracting these polar compounds often conjugated with multiple carbohydrates. Several studies demonstrated the effectiveness of employing a combination of solvents with differing polarities to extract substantial amounts of bioactive compounds (Pan *et al.,* 2015). In addition, a study has shown that extracting phenolic compounds from plants is best done by using aqueous organic solvent mixtures (Venkatesan *et al.*, 2019). Thus, a mixture design optimization was conducted using water, methanol, and ethanol.

Table 1. Influence of selected solvents on TPC and TFC contents.

Solvant	Polarity index	TPC mg GAE/g	TFC mg QE/g
Water	10.2	3.344±0.03	0.592±0.03
Acetonitrile	5.8	0.184±0.030	0.049±0.08
Methanol	5.1	1.47±0.039	0.66±0.16
Acetone	5.1	0.395±0.084	0.320±0.02
Ethyl Acetate	4.4	0.23±0.079	0.17±0.06
Ethanol	4.3	2.108±0.14	0.63±0.16
Chloroform	4.1	0.47±0.08	0.124±0.15
Dichloromethane	3.1	0.39±0.058	0.072±0.02
Toluene	2.4	0.10±0.031	0.32±0.02
Hexane	0.1	0.079±0.005	0.044±0.02

3.2. Solvent Mixture Extraction

Surface response methodology coupled with axial simplex design provides a powerful approach to optimize solvent mixtures for maximizing TPC contents, TFC contents, and other molecules with antiradical activity. Besides, analytical methods such as food and herbal medicine are often optimized using response surface methodology (RSM) (Plaza *et al,* 2018). Strati *et al*. effectively employed this approach to enhance the extraction of phenolics from *Allium ampeloprasum* (Strati *et al.,* 2018).

The findings of the effects of the solvent mixture on the antiradical activity and the contents of TPC and TFC are illustrated in Table **2**. The results revealed that pure water proved its ability for extracting high amounts of TPC, followed by the water-ethanol binary mixture in a 1:1 ratio. Concerning TFC, the equi-proportional solvent mixture "water-methanol" revealed the highest extraction, followed by the equi-proportional solvent mixture "water-ethanol". These findings could be explained by the synergistic effect between water and methanol as well as methanol. Selectivity, solubility, cost, and safety are key considerations in the selection of solvents. In this context, water emerges as a potentially green solvent due to its non-toxicity to both health and the environment. Additionally, it stands out as the safest and most cost-effective solvent (Mansouri *et al.,* 2020; Nicetin *et al.,* 2021).

According to Table **2**, the antiradical activity of ST based on the solvent mixtures used was assessed by DPPH free radical scavenging. Identically, the findings revealed that the anti-radical activity of ST extracts was importantly affected by the nature of solvent and the composition of the mixture. Indeed, among all extracts, the one obtained with the ternary mixture (16.66 water / 16.66 methanol /16.66 ethanol) showed the strongest ability to inhibit DPPH radicals flowed by pure methanol and ternary mixture (33.33 water / 33.33 methanol /33.33 ethanol). In contrast, the binary mixture “water-ethanol” produced the lowest activity. According to many studies, phenolic compounds play an important role in antioxidant activity (Mansouri *et al.,* 2020). Herb's antioxidant activity was mainly derived from phenolic compounds (Han *et al.,* 2010). In addition, polyphenols compounds can be used to enhance the economic and nutritional value of functional foods, as well as enhance their full utilization (Chater *et al.,* 2023).

Table 2. Findings of mixture tested of TPC, TFC, and DPPH and simplex axial design.

Run	Water	Methanol	Ethanol	TPC mg GAE/g	TFC mg QE/g	DPPH (%)
1	100	0	0	3.334±0.03	0.592±0.03	65.23±0.09
2	0	100	0	1.471±0.039	0.664±0.16	63.39±3.64
3	0	0	100	2.108±0.14	0.635±0.16	93.80±3.65
4	50	50	0	2.83±0.03	3.213±0.06	49.18±2.41
5	0	50	50	2.175±0.17	0.685±0.06	83.57±2.77
6	50	0	50	3.313±0.04	3.158±0.25	48.26±0.418
7	33.33	33.33	33.33	3.050±0.10	0.456±0.06	91.09±3.75
8	16.66	16.66	66.66	3.179±0.04	0.311±0.33	103.15±4.20
9	16.66	66.66	16.66	2.819±0.14	0.748±0.07	79.50±3.64
10	66.66	16.66	16.66	3.081±0.14	0.798±0.08	71.52±1.04

3.3. Analysis of Variance Test (ANOVA).

ANOVA was performed to test significance and fit the model. To determine the significance of the corresponding coefficient, p-values are used, and the smaller the p-value < 0.05, the more significant the difference (Nekhla *et al.*, 2023). F-tests were used to examine model significance. Coefficients of determination (R2) and the adjusted correlation coefficient (AdjR2) were used to determine model robustness. Analysis of linear, quadratic and special cubic models was performed for independent and response variables. Table 3 shows the TPC, TFC and free radical

scavenging activity measured for the aerial part of the plant studied. Among the models considered, the cubic model was satisfactorily reproduced for TPC (p = 0.001992), TFC (p = 0.0000) and DPPH (p = 0.0000).

