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Original Research Article

Simultaneous optimization of extraction of bioactive compounds and antioxidant activity of *Ammi visnaga* (L.) Lam aerial parts using response surface methodology

Zineb El Jabboury[1]✉*, Smail Aazza[2], Driss Ousaaid[3], Oumaima Chater[1], Uroš Gašić[4], Peđa Janaćković[5], Zora Dajic Stevanovic[6], Stefan Kolašinac[6], Meryem Benjelloun[1], And Lahsen El Ghadraoui[1]

[1]Laboratory of Functional Ecology and Environmental Engineering, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University - Fez, Morocco
[2]OLMANBGPE, Nador Multidisciplinary Faculty, Mohammed 1st University, Oujda, Morocco
[3]Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez P.O. Box 3000, Morocco
[4]Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060, Belgrade, Serbia
[5]University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia
[6]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11060 Belgrade, Serbia

ABSTRACT

In this report, different extracts from the aerial parts of *Ammi visnaga* (L.), *e.g.*, flowers, leaves, and stems were prepared using water, methanol, and ethanol. To optimize the extraction process, the design of mixtures was carried out using different extracting solvents and their combinations. The special cubic model explained the variance of the TPC and the antioxidant activity of the extracts at a level of R^2 > 95%. In general, the analysis of the model-derived response surfaces revealed that in binary mixtures (50% ethanol + 50% methanol), the yielded values of phenolic compounds and the antioxidant activity increase with the water proportion of different prepared mixtures. The ability of the quaternary mixture to extract the phenolic compounds was also positively and significantly influenced by the water content, creating a mild polar medium for the extraction of phenolic compounds. The phenolic profile of different extracts under study revealed the presence of a cocktail of active ingredients, including chlorogenic acid, caffeic acid, rutin, *p*-coumaric acid, etc. especially the flower extract of *A. visnaga* (L.).

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1. Introduction

The Umbelliferae family, also known as Apiaceae, encompasses a diverse group of plants with approximately 434 genera and nearly 3,780 species distributed across various habitats worldwide. These plants are economically significant, serving as leaf and root vegetables, herbs, spices, and ornamentals (Spinozzi et al., 2021). The family is characterized by aromatic herbs with distinctive feather-divided leaves and flowers arranged in umbels. While many species are utilized for culinary purposes, some members like poison hemlock and water hemlock are poisonous. Additionally, plants such as carrot, celery, parsley, and fennel are commonly used as vegetables, while others like anise, coriander, and cumin are valued for their herbal and spice properties (Teng et al., 2023). The Umbelliferae family's rich diversity and economic importance underscore its relevance in both traditional and modern applications (Spinozzi et al., 2021; Teng et al., 2023; Valatabar et al. 2023). *Ammi visnaga* (L.) Lam. belongs to the Umbellifereae family distributed natively in the Nil Valley, North Africa, Europe, Asia, and North America (Franchi et al., 1985; El Jabboury aet al. 2023). For centuries, humans have developed their knowledge in pharmacognosy and phytochemistry, which is considered a cornerstone of traditional and modern medicine (Mohammadhosseini et al. 2019a; Mohammadhosseini et al. 2019b; Awuchi, 2023; Chaniad et al., 2023; Theodoridis et al., 2023). Medicinal herbs are widely cultivated for many purposes including extraction of bioactive compounds with biological activities, *e.g.*, antidiabetes, antiobesity, antiinflammation, and antimicrobial effects (Nwozo et al., 2023). These plants have been used in folk medicine to treat renal colic, abdominal cramps, vitiligo, and psoriasis (Khalil et al., 2020). Medicinal plants are also considered an inexhaustible source of

✉Corresponding author: Zineb El Jabboury
Tel: +212708798109; Fax:+212708798109
E-mail address: zineb.eljabboury@usmba.ac.ma, **doi: 10.71596/tpr.2024.1107361**

phenolic compounds well-known for their biological properties (Molin et al., 2014). The beneficial properties of *A. visnaga* (L.) are attributed to its dense chemical composition. The phytochemical compounds of *A. visnaga* (L.) include pyrones, coumarins, khellin, visnagin, 4-norvisnagin, khellinol, visamminol, ammiol, and khellol (Abou-Mustafa et al., 1990; Hashim et al., 2014). The aerial parts of this plant are rich in phenolic compounds in aglycone and conjugated forms (Khalil et al., 2020). The delve into the phytochemistry of the plant (*A. visnaga* (L.)) revealed that quinic acid was the most abundant bioactive compound (9.436 mg/g) followed by other compounds, including gallic acid, protocatechuic acid, and gentisic acid (El-guourrami et al., 2023). FTIR analysis indicats that *A. visnaga* (L.) contains long-chain linear aliphatic compounds, lipids, amides, and aromatic components (Benabderrahmane et al., 2023). Robust evidence found that the lower doses of *A. visnaga* (L.) extracts did not cause any sign of toxicity and the LD_{50} for intraperitoneal and oral administration of the herb extract was found to be 3.6 and 10.1 g/kg, respectively (Jouad et al., 2002; Koriem et al., 2019).

