

Fractionation and antioxidant properties of *Ammi visnaga* umbels extracts

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Introduction

Ammi visnaga L. (Apiaceae) is a perennial herbaceous plant widely distributed in the Mediterranean region. In Morocco, the umbel is traditionally prescribed as an antidiabetic, antispasmodic, and diuretic agent (Khalil et al., 2020). The aim of the present work was to evaluate the affinity of different extracts of dried *A. visnaga* umbels toward total phenolic content and antioxidant capacity. Mixture Design Response Surface Methodology was performed to maximize phenolic compounds and antioxidants using the selected solvents from the first step. Finally, the plant material was extracted using the optimized solvent mixture, and fractionated using liquid-liquid extraction.

Materials and methods

Extraction procedure and sample preparation

The extractions were executed in triplicate conferring to the following formula: 50 mg of dried and pulverized inflorescence of *A. visnaga* were extracted for 20 minutes by sonication ultrasound-assisted extraction (UAE) 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°C.

Total phenolic content (TPC)

The total phenolic content was determined by a modification of the Folin-Ciocalteu method described by Singleton et al. (1999) as follows: 50 µL of extract was mixed with 450 µL of Folin-Ciocalteu reagent (0.2 N) for 5 minutes and then 450 µL of a Na₂CO₃ solution (75 g/L) was added. Then, all examples were reared at room temperature in the dark environment for two hours, and their absorbance was measured at 760 nm using a Jenway 6505 UV/visible, scanning spectrophotometer. The experiment was prepared in triplicates and the results are stated as mg Gallic acid equivalents (GAE)/g of dried plant.

Total antioxidant capacity/Phosphomolybdenum assay (TAC)

The total antioxidant capacity (TAC) of all samples was determined by the green phosphomolybdenum complex formation according to Prieto et al. (1999). An aliquot of 25 µL of the sample solution was united principally with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate) in Falcon 15 mL tubes. The Falcon tubes were covered and raised in a water bath at 95°C temperature and for a period of 90 minutes. Afterward, the samples were kept at room temperature and then the absorbance of the mixture was measured at 695 nm, against a blank. The experiment was conducted

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in triplicates and the results are presented as an average values expressed as g of ascorbic acid equivalents (AAE) per g of dried plant.

Fractionation

The extract was concentrated under reduced pressure at 40°C using vacuum rotary evaporator and after were suspended in water and partitioned with n-hexane, dichloromethane, chloroform and ethyl acetate. The process was repeated 3 times with each solvent. Three extracts of each solvent were pooled together and evaporated under reduced pressure using rotary evaporator and suspended in ethanol.

DPPH Free Radical-Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed as described by Aazza et al. (2011). Fifty microliters of various concentrations of samples or standards were added to 60 µM methanolic solution of DPPH (1 mL). After 60 min of incubation time (A₁), absorbance was measured at 517 nm. Absorption of a blank sample containing the same amount of methanol and DPPH solution served as the negative control (A₀). The percentage inhibition $[(A_0 - A_1/A_0) \times 100]$ was plotted against sample or standard content and IC₅₀ was determined (concentration of the extract or standard able to scavenger 50% of DPPH free radical).

Total antioxidant activity (ferric reducing antioxidant power, FRAP)

Reducing power was performed following the protocol described by (Aazza, 2011). Each sample or standard was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3,000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer.

Results and discussion

The proportions optimized according to the response surface were (Water 70%), methanol (5%) and Acetone (25%) as being the maximum inside the experimental domain, yielding maximum predicted amount of TPC (29.9197 mg Gallic acid eq./g dry plant) and TAC (153.43±1.30 mg Ascorbic acid eq./g dry plant). According to ANOVA, the special cubic model explained

the variance of the TPCs and the antioxidant activity of the extracts at a level of R² greater than 95%. In general, the amount of phenolic compounds and the antioxidant activity of the extracts increase with increasing amount of water in the methanol or acetone solvent mixture. Fractionation yielded five fractions: hexane, dichloromethane, chloroform, ethyl acetate fractions and the remaining aqueous fraction. We performed the following tests: TPC, TAC, FRAP and DPPH. The TPC showed significant differences (p<0.05) for Hexane fraction 1.36±0,090 mg GAE/g dry plant, chloroform extract 3.105±0.24 mg GAE/g dry plant, ethyl acetate extract 11.930±0.26 mg GAE/g dry plant, dichloromethane extract 14.536± 0.269 mg GAE/g dry plant. Whereas, the greatest quantity of TCP stood in the water fraction 34.25±0.398 mg GAE/g dry plant. For the DPPH, the result revealed a high antioxidant activity with IC₅₀ 8.51 µg/ml when compared to Trolox standard which has an IC₅₀ of 1.5 µg/ml. All extracts presented ferric reducing power in a dose dependent behaviour, although it was much more evident in remaining water extract with 1.344 equivalent Fe (II) /g dry plant.

Conclusion

The use of this design can be helpful to study the synergetic effect between solvent during extraction. The obtained results revealed also that the aqueous fraction exhibited the highest antioxidant activity, due to its higher phenolic compounds content. We also noticed that the increase of the solvent polarity, increase its ability to extract phenolic compounds, which can explain the increase of antioxidant activity with the increase of solvent polarity.

References

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