Table 3. Statistical evaluation of mixture model effects using ANOVA.

		SS	df	MS	F	P	R^2	R^2-Adjustd
TPC	Linear	76.58	2	38.29	14.48	0.000053	0.5176	0.4819
	Quadratic	40.49	3	13.49	10.49	0.000134	0.7913	0.7479
	Special Cubic	10.67	1	10.67	12.15	0.001992	0.8635	0.8279
	Total Adjusted	147.93	29	5.10				
TFC	Linear	9.033	2	4.51	4.8177	0.0162	0.2630	0.2084
	Quadratic	6.4408	3	2.1469	2.7304	0.0661	0.4505	0.3360
	Special Cubic	15.6871	1	15.687	113.306	0.0000	0.9072	0.8831
	Cubic	1.4409	2	0.7204	8.6789	0.00179	0.9492	0.9299
	Total Adjusted	34.34	29	1.1843				
DPPH (%)	Linear	3951.924	2	1975.962	9.549	0.00073	0.4143	0.3709
	Quadratic	1660.183	3	553.394	3.3824	0.0346	0.5883	0.5025
	Special Cubic	3281.918	1	3281.918	117.0827	0.0000	0.9324	0.91478
	Cubic	257.23	2	128.616	6.9706	0.004767	0.959	0.943904
	Total Adjusted	9538.732	29	328.922				

A global fit was applied to study the special cubic model. The degree of model fit was verified by the R2 coefficient. This coefficient expresses the proportion of total response variation predicted by the model. The closer the R2 value is to 1, the better the model fits the real data. The R2 results for the special cubic models were 0.863, 0.907 and 0.932 respectively for TPC, TFC and DPPH. This indicates that the model adequately represents the true relationship between the selected parameters. Indeed, a high R2 value indicates that the statistical model can explain a large proportion of the observed data variability, improving its reliability and predictive ability. When R2 is close to 1, the model is well suited to the experimental data, reducing prediction uncertainty (Nekhla *et al.,* 2023).

According to Table 4, all special cubic models were highly significant, as indicated by low p-values of 0.000000 and high F-values: 24.2529; 24.2529 and 61.99643 for TPC, TFC and DPPH respectively. Given the calculated F-values and probability values, the models below do not suffer from a lack of fit and are very reasonable and significant.

The model presented p-values for TPC, TFC and DPPH that are highly significant (p=0.00000), confirming the validity of the model and indicating that the model developed is reliable and accurate in predicting relevant responses.

Relationships between responses are described by polynomial models

TPC = 5.795*ethanol + 5.133*water +1.531*methanol + 10.362*ethanol*water + 16.610*ethanol*methanol – 3.762*water*methanol – 60.505*ethanol*water*methanol +0

TFC = +1.499*ethanol + 0.774*water + 0.060*methanol + 7.927*ethanol*water + 9.136*ethanol*methanol + 0.694*water*methanol - 73.359*ethanol*water* -7.174*ethanol* water*(ethanol - water) – 3.929 *ethanol*ethanol*(ethanol - methanol) + 0

% Inhibition = +65.830*ethanol + 63.991*water + 94.396*methanol - 58.156*ethanol *water - 122.68*ethanol*methanol +22.255 * water * methanol + 1061.077 * ethanol * water * methanol + 1 1.992 * ethanol * water * (ethanol-water)-146.785 * ethanol * methanol * (ethanol-methanol) +0

3.4. Contour plots analysis

Three dimensional (3D) interaction contour plots are presented in Figures 1, 2, and 3 (A and B) based on interactions between water, ethanol, and methanol. Correspondingly, hydromethanolic (1/2methanol and 1/2 water) and hydroethanolic (1/2 ethanol and 1/2water) combinations are the most efficient binary interactions for removing both the highest amounts of TPC and TFC. In contrast, the lowest TPC and TFC extraction was observed in methanol. In the same vein, the results are in accord with previous studies, showing that methanol-water binary combination is the most efficient for the extraction of polyphenols (El ksibi *et al.,* 2015). TPC and TFC amounts were found to be influenced by the solvent system. Numerous factors have been shown to influence TPC, such as geographical and climatic factors genetic factors, plant maturity, and storage time (Generalić *et al.,* 2012; Benhouda *et al.,* 2014).