The health benefits of *A. visnaga* (L.) have been investigated by several studies highlighting its antidiabetic, antihyperlipidemic, anticancer, antibacterial, and antifungal activities (Koriem et al., 2019). Bioactive compounds are found in different amounts in natural products and used extensively in pharmaceutical and food industries. Thus, the main challenge is likely to find the most appropriate method(s) to obtain the highest yield of these compounds well-known for their pharmacological properties (Lin et al., 2018). Different techniques were used to evaluate their ability to extract the maximum content of phenolic compounds comprising mild methods like ultrasound-assisted extraction (UAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), pulsed electric field (PRF) pretreatment, ohmic heating (OH) pretreatment, and cold plasma (CP) pretreatment as well as conventional methods, *e.g.*, solvent extraction, soxhlet extraction, squeezing or cold pressing, and steam distillation (Ebrahimi and Lante, 2022). Recently, several investigations have been carried out to examine efficient extraction techniques to reduce extraction time, energy and costs, along with organic solvent consumption (Lopez-Avila and Luque de Castro, 2014). It has been proved that flavonoids and terpenes are both successively extracted using ethyl alcohol and ethyl acetate, while polyphenols can be extracted by a mixture of acetone, methanol, and ethanol (Alberti et al., 2014). The extraction optimization using different extracting solvent mixtures could provide promising findings (Pourbasheer et al. 2014; 2017). The design of experiments has highlighted its importance for the extraction optimization process (Cavalcanti et al., 2021). Response surface methodology (RSM) is a valuable tool extensively utilized to optimize the extraction of phenolic compounds. It aids in predicting the most suitable combination of extracting solvents, thereby minimizing time, solvent usage, and reducing the need for extensive benchwork (Cavalcanti et al., 2021).

In the present report, response surface methodology was used to optimize the extraction of phenolic compounds and antioxidant activity using three complementary assays, namely HCA, TAC, and CUPRAC as well as the phenolic profile from the aerial parts of Moroccan A. *visnaga* (L.) collected from Taounate region.

2. Experimental

2.1. Plant material

The wild fresh plant (Fig. 1) was collected in the Taounate region of Morocco (34°33'47'N, 4°39'34'W) in April 2020, according to the guidelines described by the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and him Flora (IUCN, 1989). The plant parts, viz., flowers, leaves, and stems were separated and subsequently dried at 40 ºC and finely ground before extraction process. The plant was identified by the Team of the Department of Botany and Vegetal Ecology of the Scientific Institute, Rabat. A representative sample of the plant material (*A. visnaga* (L.)) was deposited at the herbarium of the same department under voucher number RAB114158.

Fig. 1. The photograph of *Ammi visnaga* (L.).

2.2. Extraction procedure and sample preparation

The extraction was done in triplicates using three pure solvents, water, ethanol, and methanol, and their mixtures, according to the following procedure. In this context, 50 mg of dried and pulverized inflorescence of *A. visnaga* (L.) were extracted for 20 min by sonication with 1 mL of solvents mixture. The extracts were centrifuged for 15 minutes at 6000 rpm, and the supernatants were recuperated and stored at 4 ºC (El Jabboury et al., 2022).

2.3. Evaluation of solvent impacts by simplex axial design

Two dissimilar categories of standard designs are usually used for the extraction experimentation with combinations involving (i) Simplex-centroid design, and (ii) Simplex-lattice design. Both designs assess the triangular reply surface at the vertices (*i.e.*, the corners of the triangle), then the centroids (sides of the triangle) (Montgomery, 2013). In the simplex-centroid design, different conditions tested a triangle with pure components in the vertex, representing 100% of each single solvent. Central points on every side express permutations of the binary blends (1/2: 1/2: 0; 1/2: 0: 1/2;

0: 1/2: 1/2), and, the medium point as a ternary mixture (1: 1: 1). This scheme is from time to time increased with internal points (axial ones) expressing 2/3 of one of the targeted solvents and 1/6 for the others (Fig. 2), also known as simplex axial design (SAD) (Sampaio et al., 2015). To boost the extraction procedure, a mixture design was developed as presented in Fig. 1. The simplex-centroid design coupled with axial points in three replicates was chosen to determine the solvent combination of water (W), ethanol (E) and methanol (M). Fig. 2 presents all tested conditions. This design, permitted the evaluation of linear (W, E and M), quadratic (WE, WM, and EM), and special cubic (WEM) models for the response under study.

2.4. Total phenolic content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method described by Hasperué et al. (2016). In brief, 50 µL of the extract was mixed with 450 µL of Folin-Ciocalteu reagent (0.2 N) for 5 min, and then 450 µL of a Na_2CO_3 solution (75 g L^{-1}) was added. All samples were incubated at room temperature in dark condition for 2 h and their absorbance was read at 760 nm in a Jenway 6505 UV/visible, scanning spectrophotometer. The calibration curve was obtained using gallic acid over the concentration range of 0-250 µg/mL. The experiment was tested in triplicates and the results were expressed as mg GAE/g of dried plant.

2.5. Total dihydroxycinnamic acid derivative content (HCA)

The total HCA content was estimated using the method described by Fraisse et al. (2011). Total HCA content in the extract was determined from the calibration curve with chlorogenic acid (CGA) as standard. Results were expressed as milligrams of CGA equivalents (CGAE) per g of dried weight.

2.6. Determination of antioxidant activity

2.6.1. Total antioxidant capacity (TAC)

This assay was performed according to the procedure described by Yilar et al. (2020). Accordingly, a calibration curve was prepared using different concentrations of ascorbic acid as standard and the relevant results were expressed as mg of ascorbic acid equivalents (AAE) per g of DW.
In each method, all samples were analysed in triplicates (n = 3). Absorbance of the resulting solution was measured using a UV/visible spectrophotometer.

