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Extraction and purification of γ-Pyrones (khellin and visnagin) from *Ammi visnaga* Lam. plant

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Introduction

In Iran, medicinal plants have a wide distribution and diversity in terms of geographical location, climate variability, which the necessity of researching them as a valuable source that contains a wide range of effective medicinal substances is of particular importance.

Ammi visnaga Lam. from umbelliferae family is one of the valuable indigenous medicinal plants in the country. It is an annual herbaceous plant with a stem height of 0.8-1 meter. It has leaves with many cuts and cotton shape and white and complex flowers. The effective medicinal substance γ-pyrones/furanochromones is one of the main compounds of this plant, which mainly contains khellin and visnagin.The most important medicinal effects of Ammi visnaga extract are being the vasodilator of the peripheral and cardiac vessels, which are used in the treatment of cardiovascular diseases, and with the relaxing effect on the smooth muscles, it causes the vasodilator of the respiratory tracts, and therefore, they are effective in the treatment of asthma and angina pectoris. Recently, its therapeutic effect on diseases such as vitiligo and psoriasis has also been confirmed. In addition, due to the low toxicity and lack of accumulation of γ-pyrones in the body, this effective medicinal substance can be included in the ranks of valuable drugs for the treatment of the above-mentioned diseases.

Our main goal in this research is to extract and purify the active ingredient γ-pyrones (mainly containing khellin and visnagin) from Ammi visnaga Lam. It is native to Iran, which has been done using recrystallization chromatography methods and analyzed with the methods listed in WHO 2011&1998 (world health organization) reference and spectrometry.

In this research, khellin and visnagin were separated and purified by column chromatography, recrystallization and thin layer chromatography methods, and were identified by using their melting point and FTIR (Fourier transform

infrared) spectrometry and comparing with standard samples. The efficiency and degree of purity of both khellin and visnagin have been calculated using HPLC (high performance liquid chromatography) and compared with their standard samples.

Methods and Materials

Ammi visnaga Lam. was collected from the northern regions of Iran, Gilan, Lushan and Rostam Abad in late September.

In this research, two parts of the stem and seed of the plant have been used to extract the active ingredient using a modified method (Moubasher & Barakat 1950). For separation and purification has been used from a chromatography column with dimensions of 30×1.4 cm, filled with aluminum oxide and recrystallization method. Then the collected fractions were detected using thin layer chromatography on TLC plates with Silica gel 60 F254 staining specifications (El-Shibini et al., 1969) and pure samples of khellin and visnagin were identified by using a UV-365nm detector and measuring R_f (Wagner et al. al., 1996 & 1984) and also determining their melting point.

Statistical analysis was performed using one-way ANOVA method on different organs of the plant and the significance of the results of these tests was evaluated with p-value<0.05. Also, all experiments were performed with three repetitions and their results were reported as Mean±SD using Excel 2003.

The measurement of humidity by gravimetric method, measurement of total ash, ash insoluble in acid, ash soluble in water, and measurement of heavy metals (lead and cadmium) were performed using the methods listed in WHO.

FTIR spectrometry method has been used to determine the functional groups in khellin and visnagin samples in comparison with their standard samples. Also, HPLC method has been used to determine the amount and degree of purity of khellin and visnagin in the extract.

Results & Discussion

The results obtained from the tests conducted on the extract showed that the moisture content with an average of 3.6% was lower than its permissible limit (maximum 5%) and total ash, ash insoluble in acid and ash soluble in water of the extract were also with an average of 215 mg/g, 25 mg/g and 35 mg/g were obtained. Also, the amount of lead and cadmium in the extract was lower than the permissible limit with an average of 2.42 ppm and 0.211 ppm respectively (maximum 10 ppm for lead and maximum 0.3 ppm for cadmium, respectively).

The results obtained from the results of FTIR spectrometry and the determination of the melting point of khellin and visnagin have a Considerable compliance with their standard samples. It also showed an almost two-fold increase in their amounts in plant seeds compared to plant stems, which were 1.114, 0.326 and 0.54, 0.172 mg per gram of sample, respectively. The agreement of the results of this research with the research done by Franchi (Franchi et al., 1985) indicates that during flowering and seed production, γ-Pyrones (mainly khellin and visnagin) are often found in the places of flower production in the fruit or seed of the plant. and therefore the increase in its amount can be due to the growth stage of the plant, which is considered one of the important and influential factors in determining the amount of γ-Pyrones in the plant (Sellami et al., 2013).

Also, the HPLC chromatogram obtained from extract was completely consistent with the chromatograms of the standard samples of khellin and visnagin showed sharp, symmetrical peaks with high resolution at retention time 8.617 and 10.750 minutes respectively, similar with the standard samples. This similarity confirms the purity of khellin and visnagin extracts compared to their standard samples. The mean contents of khellin and visnagin in the extract were

1.114 mg/g and 0.326 mg/g and purity of 90.16% and 79.62% respectively. The results obtained from HPLC analysis in this research with studies by Bishr (Bishr et al., 2016), Badr (et al., 2015), Kamal (Kamal et al., 2015), Alqasoumi (Alqasoumi et al., 2014), Shinde (Shinde & Laddha, 2014) has shown a good agreement.

Conclusion

The results obtained in this research have shown the appropriateness of selective extraction and purification methods. Therefore, as a valuable medicinal substance, it can be used in the country's pharmaceutical industry and it is possible to replace it with chemical drugs with similar therapeutic effects.

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