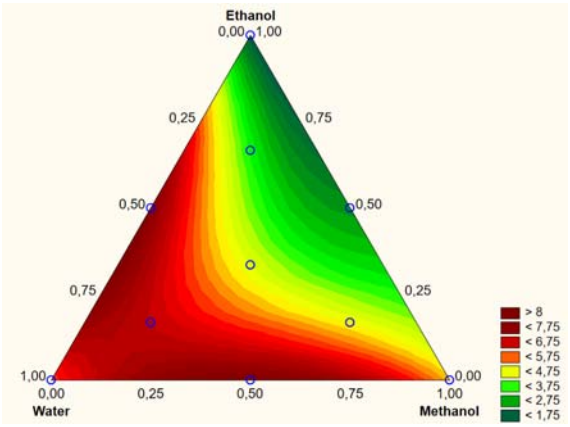

Figure 1A. The Special Cubic Model predicts TAC using water, ethanol, and methanol mixture (2D).

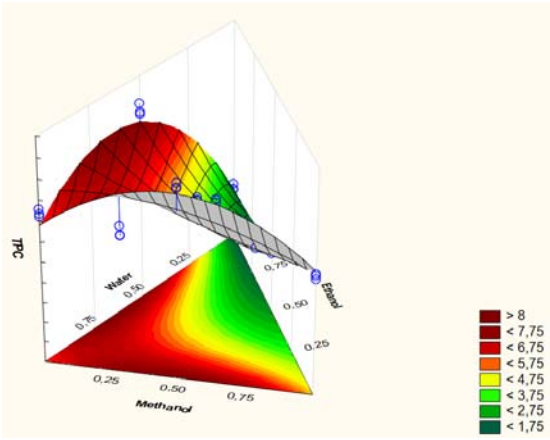

Figure 1B. The Special Cubic Model predicts TAC using water, ethanol, and methanol mixture (2D).

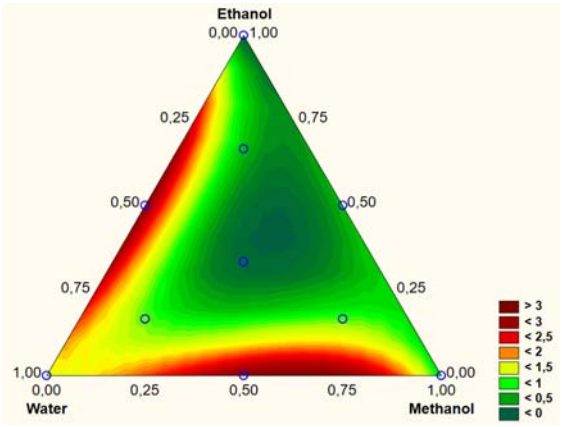

Figure 2A. The Special Cubic Model predicts TFC using water, ethanol, and methanol mixture (2D).

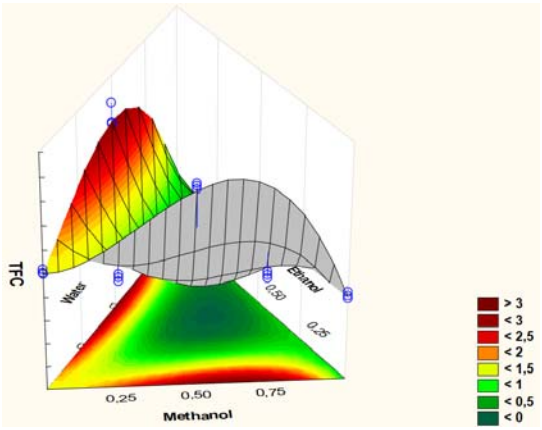

Figure 2B. The Special Cubic Model predicts TFC using water, ethanol, and methanol mixture (2D).

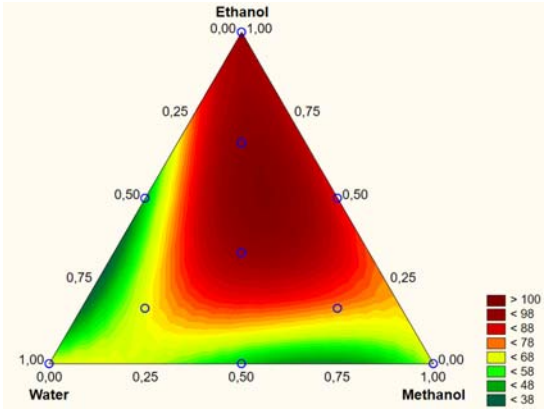

Figure 3A. The Special Cubic Model predicts DPPH using water, ethanol, and methanol mixture (2D).