2.6.2. CUPRAC assay

Further elution using hexane-CH_2Cl_2 (95:5, *v/v*) afford a white powder of (**4**)**,** yield 10.6 mg; m.p. 302-304 °C; ^{13}C-NMR (CD_2Cl_2; 125MHz): δ 154.8 (C-20), 107.1 (C-30), 80.9 (C-3), 55.4 (C-5), 51.0 (C-9), 48.8 (C-18), 41.9 (C-14), 41.2 (C-8), 39.6 (C-19), 38.8 (C-16), 38.7 (C-13), 38.2 (C-22), 38.1 (C-1), 37.7 (C-4), 37.0 (C-10), 34.7 (C-17), 33.9 (C-7), 27.9 (C-23), 26.6 (C-15), 26.1 (C-12), 25.6 (C-29), 25.5 (C-21), 23.6 (C-2), 21.4 (C-11), 19.8 (C-28), 18.1 (C-6), 16.4 (C-24), 16.3 (C-26), 15.8 (C-25), 14.7 (C-27) (Trinh et al., 2008).

2.7. Phenolic profile

The dionex Ultimate 3000 UHPLC system (ThermoFisher Scientific, Bremen, Germany) equipped with a diode array detector (DAD) and TSQ Quantum Access Max triple-quadrupole (QQQ) mass spectrometer (ThermoFisher Scientific, Basel, Switzerland) was used to delve into the phytochemistry of different parts of *A. visnaga* (L.). Elution was performed at 40 °C on a Syncronis™ C18 column (100 × 2.1 mm) with 1.7 µm particle size (ThermoFisher Scientific, Fair Lawn, NJ, USA). Water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) were used as the mobile phase following gradient elution: 5% B in first min, 1-14 min 5-95% B, 14-14.2 min 95-5% B and 5% B until the 20th min (Skorić et al., 2022). Full scanning (FS), product ion scanning (PIS) and neutral loss scanning (NLS) modes were conducted for qualitative analysis. Xcalibur software (version 2.2) was used for the instrument control, data acquisition, and analysis. Compounds were identified by direct comparison with commercial standards and literature data. The total amount of each compound was evaluated by calculation of peak areas (Skorić et al., 2022).

2.8. Statistical analysis

The models described above, utilizing a 3rd-degree polynomial function, were used in surface matching. The effect of S-LD on TPC and antioxidant activity was analyzed using the least square multiple regression method. According to this experiment, typical multiple regression equations were used as follows:

Linear model: $y = b_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3$ (Eqn. 1)

Quadratic model: $y = b_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3 + b_{12} \times x_1 \times x_2 + b_{13} \times x_1 \times x_3 + b_{23} \times x_2 \times x_3$ (Eqn. 2)

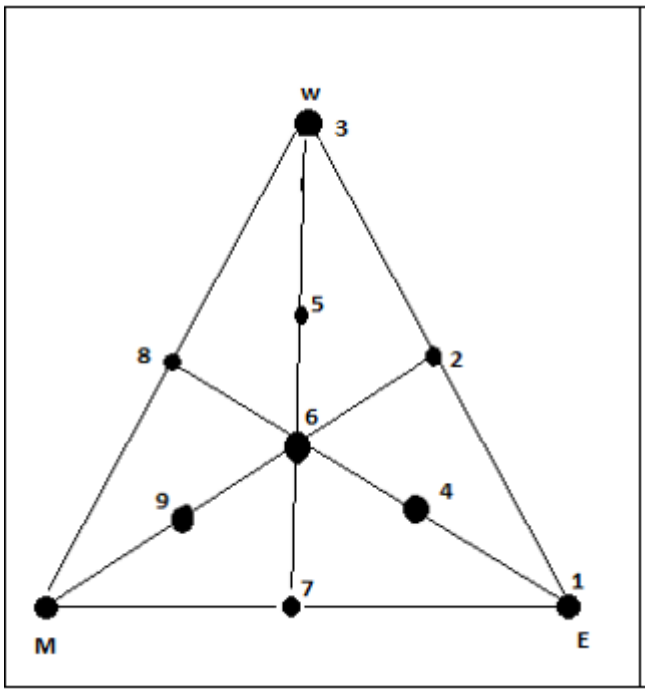
Special cubic model: $y = b_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3 + b_{12} \times x_1 \times x_2 + b_{13} \times x_1 \times x_3 + b_{23} \times x_2 \times x_3 + b_{123} \times x_1 \times x_2 \times x_3$ (Eqn. 3)

The following parameters: Sum of square (SS), mean of square (MS), df (degree of freedom), test F, *p*-values, R-square (R^2), R-square adjusted (R^2_{adj}). were considered when selecting the appropriate statistical model.
The analysis of variance (ANOVA) was applied to determine the fitness of the multiple regression model ($p < 0.05$) and to evaluate the significant effects of variables and the relevant interactions. The analyses were performed using the free version of STATISTICA version 10 software.

