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PHYTOCHEMICAL SCREENING BY USING TLC AND GC-MS METHODS FOR QUALITATIVE DETERMINATION OF COMPOUNDS IN *AMMI VISNAGA* L. EXTRACT

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Abstract

This study aimed to extracted and purified of khellin extract from *Ammi visnaga* L. grown in Iraq. Whole plant were dried, mashed, and then underwent extraction. The plant samples were subjected to n-hexane for nine hours, and then it were extract with methanol by soxhlet. The present study was included three parts, the first part was extraction method for isolation of khellin and second includes the development of a rapid analytical method for detection of khellin using GC-MS. The last part is involving separation of khellin by using thin layer chromatography TLC with different solvent systems. The overall, 23 compounds were identified in methanolic extract of *A. visnaga* were analyzed by GC-MS; and the four major compounds found to be khellin (28.391%), viznagin (25.606%), edulisin III (5.683%) and (z)-cnidimine (5.241%). Finally, we separated the compounds by TLC technique by using three different solvent systems and compared it with standard of khellin.

Key words: *Ammi visnaga* L. Phytochemical screening, GC-MS, khellin, TLC-separation

Introduction

Ammi visnaga L. it's a member of the family Apiaceae (Umbelliferae). It is an annual or biennial plant rising from a taproot erect, the maximum height of about 1.5 m. The root is like the root of the carrot with tangled leaflets (Jaradat *et al*., 2015). The inflorescence is a compound umbel of white flowers and highly swollen at the base, later on, it becomes woody and used as toothpicks. The fruit is a compressed oval-shaped structure consisting of two mericarps and around 3 mm in length, somewhat resembling a caraway (Al-Snafi, 2013). These fruits are used for medicinal use Fig. 1. It grows in the Middle East, Europe and North Africa. Called traditionally by many names like, bisnaga, toothpick-plant, toothpick weed (since it used as tooth pick) and khella (Al-Mayah *et al*., 2016). In Iraq *A. visnaga* grow in Sulimania, Erbil, Mosul, Kirkuk, Baghdad and Basrah (Chakravarty, 1976). *A. visnag* used traditionally for many conditions like kidney stone, respiratory condition, mild angina, as diuretics, topically for psoriasis and vitiligo (Hashim *et al.,* 2014).

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There are many pharmacological activity for khella like cardio protective, antimicrobial, antispasmodic, melanoprotective, anti-urolithiatic hypoglycaemic, and neuroprotective activities (Alam *et al*., 2018). The vasodilation, antispasmodic and muscle relaxant effect are well established and documented in pharmacopeia (WHO, 2007). Phytochemistry studies on the *A. visnaga* shows the presence of different groups of chemical constituents. The quantities and presence of these significant metabolites depend on which parts of the plant analyzed. Furthermore, in which conditions the plants grow. The uses of different bio-regulators also effects of metabolites in *A. visnaga* (Hashim et al., 2014). There are many chemical compounds in *A. visnaga*, the major is a group of γ-pyrones which are furanochromone derivatives the main are khellin and visnagin Fig. 2. These two compounds have much reported pharmacological activity like vasodilation (Alam et al., 2018). Khellin have renoprotective, antibacterial and cytotoxic activity and others (WHO, 2007).

Regarding the cytotoxic activity, it has been found that khellin showed weak activity against HT-29

Fig. 1: A. *visnaga* field in Sulaymaniyah Governorate shows the inflorescence of plants.