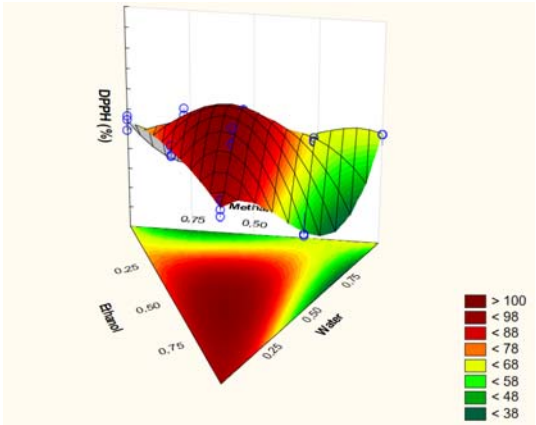

Figure 3B. The Special Cubic Model predicts DPPH using water, ethanol, and methanol mixture (2D).

The optimum solvent system was determined to have a significant effect on the TPC. The solvent system was found to have a significant impact on TPC extraction efficiency. Furthermore, the solvent system was also shown to have a significant effect on the antioxidant capacity of the extract. According to the contour graph, the high level of antioxidant activity was produced by pure ethanol, while a low level was produced by pure water and methanol. In the same vein, the highest antioxidant capacities occur between the positions: ethanol (100%), methanol; ethanol (50%; 50%), water; and ethanol (50%; 50%).

Based on the results of the desirability analysis (as presented in Fig 4A and 4B), the best solvent combination to extract both TPC and TFC is a binary interaction of water and methanol (50%; 50%). The best solvent combination to extract both TPC and TFC is a binary interaction of water and methanol (50%; 50%). The great solvent interactions to extract secondary metabolites with efficiencies of antioxidants are tertiary combination of water-ethanol-methanol with 18.75 % of water, 25 % of methanol, and 56.25 % of ethanol.