3. Results and Discussion

3.1. Total phenolic content

The results of total phenolic compounds obtained for extracts of the four parts of *A. visnaga* (L.) are given in Table 1.
The process of recovering the phenolic compounds in multiple samples is persuaded by the solubility of these compounds and the polarity of the solvent applied in



	Ethanol	Water	Methanol
1	0.00	0.00	100.00
2	0.00	50.00	50.00
3	0.00	100.00	0.00
4	16.67	16.67	66.67
5	16.67	66.67	16.67
6	33.33	33.33	33.33
7	50.00	0.00	50.00
8	50.00	50.00	0.00
9	66.67	16.67	16.67
10	100.00	0.00	0.00

Fig. 2. Simplex axial design (SAD) (W: Water, M: Methanol, E: Ethanol).

Table 1
Total phenolic contents obtained for extracts the four parts of *Ammi visnaga* (L.).

Number	Ethanol	Methanol	Water	TPC mg GAE/g		
				Flower	Stem	Leaf
1	0.00	50.00	50.00	47.47±0.97	25.49±0.17*	27.63±0.07
2	0.00	100.00	0.00	39.25±1.19^{a}	28.61±0.06*	31.22±1.06
3	16.67	16.67	66.67	25.57±1.08^{ab}	14.55±0.14^{ab*}	17.91±0.38^{ab}
4	16.67	66.67	16.67	41.44±1.89^{c}	28.24±0.34^{c*}	28.39±0.84^{c}
5	33.33	33.33	33.33	34.21±0.16^{ac}	19.96±0.99^{d*}	24.31±0.48
6	50.00	0.00	50.00	38.75±0.36^{ac}	25.51±0.11^{c*}	31.29±0.59^{ce}
7	50.00	50.00	0.00	49.71±0.12^{bcde}	32.09±0.44^{ce*}	24.41±0.56**
8	66.67	16.67	16.67	19.01±1.1^{abde}	13.67±0.06^{abcd*}	11.81±0.38^{abde*}
9	100.00	0.00	0.00	29.73±1.91^{abd}	22.24±1.59*	20.29±2.50^{bde}
10	0.00	0.00	100	10.90±1.10 ^{abcde}	8.34±0.39^{abde}	5.84±0.27^{abcde}
All Runs				33.60±11.99^{a}	21.87±7.38*	22.31±8.17^{be}

the extraction process (Sulaiman et al., 2011). The results obtained showed that the total phenolic compound (TPC) ranged from 8.34 ± 0.39 to 32.09 ± 0.44 mg GAE/g for dry stem. However, these amounts varied greatly among flower extracts between 10.90±1.10, and 49.71 ± 0.12 mg GAE/g, and from 5.84 ± 0.27 to 31.29 ± 0.59 mg GAE/g in the leaves. This highlights the influence of the extracting solvent. A previous report has shown that the TPC amount found in *A. visnaga* (L.) ranges from 76.10 to 195.30 mg GAE/g (Aourabi et al., 2021). The obtained results are lower than those reported previously (Sarra et al., 2018; Aourabi et al., 2021). This variability is highly related to environmental factors such as ecolimatic conditions, drought, salinity, viruses among others (de Carvalho et al., 2018; Vaughan et al., 2018).

3.2. Analysis of variance (ANOVA)

Table 2, also at the model shows the significant effect of the processing variables on the TPC (p < 0.001). In

general, the effect of all processing variables was determined to be positive, which indicated that the increase in each solvent level provided an increase in total phenolic content. Optimization of TPC extraction by simplex lattice mixture design from *Prunus mahaleb* L. showed the same effect of solvent likewise our finding (Ozturk et al. 2014).

The linear model explained the variance at the level of R^2 with a frequency of (R^2 0.65 and 0.62 R^2adj), (R^2 0.65 and 0.62 R^2adj), (R^2 0.57; 0.54 R^2adj) and (R^2 0.25; 0.20 R^2adj), for leaves, flowers, stem, and roots, respectively. Expanding from linear to quadratic model improved the fitness for regression analysis (Table 3).

Table 2
Analysis of variance results for different statistical models.

Model	SS Effect	df Effect	MS Effect	F	p	R^2	R^2_{adj}
leaves							
Linear	1263.19	2.00	631.59	25.41	0.00	0.65	0.62
Quadratic	522.09	3.00	174.03	28.05	0.00	0.92	0.90
Special Cubic	77.62	1.00	77.62	25.06	0.00	0.96	0.954
Total Adjusted	1934.15	29.00	66.69				
Flowers							
Linear	3045.93	2.00	1522.96	36.67	0.00	0.73	0.71
Quadratic	952.49	3.00	317.49	45.18	0.00	0.96	0.95
Special Cubic	0.41	1.00	0.41	0.057	0.008	0.96	0.94
Total Adjusted	4167.06	29.00	143.69				
Stems							
Linear	909.58	2.00	454.79	18.27	0.00	0.575	0.54
Quadratic	624.02	3.00	208.0	104.41	0.00	0.970	0.96
Special Cubic	11.25	1.00	11.250	7.077	0.00	0.977	0.97
Total adjusted	1581.42	29.00	54.532				

Table 3
Coefficients of the overall fitness for the regression model ($p < 0.05$).

