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Supercritical fluid extraction of γ-Pyrones from *Ammi visnaga* L. fruits

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ABSTRACT

Extraction with supercritical fluid technique has proved to be effective in many applications including extraction and separation of various active principals from medicinal plants. It was used due to its advantages especially safety, specificity, selectivity and ease of component recovery.

Ammi visnaga, L. belongs to the family Apiaceae. The fruits are used specifically for the treatment of kidney stones depending on its γ-Pyrones (mainly khellin and visnagin) [2]. The supercritical fluid extraction technique of khellin and visnagin was investigated and the operating conditions for their extraction were optimized. The effect of different pressure (150, 200, 300, 400 and 500 bars), temperature (35, 40, 45, 50 and 55 °C), and particle sizes of the raw material (0.5, 1, 1.4 mm and entire fruits) on the extract yield was studied under dynamic conditions for extraction for a run time of 90 min. Optimum supercritical extraction condition was found to be 200 bars at 45 °C and optimum particle size was found to be 1.4 mm. The yield is yellowish white bitter powder and measures 1.74% w/w relative to the dried weight of the fruits containing 38.414% w/w average γ-Pyrones content of which 29.4%w/w khellin, and 9.014%w/w visnagin.

The obtained extracts were analyzed by reversed phase HPLC.

1. Introduction

Ammi visnaga L. belongs to the family of Apiaceae. *Ammi visnaga* is known under a diversity of names including Bishop's weed, Khella and Toothpick weed or herb [2].

Ammi visnaga fruit preparations, such as teas prepared from crushed or powdered seeds, have traditionally been used in the Middle East to ease urinary tract pain associated with kidney stones and to promote stone passage [3]. Visnagin and khellin are among the main compounds of *Ammi visnaga* fruits, belonging to the category of furanochromones (2–4%). Many properties have been attributed to *Ammi visnaga* and its constituents such as antibacterial, antifungal, antiviral, antidiabetic, anti-inflammatory and neuroprotective [4–8]. The most prominent effects of *Ammi visnaga* extract and its compounds are being peripheral and coronary vasodilator and antispasmodic, where it relaxes the smooth muscles; also it acts as bronchodilator and for urolithiasis [9–14]. Visnadin, khellin and visnagin are thought to be responsible for the calcium channel blocking activity [10–14]. Vasodilatory and antispasmodic effects on the urinary tract could possibly explain the usage for urolithiais, acting as expulsive therapy, thereby facilitating and reducing time for passage of smaller stones. Khan et al. reported a reduction of oxalate and calcium content in rat kidneys after administration of *Ammi visnaga* tea preparation [15].

Solvent extraction methods were applied to extract the active ingredients (γ-Pyrones mainly khellin and visnagin) from *Ammi visnaga* fruits. The solvent used are methanol, ethanol and hydroethanol. Actually, the solvent methods have many disadvantages such as toxicity, health hazards, unfriendly to environment, high cost and unavailability. Therefore, we shifted to an advanced method to extract the (γ-Pyrones from *Ammi visnaga* fruits; that is supercritical fluid carbon dioxide extraction technology (SCFE).

SCFE technology has been replacing the traditional solvent extraction methods as it overcomes the drawbacks of using the solvents; in addition it has many other advantages such as it gives an extract free from residual solvents and produces uniform stable extract. Supercritical fluid extraction technique is more selective, efficient, cheap, and afford mean for simple recovery of the compounds without degradation [1,16–22].

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The objective of the present work was to investigate the optimum condition for supercritical fluid extraction of γ-Pyrones from the fruits of *Ammi visnaga* L. The obtained γ-Pyrones extract was analyzed by reversed phase HPLC.

2. Materials and methods

2.1. Materials

Ammi visnaga fruits samples were supplied by Mepaco-Medifood Pharmaceutical Company, Egypt. Khellin and Visnagin reference standards were purchased from Sigma Aldrich, Germany. HPLC grade Methanol and Water were acquired from Merck, Germany.

2.2. Equipments

Lab-scale supercritical fluid extraction unit, ISCO SFX™ 220, ISCO Inc., USA. Ultra-Fast Liquid Chromatography (UFLC), Shimadzu Model Prominence LC-20 ADXR, equipped with auto sampler, SIL-20ACXR and PDA detector (Shimadzu Model: SPD-M20A), Kyoto, Japan.

2.3. Experimental

2.3.1. Supercritical fluid extraction method

Optimization trials were conducted on lab-scale SCFE equipment through changing or fixing parameters to get the most optimum extraction procedure; these parameters were:

- Pressure: the critical pressure (P_c) was changed from 150, 200, 300, 400 and finally 500 bars; together with fixing temperature and particle size all over the trials.
- Temperature: the critical pressure (P_c) and particle size were fixed; with changing critical temperature from 35, 40, 45, 50 and 55 °C.
- Particle size of the sample: optimum critical pressure (P_c) and temperature (T_c) were fixed; then different particle sizes were used i.e. entire fruit and crushed fruit (1.4, 1 and 0.5 mm).