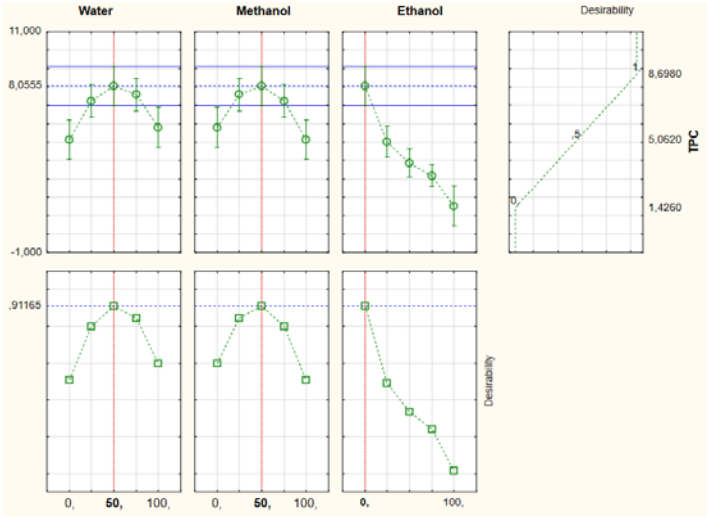

Figure 4A. Desirability results of TPC

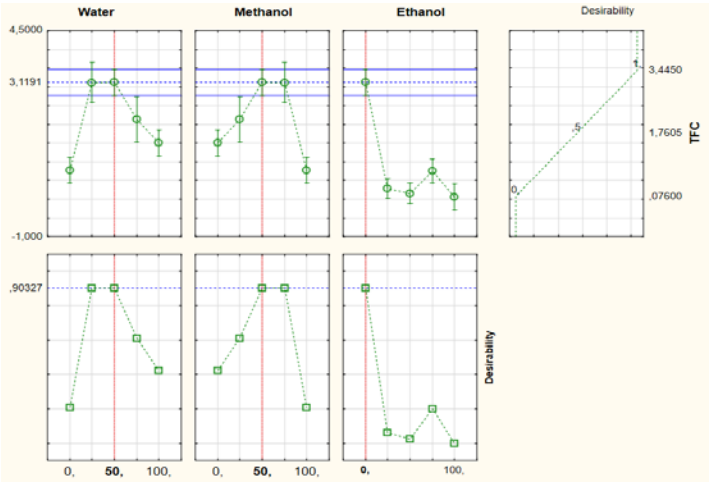

Figure 4B. Desirability results of TFC

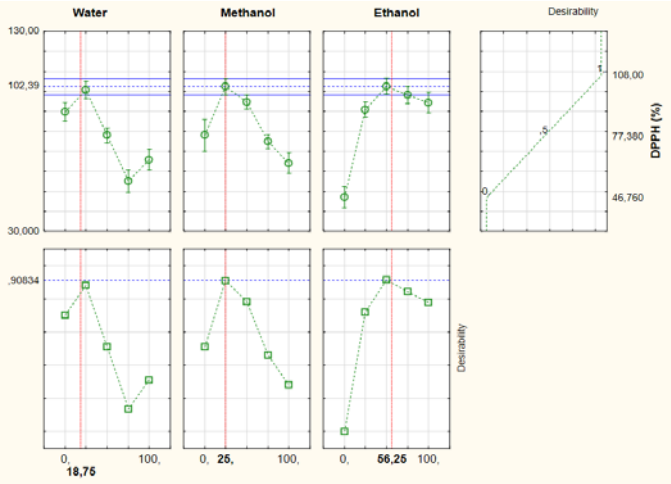

Figure 4C. Desirability results of DPPH

3.5. Pareto Chart Analysis

A standardized Pareto chart was used to assess the relative relevance of main effects and their interactions with statistical significance (p 0.05). Figure 5 shows the effect of independent variables (solvent mixtures) and their interactions on TPC, TFC recovery and TS antioxidant activity. At the 0.05 level, which extends beyond the reference line, the effect is significant. According to Figure 5A, water (A) and methanol (B) are the most influential factors on TPC extraction from TS, and the following solvents: (AC) water∗ ethanol > (AB) water ∗ methanol > (ABC) water ∗ methanol ∗ ethanol showed the most significant effect. According to Figure 5B, the parameters with a greater normalized effect on the extraction of TFC from TS were classified as follows: (ABC) water ∗ methanol ∗ ethanol > (AC) water ∗ ethanol > (AB) water ∗ methanol > (A) water > (B) methanol. Whereas in the case of antioxidant activity (DPPH) evaluation (Figure 5C), methanol (C), water (A) and ethanol (B) showed the most significant effect, followed by the following ternary interaction: (ABC) water∗ ethanol ∗ methanol and finally the binary interactions: (AC) water ∗ ethanol > AB) water ∗ methanol.

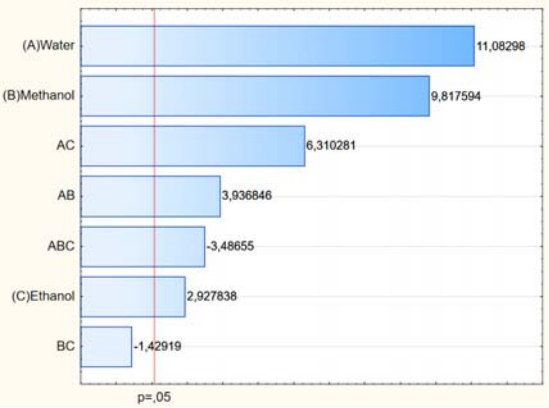

Figure 5A. TPC standardized effects Pareto chart analysis.

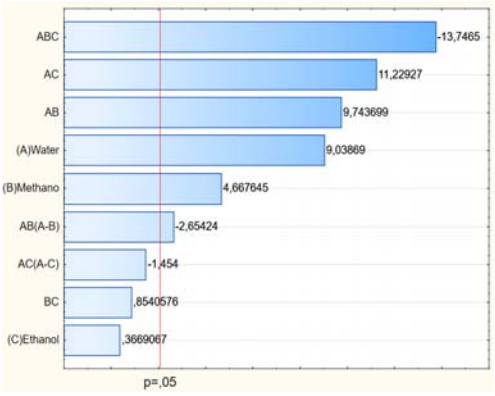

Figure 5B. TFC standardized effects Pareto chart analysis.

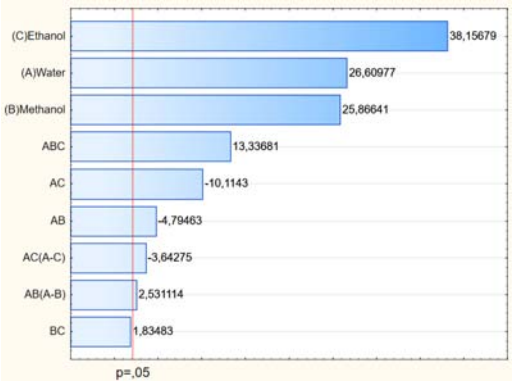

Figure 5C. DPPH standardized effects Pareto chart analysis.