	SS	df	MS	F	*p*
Leaves					
Model	1862.92	6.00	310.49	100.24	0.00
Total Error	71.24	23.00	3.10		
Lack of Fit	52.57	3.00	17.52	18.77	0.01
Pure error	18.67	20.00	0.93		
Total adjusted	1934.16	29.00	66.70		
Flowers					
Model	3998.84	6.00	666.47	91.12	0.00
Total Error	168.22	23.00	7.31		
Lack of Fit	141.27	3.00	47.09	34.95	0.02
Pure error	26.95	20.00	1.35		
Total adjusted	4167.06	29.00	143.69		
Stems					
Model	1544.86	6.00	257.48	161.96	0.00
Total Error	36.56	23.00	1.59		
Lack of Fit	28.51	3.00	9.50	23.62	0.00
Pure error	8.05	20.00	0.40		
Total adjusted	1581.42	29.00	54.53		

Whereas, the best fitness was found in the special cubic model in which the coefficients of determination were also improved. This special cubic model also explained (R^2 0.96 and 0.95 R^2adj), (R^2 0.96 and 0.95 R^2adj), (R^2 0.98; 0.97 R^2adj) and (R^2 0.91; 0.89 R^2adj), of the variance and adjusted it for leaves, flowers, stem, and roots, respectively. similar results have been described by Baj et al. (2018). This evaluation may indicate a statistically significant interaction between three-component systems which could be explained by the higher complexity of the model and the interactions that may occur between the three selected solvents.

Due to a higher coefficient of determination, a special cubic model was chosen to further evaluate the statistical impact of the composition of the solvent mixture installed on the total phenolic content of extracts from different parts of the plant. The regression models for the experiment are shown below:

TPC-Leaves = +6.41×x + 17.86×y + 26.66×z + 0.809×x×y + 29.907×x×z + 31.79×y×z + 163.18×x×y×z+0 (Eqn. 4)

TPC-Flower = +11.19×x + 26.70×y + 45.80×z + 5.958×x×y + 79.32×x×z + 9.85×y×z - 11.92×x×y×z + 0 (Eqn. 5)

TPC-Stem = +9.03×x + 14.38×y + 24.91×z + 9.92×x×y + 60.90×x×z + 32.84×y×z - 62.12×x×y×z + 0 (Eqn. 6)

In the above equations, the symbols X, Y and Z respectively stand for ethanol, methanol and water. Furthermore, in all four parts of the plant, TPC was positively and linearly influenced by methanol (y) and water (z), respectively. The obtained results show that ethanol (x) has the lowest coefficient; and the smallest proportion of TPC.

In the group of binary interactions, the use of ethanol reduced the extraction ability of methanol (xy), without affecting the extracting power of water (z). The ternary interaction (xyz) showed a synergistic effect between the components of the mixture for leaves and roots, while it displayed an antagonistic effect on TPC extraction from flowers and plant stems.

3.3. Surface analysis

3.3.1. TPC from leaves

Total phenolic compound was determined to evaluate the strength of the extracting solvent used on different parts of *A. visnaga* (L.). The special cubic model gave a satisfactory value to determine the coefficient (R^2 = 0.963, R^2adj = 0.954), and all coefficients were significant at the 95% confidence level.

The response surfaces for TPC, which were obtained from leaves by mixture design for the percentage composition of methanol, water, and ethanol, are illustrated in Fig. 3A and Fig. 3B. As seen, water was the best solvent for the TPC extraction followed by methanol, whereas ethanol extracted the lowest amount of TPC. According to Fig. 3 (A and B), in binary mixtures, adding water to ethanol and methanol increases its power to extract TPC. The highest amount was extracted by ternary mixtures.

The best solvent mixture given by the analyzed program for optimal TPC extraction from the leaves of *A. visnaga* (L.) consisted of 50% of water, 40% methanol, and 10% of ethanol.

3.3.2. TPC from flowers

The quadratic and special cubic models explained better the variance in TPC content at the level of R^2 (0.96), indicating the well fitness of the proposed model. Thus, it is capable of proper predicting of the behavior of the mixture.

The response surfaces for TPC, which were obtained from flowers by mixture design as a function of the percentage composition of water, methanol, and ethanol, are illustrated in Fig. 4 (A and B).

In the linear interactions, water served as the best extracting solvent followed by methanol and ethanol. Moreover, the analysis of the binary interaction shows that TPC increases with the increase in water in the two solvents. The best binary interaction occurs between water and ethanol. The ternary interaction did not have a good impact on TPC extraction. The highest extract amounts were found to occur with aqueous and ethanol between 50 and 90%. It could be also inferred that water addition to ethanol and methanol could increase their power to extract TPC from flowers.

3.3.3. TPC from stems

The surface fitting for special cubic model is shown in Fig. 5 (A and B). Accordingly, water has better linear effect on TPC extraction compared to methanol and ethanol. The increasing water ratio in the binary mixture with methanol and ethanol, increases the polarity of the solvent mixtures and enhances its ability to extract phenolic compounds. The best results are seen when the water content in the mixture reaches 50%. That amount returns to decrease when water amount overtakes 75%.

3.4. Pareto chart analysis

Pareto charts show each effect and the combination of effects by a bar in decreasing order of significance. From a graphical viewpoint, this helps us visualize the influencing variables and their degree of influence. The Pareto diagrams present a ranking of the most significant factors to the significant ones, with a significance limit of the *p*-value of 5.0%. A factor with a negative effect has a higher value for the low level than the value for the high level (Chemistry and Duret 2012). Fig. 6 (Fig. 6-1, Fig. 6-2, and Fig. 6-3) shows the Pareto chart of the effects of the studied variables on the polyphenol content. Water was the solvent that influenced mostly and positively phenolic extraction from different parts of plants, followed by methanol. Ethanol was the third parameter that showed a significant positive effect for the extraction from *A. visnaga* (L.) leaves, while the binary integration between water and methanol (AC) comes in the third place, also with a positive effect for leaves, stem, and roots.