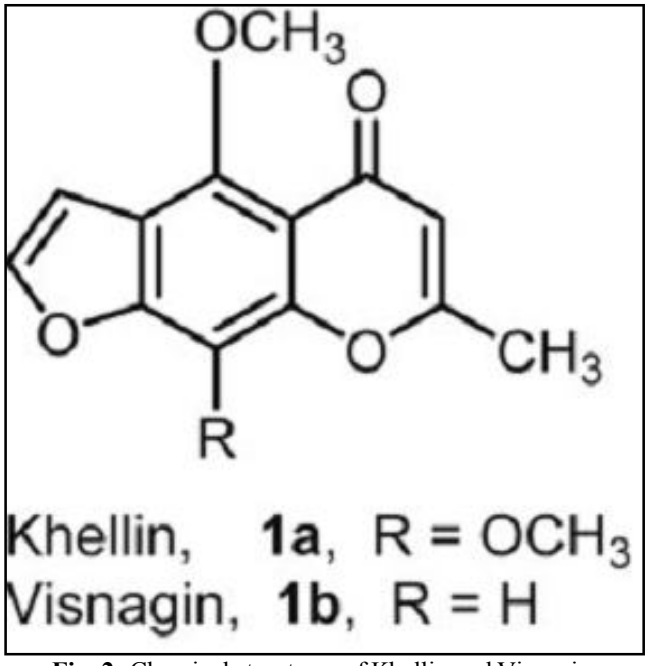

Fig. 2: Chemical structures of Khellin and Visnagin.

(colorectal cancer), MCF-7 (breast cancer), HEp-2 (larynx cancer) and MKN-45 (gastric cancer). And mild to moderate activity against hepatocarcinoma cell line (HepG2) (Beltagy & Beltagy, 2015).

The aim of our study is too extracted of khellin from *A. visnaga*. grown in Iraq, and purification of it by TLC. Then determine qualitatively the constituents in *A. visnag*a extract and distinguish compounds in methanol extracts by using GC-MS.

Materials and Methods

Samples collection

An intensive survey was carried out during July 2019 in the north of Iraq in Sulaymaniyah Governorate for the collection. Then plants were identified and collected. It was washed to get rid contaminants than the plant was air-dried in the shade for several days at room temperature.

Chemical study

The Chemical study was carried out in the department of pharmacognosy and medicinal plants in college of pharmacy university of Basra.

Preparing materials

The whole plant was washed and then dried at room temperature until the whole plant became well dried. After the drying, the plant's materials were grinded (inflorescence of plants) into a fine powder by using the blender and transferred into special containers with proper name labeling for future use.

Khellin extraction

For the khellin extraction was performed by the soxhlet extraction method. This extraction was done by taking 50gm of dried plant powder and packed into a thimble and extracted with 300ml of n-hexane. The extraction process continues for nine hours by soxhlet until the color of the solvent in the siphon tube of an extractor becomes colorless. The defatted crude plants dried in the hood and undergo extraction with 300ml methanol by soxhlet until the color in the siphon arm became clear. This process takes 12 hours of operation. Collected extract stored in a dark container in the refrigerator for further use.

Phytochemical screening by chemical tests

Horstmann's detection: The plant residue treated with few drop of alcoholic m-dinitrobenzene, and 15% potassium hydroxide absence of violet color (Karawya, 1970).

Shinnoda's detection: Pieces of magnesium bar and concentrated hydrochloric acid were mixed with alcoholic plant extract after some minutes, pink to orange color appeared (Gul *et al.,* 2017).

6- (GC-MS) analysis

Detection and isolation of different ingredient in the methanolic extract done by using a mass spectrometer Agilent gas chromatograph equipped and coupled to a mass detector Agilent 5977A spectrometer with a HP-5MS (5% Phenyl methyl siloxen), 30m ×250 Um × 0.25 mm ID of capillary column. The temperature of injector was 40^0C maintained for 5 min then raised gradually to 310^0C at rate of increment 100\min helium gas 99.99% used as mobile phase at flow rate of 1ml\min. an injection volume of 3ml was employed (split ratio of 75 : 1). Mass spectra were taken at 70 ev; a scan interval of 4min and

fragments from 45 to 450 Da. The solvent delay was 0 to 5min and the total GC-MS running time was 30min. The samples were injected in split mode as (79 : 1) Mass spectral scan range was set at 30 to 60 0(m/z).