The results found showed that the type of solvent could have an effect on the amount of TPC (Figure 6). Several studies have revealed that different parameters can influence TPC, namely geographical and climatic conditions (Skroza *et al.,* 2012), genetic factors, plant maturity stage and storage period (Benhouda *et al.*, 2014),

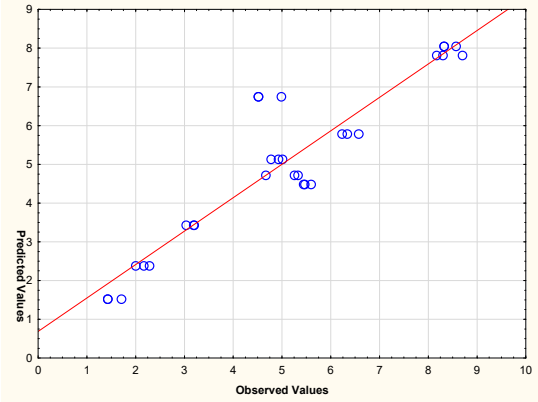

Figure 6A. Actual values (from experiments) compared to predicted values (from the model) (TPC)

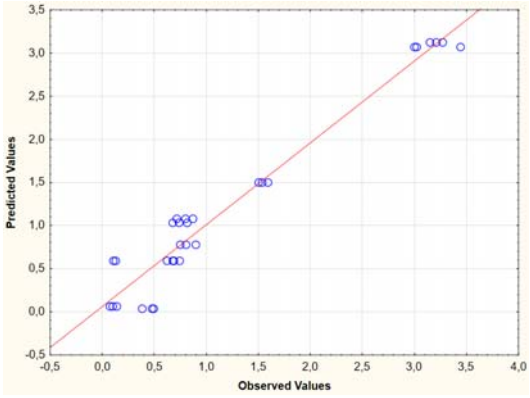

Figure 6B. Actual values (from experiments) compared to predicted values (from the model) (TFC)

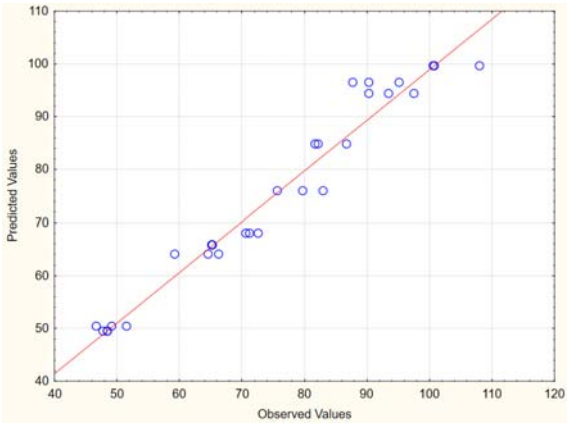

Figure 6C. Actual values (from experiments) compared to predicted values (from the model) (DPPH)

3.6. Principal Components Analysis

Principal components analysis (ACP) can be used to summarize variability in complex data sets that are difficult to interpret (Nićetin *et al.,* 2021). The degree of correlation between two vectors is determined by the angle at which they subtend to each other in this analysis. To assess the correlation between the different variables, principal component analysis was performed. The first factor (factor 1) explained 55.20% of the total variability, while the second factor (factor 2) explained 25.50%. As a result, the first two factors were able to explain 80.7% of the total variability. Figure 7 shows the correlations between the PCA components and the initial variables. Water correlates with total polyphenol and flavonoid content, while ethanol correlates with antioxidant activity (DPPH). This explains why increasing the amount of water in the extraction system enhances its ability to extract phenolic compounds and flavonoids. Similarly, the presence of ethanol favors the extraction of molecules with antioxidant capacity. The same conclusion was reached using Pareto analysis.

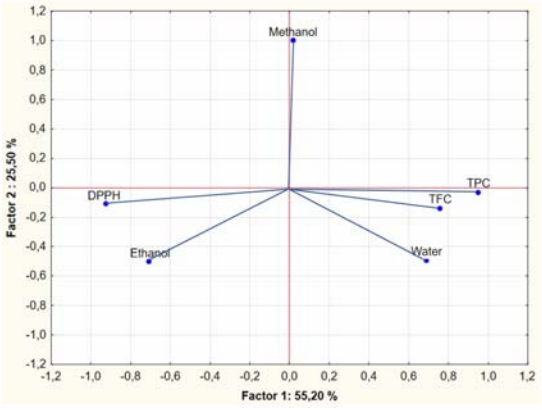

Figure 7: Principal components analysis (ACP).

4. Conclusion

This is the first study to optimize phenolic contents and other molecules with ST's antiradical activity, showing that this waste has the potential to be a useful source of bioactive compounds. Pure water dissolves polyphenols in the plant studied more effectively than other and/or mixture solvents, which is a function of their polar properties. Combined with their ability to cover a wider range of polarity and their synergistic interactions, water and methanol create an ideal solvent environment for flavonoid extraction. As a result of the synergistic interactions between ethanol (66.66%), methanol (16.66%) and water (16.66%), the ternary mixture is found to optimize the extraction of antioxidant compounds from a wide range of molecules. Due to the richness of its metabolites, *Salvia tingitana* can be utilized in a variety of fields, particularly pharmaceuticals. Interestingly, STs' bioactive potential could be further explored through future studies exploring the sustainability and scalability of extraction methods, as well as testing their bioactivity in in vivo and in vitro models

Availability of Data and Materials

Not applicable.