Table 4 displays the obtained results of the antioxidant activity. The analysis of the obtained experimental amounts was in high concordance with the predicted amounts, suggesting that this statistical tool revealed its efficacy to choose the most appropriate solvent

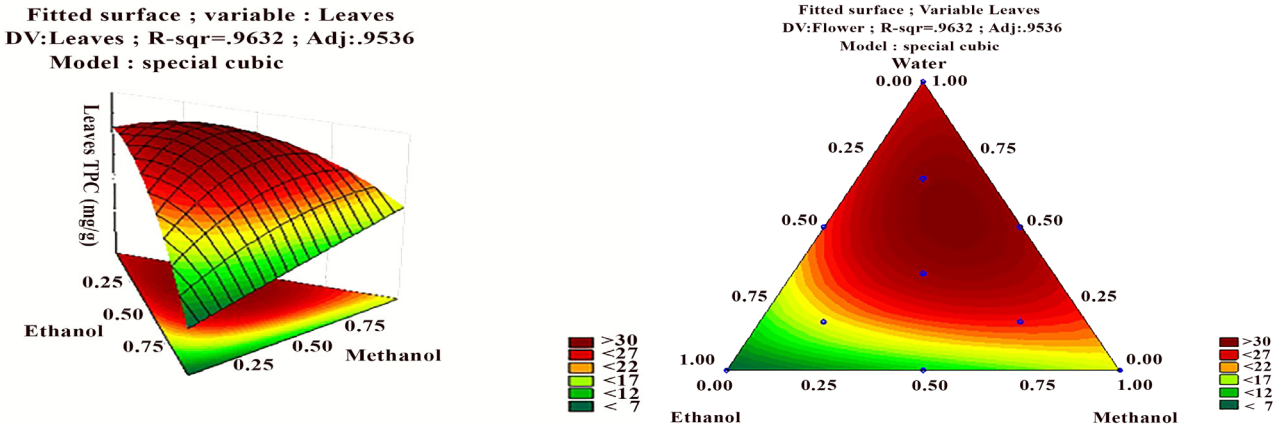


Fig. 3. Response surface contour (A: left) and response surface (B: right) plots of the special cubic model predicted TPC as a function of the ethanol, water and methanol proportions.

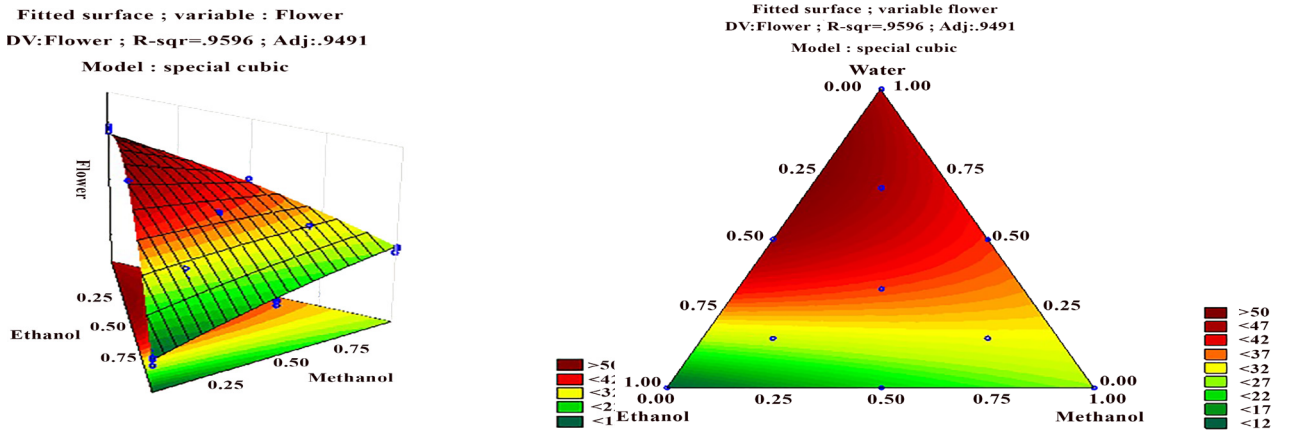


Fig. 4. Response surface contour (A: left) and response surface (B: right) plots of the special cubic model predicted TPC as a function of the ethanol, water and methanol proportions.