TLC qualitative analysis:

Silica gel plates' (10 × 10 cm. and 0.25 mm. thick) dried and then spotted with methanolic extract of *A. visnaga* plants with 13 spots and 0, 1% w\v standard solution of khellin in methanol as single spot.

Using three different solvent system

1- Toluene: Ethyl acetate: formic acid (6:2:2)

2-Ethyl acetate: hexane: methanol: (6:2:2)

3- Ethyl acetate: chloroform: methanol (2.4:2.1:0.5)

20 ml of mobile system poured in TLC gar with filter paper. The system closed for 5 min then spotted TLC plate inserted carefully in the gar allowing the mobile phase to dissolve the spots and start the separation process.

Table 1: High percentage of chemical compounds in methanolic extract of *A. visnaga* plant.

N.	Compound Name	R.T.	Pick high	Corr. max %	% of total
1	Visnagin	23.224	2764	90.19%	25.606%
2	Khellin	25.023	3019	100.00%	28.391%
3	Edulisin III	28.539	3526	20.02%	5.683%
4	(Z)-Cnidimine	29.047	3620	18.46%	5.241%

Separation khellin:

The straight line of spots aligned with the khellin spot catted and then scratched the silica powder. The powder

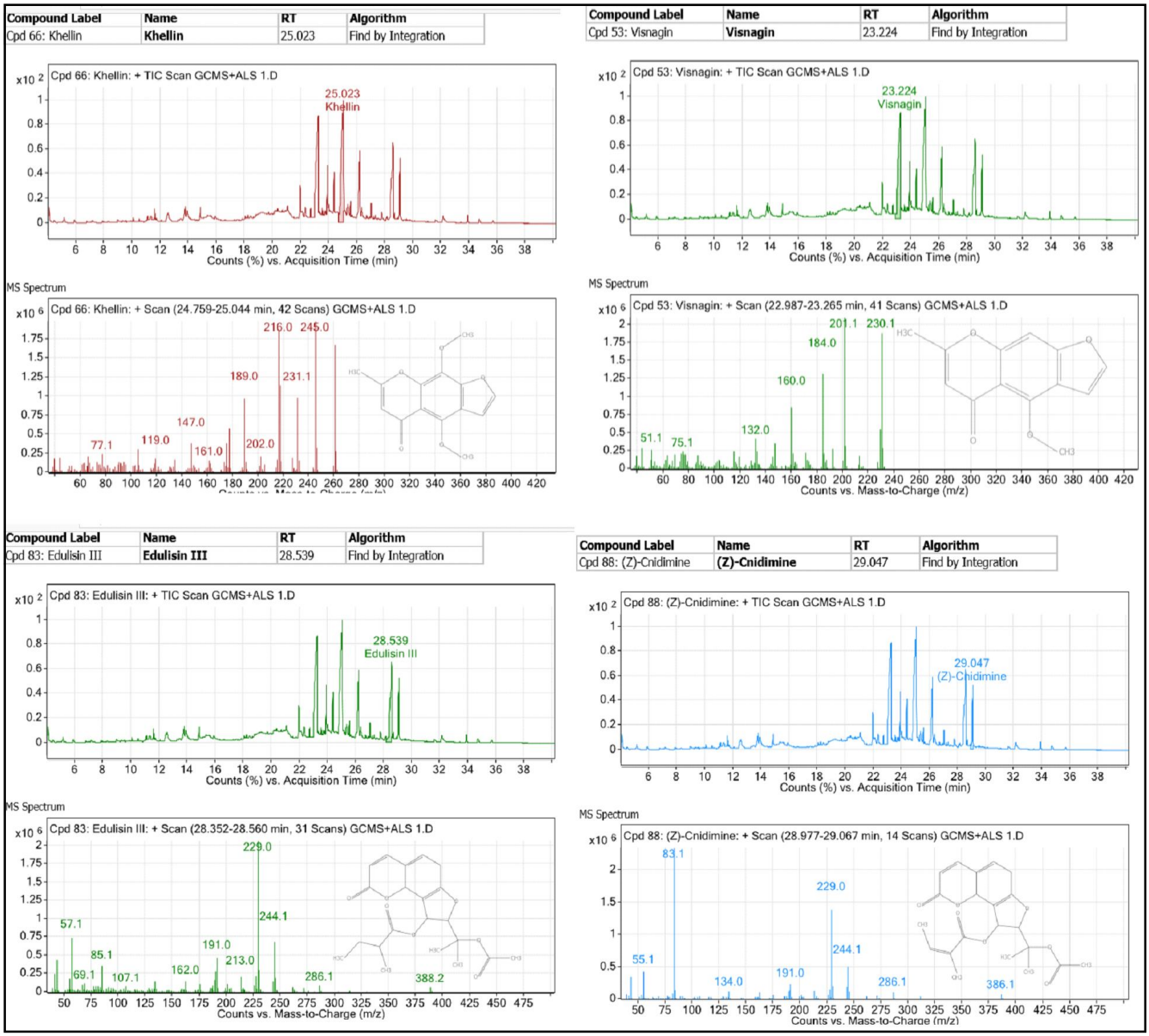

Fig. 3: GC-MS for *A. visnaga* methanolic extract.

then immersed in methanol to dissolve khellin. The solution undergoes centrifugation at 60 r\s for 5 min to separate the unresolved silica gel clear solution decanted and allow drying. Yellowish white crystal obtained.

We obtained crystal dissolved in methanol and then analyzed with standard khellin solution by TLC using two different solvent systems to ensure complete separation.

Results and Discussion