Funding

This study was supported by CNRST under grant number PPR/2015/35.

Conflict of Interest

The authors declare no conflict of interests, financial or otherwise.

References

Aazza S. 2021. Application of multivariate optimization for phenolic compounds and antioxidants extraction from Moroccan Cannabis sativa waste. *J Chem*., **2021**: 1-11.

Asadollahi M, Firuzi O, Jamebozorgi F H, Alizadeh M and Jassbi AR. 2019. Ethnopharmacological studies, chemical composition, antibacterial and cytotoxic activities of essential oils of eleven Salvia in Iran. *J HerbMed*., **17**: 100250.

Aşkun T, BAŞER K, Tümen G and Kürkcüoğlu M. 2010. Characterization of essential oils of some Salvia species and their antimycobacterial activities. *Turk J Biol.*, **34(1)**: 89-95.

Azzane A, Amssayef A and Eddouks, M. 2022. Salvia aucheri Exhibits Antihypertensive Activity in Hypertensive Rats. *Cardiovasc Hematol* Agents *Med Chem.*, **21**: 167-176.

Azzane A and Eddouks M. 2022. Antihyperglycemic, Antihyperlipidemic, and Antioxidant Effects of *Salvia Tingitana* in Streptozotocin-Induced Diabetic Rats. *Cardiovasc Hematol Disord Drug Targets*., **22 (2):** 118–127.

Azzane A, Farid O and Eddouks M. 2023. Antihyperglycemic and Antidyslipidemic Effects of *Artemisia arborescens* Aqueous Extract on Streptozotocin-induced Diabetic Rats. *Cardiovasc Hematol* Agents *Med Chem.*, **21(2):** 120-138.

Benhouda A and Yahia M. 2014. Toxicity, analgesic and anti-pyretic activities of methanolic extract from Hyoscyamus albus' leaves in albinos rats. *Int J Pharm PharmSci*, **6(3):** 121-127.

Bisio A, Ciarallo G, Romussi G, Fontana N, Mascolo N, Capasso R and Biscardi D. 1998. Chemical composition of essential oils from some Salvia species. *Phytother Res*., **12:** 117-S120.

Bouaziz M, Dhouib A, Loukil S, Boukhris M and Sayadi S. 2009. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. *Afr J Biotechnol.*, **8(24).**

Chansriniyom C, Nooin R, Nuengchamnong N, Wongwanakul R, Petpiroon N, Srinuanchai W, Chantarasuwan B, Pitchakarn P, Temviriyanukul P, Nuchuchua O. 2021. Tandem mass spectrometry of aqueous extract from *Ficusdubia*sap and its cell-based assessments for use as a skin antioxidant. *Sci Rep.,* **11(1):** 16899.

Chater O, El ghadraoui L, Harrach A and Aazza S. 2023. Extractive optimization of antioxidants and phenolic compounds from Anacyclus pyrethrum. *Not Sci Biol*., **15(4)**: 11616-11616.

El Baakili A, Fadil M and Es-Safi NE. 2023. Ultrasonic-assisted extraction for phenolic compounds and antioxidant activity of Moroccan Retama sphaerocarpa L. leaves: Simultaneous optimization by response surface methodology and characterization by HPLC/ESI-MS analysis. *Heliyon*., **9(6).**

El Ksibi I, Slama RB, Faidi K, Ticha MB and M'henni MF. 2015. Mixture approach for optimizing the recovery of colored phenolics from red pepper (Capsicum annum L.) by-products as potential source of natural dye and assessment of its antimicrobial activity. *Ind Crop Prod*., **70**: 34-40.

Garcia LMZ. Oliveira TF, Soares PK, Bruns RE and Scarminio IS. 2010. Statistical mixture design—Principal component determination of synergic solvent interactions for natural product extractions. *Chemometr Intel Lab Sysi.*, **103(1):** 1-7.

Generalić I, Skroza D, Šurjak J, Možina S, Ljubenkov I, Katalinić A, Šimat V and Katalinić V. 2012. Seasonal variations of phenolic compounds and biological properties in sage (Salvia officinalis L.). *Chem Biodivers*., **9(2)**: 441-457.

Han L, Gao X, Xia T, Zhang X, Li X and Gao W. 2019. Effect of digestion on the phenolic content and antioxidant activity of celery leaf and the antioxidant mechanism via Nrf2/HO-1 signaling pathways against Dexamethasone. *J Food Biochem*., **43(7)**:12875.

Kelen M and Tepe B. 2008. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. *BioresourTechnol*., **99(10)**: 4096-4104.