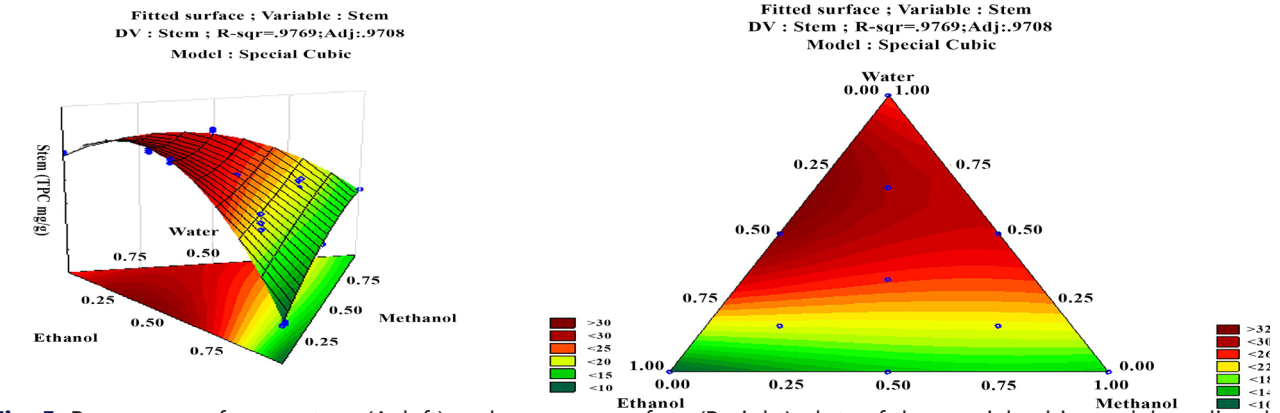


Fig. 5. Response surface contour (A: left) and response surface (B: right) plots of the special cubic model predicted TPC as a function of the ethanol, water and methanol proportions.

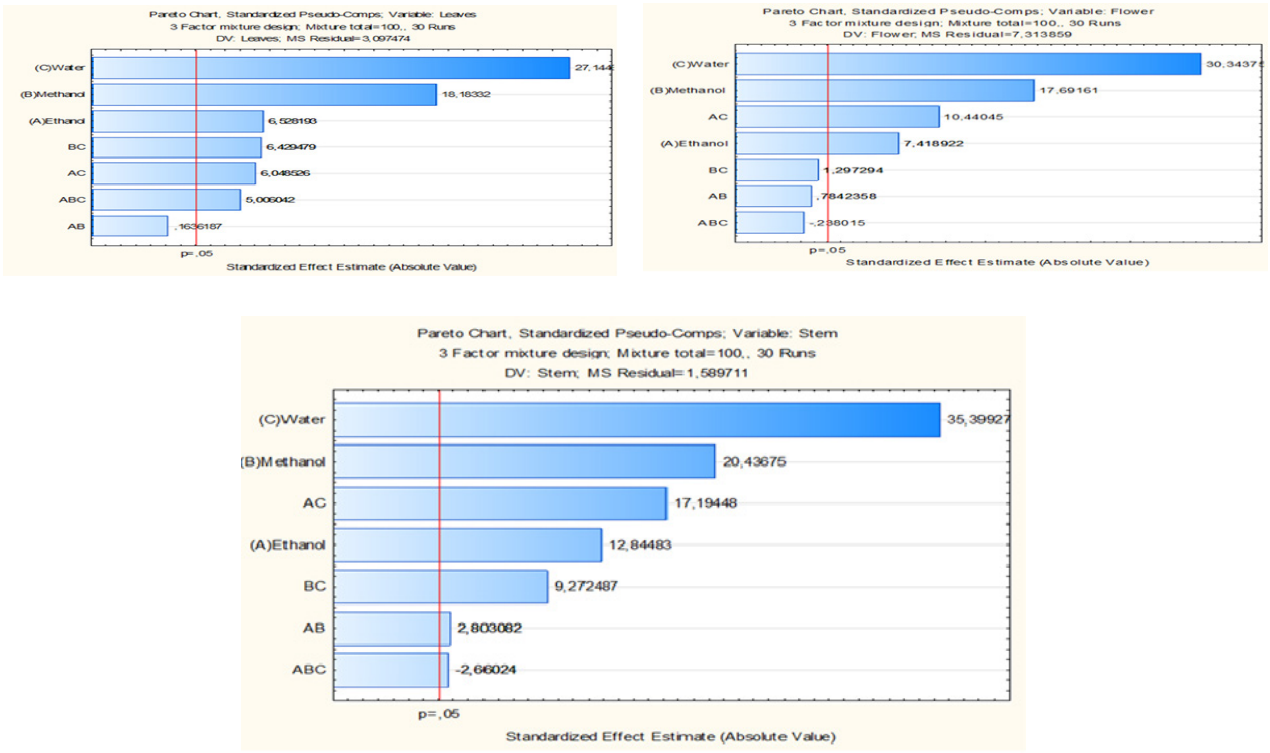


Fig. 6. Pareto charts of the standardized effects on TPC from leaves (A: Above left), flowers (Above right B), and stems (C: Below).

Table 4
The antioxidant activity of extracts of different AV parts.

	TPC mg GAE/g	TAC mg GAE/g	CUPRAC mg AAE/g	HCA mg CGAE/g
Flower	49.71±0.12	62.07±2.98	0.99±0.05	11.39±0.39
Stem	32.09±0.44a	48.63±3.69 a	0.93±0.09	4.06±0.61 a
Leaf	31.29±0.59 a	29.71±0.29 ab	1.09±0.02	4.85±0.73 a

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$).

combination. The flower extract contained the highest TPC (49.72 ± 0.12) compared to other studied extracts. The same extract exhibited the highest antioxidant ability examined by TAC and HCA with values of 62.07 ± 2.98 mg/g GAE and 11.39 ± 0.39 mg/g CGAE, respectively.

3.5. Phenolic profile of optimized extraction of different parts of *A. visnaga*

Table 5 shows the findings of the determination of the phenolic profile of different optimized extracts of *A. visnaga* (L.), including stem, leaves and flowers. The phenolic profile was determined using HPLC to provide a scientific basis and the variability of phytochemistry of different parts of Moroccan *A. visnaga* (L.) widely used in the traditional pharmacopeia. Quantification and determination of the phenolic profiles of different parts revealed 21 compounds with different levels in the plant parts under study.