2.3.1.1. Selection of optimum pressure. Fixed weight samples of crushed *Ammi visnaga* fruits on sieve 1.4 mm (fixed particle size) were used for each trial; to be filled in the 7 ml extraction column. The effect of five different extraction pressures (150, 200, 300, 400, and 500 bars) was investigated using fixed temperature (T_c, 45 °C) together with constant flow rate of the fluidized carbon dioxide over a fixed time ranges of 1.5 h under dynamic mode of extraction. Each experiment was repeated three times and the average of the outcome was recorded.

2.3.1.2. Selection of optimum temperature. Fixed weight samples of crushed *Ammi visnaga* fruits on sieve 1.4 mm (fixed particle size) were used for each trial; to be filled in the 7 ml extraction column. The effect of five different extraction temperatures (Tc, 35, 40, 45, 50 and 55 °C) was investigated using fixed pressure (P_c, 200 bars) together with constant flow rate of the fluidized carbon dioxide over a fixed time ranges of 1.5 h under dynamic mode of extraction. Each condition was repeated three times and the average of the outcome was recorded.

2.3.1.3. Selection of optimum sample particle size. In a similar way, in each experiment, a fixed weight of crushed *Ammi visnaga* fruits is to be filled in the 7 ml extraction column. The effect of different particle sizes of the material used (entire fruits, 1.4 mm, 1 mm and 0.5 mm) was investigated using fixed optimum extraction temperature (T_c, 45 °C), fixed optimum pressure (P_c, 200 bars) and were fixed and constant flow rate of the fluidized carbon dioxide over a fixed time ranges of 1.5 h under dynamic mode of extraction. Each experiment was repeated three times and the average of the outcome was recorded.

2.3.2. Optimization of the supercritical fluid extraction condition

In order to find out the effect of different extraction pressures and effect of different extraction temperatures on the yield of γ-Pyrones with various particle sizes mentioned above; extraction pressures around the optimum (P_c) obtained with particle size 1.4 mm, viz: (150, 200 and 300 bars) and the effect of extraction temperatures around the optimum (T_c) obtained with particle size 1.4 mm, viz: (40, 45 and 45 °C) were investigated alongside with the different sizes (entire fruits, 1.0 mm and 0.5 mm).

2.3.2.1. Effect of different extraction pressures on the yield of γ-Pyrones from Ammi visnaga fruits at three different particle sizes. In all experiments, a fixed weight sample of *Ammi visnaga* fruits of three different particle sizes (entire fruits, 1.0 mm and 0.5 mm) were separately used and to be filled in the 7 ml extraction column. Three different extraction pressures (150, 200 and 300 bars), fixed temperature (T_c,45 °C), constant flow rate of the fluidized carbon dioxide over a fixed time ranges of 1.5 h under dynamic mode of extraction were chosen. Each condition was repeated three times and the average of the outcome was recorded.

2.3.2.2. Effect of different extraction temperatures on the yield of γ-Pyrones from Ammi visnaga fruits at three different particle sizes. In all experiments, a fixed weight sample of *Ammi visnaga* fruits of three different particle sizes (entire fruits, 1.0 mm and 0.5 mm) were separately used and to be filled in the 7 ml extraction column. Three different extraction temperatures (40, 45 and 50 °C), fixed pressure (P_c, 200 bars), constant flow rate of the fluidized carbon dioxide over a fixed time ranges of 1.5 h under dynamic mode of extraction were chosen. Each condition was repeated three times and the average of the outcome was recorded.

2.3.3. HPLC analysis of the obtained extracts

2.3.3.1. Chromatographic conditions. The γ-Pyrones content of *Ammi visnaga* extracts obtained by supercritical carbon dioxide fluid extraction were analyzed by liquid chromatography (UFLC) (Shimadzu Model Prominence LC-20 ADXR), equipped with auto sampler, SIL-20ACXR, PDA detector (Shimadzu Model: SPD-M20A); equipped with a Hypersil C18 column, (Thermo BDS, 5 μm, (250 × 4.6 mm); SN: 10185576, USA). Column temperature was 40 °C. The mobile phase consists of methanol: water (50: 50)% at flow rate 1.5 ml/min. Detection wavelength was 245 nm and the injection volume was 20 μl.

2.3.3.2. Standard solution preparation. Twenty milligrams of standard khellin were weighed accurately and transferred into 100 ml volumetric flask. The standard khellin was dissolved in methanol till volume; then sonicate for 20 min to obtain a concentration of 20 mg/dl. The standard solution was filtered over 0.45 μm syringe filter prior to injection.

2.3.3.3. Sample preparation. Fifteen milligrams of the obtained *Ammi visnaga* extracts were weighed accurately and transferred into 20 ml volumetric flask. The extract was dissolved in methanol till volume; then sonicate for 20 min to obtain a concentration of 75 mg/dl. The obtained solution was filtered over 0.45 μm syringe filter prior to injection.

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