Khiyari MEA, Kasrati A, Jamali CA, Zeroual S, Markouk M, Bekkouche K, Wohlmuth H, Leach D and Abbad A. 2014. Chemical composition, antioxidant and insecticidal properties of essential oils from wild and cultivated Salvia aucheri subsp. blancoana (Webb. &Helder)), an endemic, threatened medicinal plant in Morocco. *Ind Crop Prod*., **57**:106-109.

Li M, Li Q, Zhang C, Zhang N, Cui Z, Huang L and Xiao P. 2013. An Ethnopharmacological Investigation of Medicinal Salvia Plants (Lamiaceae) in China. *Acta Pharm Sin. B*., **3 (4):** 273–280.

Louli V, Ragoussis N, Magoulas K. 2024. Recovery of phenolic antioxidants from wine industry by-products. 2004. *Bioresour. Technol.,* **92(2):** 201-208.

Lu Y. and Foo LY. 2022. Polyphenolics of Salvia—a review. *Phytochem*., **59(2)**: 117-140.

Mansouri I, Laaroussi H, El Ghouizi A, Lyoussi B and El Arabi, I. 2020. Phytochemical content and antioxidant activity of flesh fruits Rosa canina extracts collected from AitAyachMidelt. *Indian J Agric Res*., **54(3)**: 373-377.

Martino LD, Roscigno G, Mancini E, Falco ED and Feo VD. 2010. Chemical composition and antigerminative activity of the essential oils from five Salvia species. *Molecules*., **15(2)**: 735-746.

Nekhla H, Aazza S, Tarmoun K, El Hanafi L, Lahrizi L, Squalli W, Ahmed Harrach A and El Ghadraoui L. 2023. Optimization of Polyphenol Extraction from Three Parts of Chamaeropshumilis L.(Leaves, Fruit And Pulp) Through Response Surface Methodology. *Chem Afr.*, **6(3)**:1191-1200.

Nićetin M, Pezo L, Filipović V, Lončar B, Filipović JS, Šuput D and Knežević V. 2021. The effects of solution type temperature and time on antioxidant capacity of osmotically dried celery leaves. *Therm Sci*., **25(3):** 1759-1770.

Oliveira A, Padilha C, Gonzales Ortega G, and Petrovick P. 2001. Achyrocline satureioides (Lam.) DC. (marcela), Asteraceae, avaliaçãocomparativa da drogavegetal e estudospreliminares de otimização da extração. *Caderno de Farmácia. Porto Alegre.,* **17**: 33-38.

Pan Y, Zhang J, Shen T, Zuo ZT, Jin H, Wang YZ and Li WY. 2015. Optimization of ultrasonic extraction by response surface methodology combined with ultrafast liquid chromatography–ultraviolet method for determination of four iridoids in Gentianarigescens. *J Food Drug Anal*., **23(3)**: 529-537.

Plaza M and Marina ML. 2023. Pressurized hot water extraction of bioactives. *TrAC Trends Anal Chem*., **166**: 117201.

Saravana Pandian P, Sindhanaiselvan S, Subathira A and Saravanan SA. 2023. correlative algorithmic optimization study for an integrated soft computing technique in aqueous two-phase protein extraction from Litopena eusvannamei waste. *Biomass Convers Biorefin*., **13(18):** 16819-16833.

Sharifi-Rad M, Ozcelik B, Altın G, Daşkaya-Dikmen C, Martorell M, Ramírez-Alarcón K, Alarcón-Zapata P, Morais-Braga MFB, Carneiro JNP, Alves Borges Leal Al. 2018. Salvia Spp. Plants-from Farm to Food Applications and Phytopharmacotherapy. *Trends Food Sci Technol*., **80**: 242–263.

Skroza D, Surjaka J, Mozinab SS. 2012. Seasonal variations of phenolic compounds and biological properties in sage (*Salvia officinalis* L.). *Chem Biodivers* **9(2):** 441–57

StatSoft I. 2011. Statistica (data analysis software system).

Strati IF, Kostomitsopoulos G, Lytras F, Zoumpoulakis P, Proestos C and Sinanoglou V. 2018. Optimization of polyphenol extraction from Allium ampeloprasum var. porrum through response surface methodology. *Foods*., **7(10)**: 162.

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M and El-Elimat T. 2007. Antiradical activity and total phenolic content of selected Jordanian plant species. *Food Chem*., **104(4)**: 1372–1378

Venkatesan T, Choi Y. W and Kim YK. 2019. Impact of different extraction solvents on phenolic content and antioxidant potential of Pinusdensiflora bark extract. *BioMed Res Int.*, **2019 (1)**: 3520675.

Zhang QW, Lin LG and Ye WC. 2018. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin med.,* **13**: 1-26.