Treatment of the metabolomics profile of different parts of *A. visnaga* (L.) revealed high variability. The most prevalent phenolic compound was chlorogenic acid, which was found in varied concentrations in the flower, stem, and leaf, being 222.24, 146.34, and 16.75 µg/g, respectively. In the second rank, isorhamnetin-3-*O*-rutinoside registered the following values 130.88, 46.66, and 18.14 µg/g for flower, stem, and leaf, respectively. The levels of caffeic acid in different parts under study were 76.83, 5.67, and 18.45 µg/g, respectively. The flowers of *A. visnaga* (L.), which constituted the majority of the plant under investigation, exhibited the highest concentration of individual phenolics identified through UHPLC-DAD analysis (Table 5). These findings will be of crucial importance for further experimental investigations to determine the biological properties of *A. visnaga* (L.) as well as to enrich the Moroccan databases about phytochemicals of the plant under study.

noteworthy that the floral extract was the extract with the highest concentration of phenolic components, which are closely associated to its antioxidant properties

Table 5
Phenolic compounds of optimized extraction of different parts of *Ammi visnaga* (L.).

Phenolic compounds	Flower	Stem	Leaf
	µg/mg		
Chlorogenic acid	222.24	146.34	16.75
Isorhamnetin 3-*O*-rutinoside	130.88	46.66	18.14
Caffeic acid	76.83	5.67	18.45
Isorhamnetin 3-*O*-glucoside	71.39	41.70	15.95
Kaempferol 3-*O*-glucoside	50.95	2.72	4.74
Rutin	46.90	4.31	15.55
p-Coumaric_acid	20.51	1.89	3.34
Quercetin 3-*O*-glucoside	19.63	4.21	31.48
Isorhamnetin	19.13	1.50	11.32
Neochlorogenic acid	14.42	4.51	9.53
Kaempferol	8.91	0.32	0.91
Quercetin 3-*O*-rhamnoside	3.81	NF	0.05
Dihydroquercetin	1.47	NF	NF
Quercetin	0.20	NF	1.33
p-Hydroxybenzoic acid	0.78	0.87	1.57
Luteolin	NF	0.21	NF
Phlorizin	NF	0.02	NF
Myricetin	NF	0.08	NF
Eriodictyol	NF	0.07	NF
Naringenin	0.08	0.02	NF
Hispidulin	0.03	0.04	NF
SUM (µg/mg)	688.16	261.14	149.12

NF: Not found

(Table 4). Numerous studies have been published to assess the phytochemical profile of *A. visnaga* (L.), and they revealed that many components of the plant were identified in varying concentrations, including γ-pyrones, coumarin, khellin, visnagin, 4-norvisnagin, khellinol, visamminol, ammiol, and khellol (Hashim et al., 2014; Khalil et al., 2020). The accumulation of phenolic compounds is not distributed equally through different parts of the plants (Padda and Picha, 2007; Jia et al., 2022). Roots appear to have the lowest amounts of phenolic compounds than other parts of the plant under study (Jung et al., 2011). The aerial parts, especially leaves of *A. visnaga* (L.), contain significant quantities of phenolics than other parts of the plants, which are used to make tea due to their high radical scavenging activity (Islam et al., 2002; Oki et al., 2002; Jung et al., 2011).

Zaher et al. (2022) detected 46 individual phenolic compounds, including edulisin III, binapacryl, khellin, and visnagin, which represent 89.89% of the chemical composition of *A. visnaga* (L.). Other studies documented that the most abundant phenolics were quercetin, rhamnocitrin, rhamnetin and rhmnazin (Harborne and King, 1976; Khalil et al., 2020). The findings of this work are in high concordance with results evoked by Activity et al. (2011). In fact, *A. visnaga* (L.) is a dense source of bioactive compounds that act their positive impacts synergistically by affecting several physiological functions.

4. Concluding remarks

In the present study, optimization of the bioactive compound extraction from different aerial parts of *A. visnaga* (L.) was performed using response surface methodology. This mathematical tool increased the recovery of extracts with considerable amounts of phenolics and remarkable antioxidant activity. The special cubic model was adequate for the experimental results of specific tests. The analysis of the model-derived response surfaces revealed that in binary mixtures (50% ethanol and 50% methanol), the yielded values of phenolic compounds and the antioxidant activity increase with the water proportion of different prepared mixtures. Therefore, flowers contained the highest amounts of bioactive compounds, *e.g.*, chlorogenic acid,

isorhamnetin-3-*O*-rutinoside, caffeic acid, isorhamnetin 3-*O*-glucoside, and other compounds in remarkable quantities. Otherwise, the obtained findings constitute a primary step to recuperate the highest amount of bioactive components with considerable antioxidant activity for further experimental investigations.

Author contribution statement

Conceptualization and literature search were performed by Zineb El Jabboury, Smail Aazza and Lahsen El Ghadraoui. The first draft of the manuscript was prepared by Zineb El Jabboury and Driss Ousaaid. Uros gasic, Peda Janackovic, Zora Stevanonovic, Stefan Kolasinac performed the phytochemical profile. Oumaima Chater, Meryem Benjelloun critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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