Khellin extraction: depending on solubility of khellin which is, 25 mg/100 ml of water, 2.6 g/100 ml of methanol,1.25 g/100 ml of isopropanol, 0.5g/100 ml of ether , Which increased by heating (Florey, 1981). The percentage yield varies depending on the type of solvent used, the best yield obtained from methanolic extract.

Phytochemical screening by chemical tests:

Horstmann's detection: The color produced by reacting khellin or visnagin with m-dinitrobenzene confirm complete extraction of khellin and visnagin.

Shinnoda's detection: in this detection the pink to orange color showed the presence of flavonoid in methanol extraction (Gul *et al.,* 2017).

(GC-MS) analysis: The identification of the components of *A. visnaga* extract was detected by GC-MS analysis. The major four compounds in this extract were identified in the methanolic extract of *A. visnaga* representing in table 1.

The results of this study discovered that the highest percentage compounds in methanol extracts of *A. visnaga* include khellin, visnagin, edulisin III & (z)-cnidimine Fig. 3, while the least percentages included different compounds. Compared to other studies for *A. visnaga* in different countries it contained Y- pyrones (furanochromone up to 4%) the main compounds khellin 10.3-1.29, Visnagin (0.05 0.20 %), khellinol, ammiol khellol and Khellimin, the yield of compounds differ between countries may be due to weather and environment and other conditions like water, soil, light, etc (Al-Snafi, 2013).

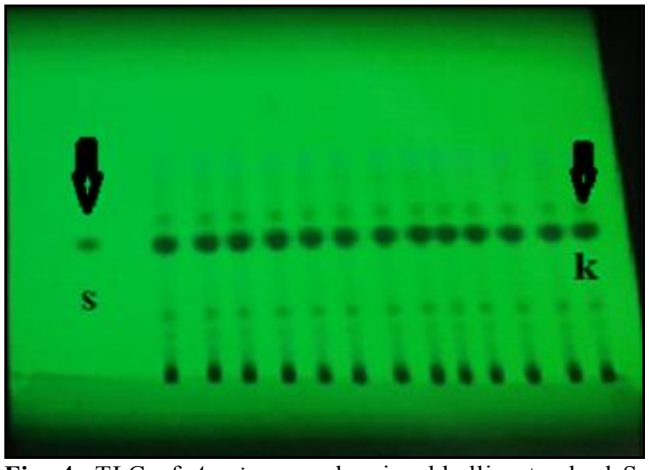

Fig. 4: TLC of *A. visnaga* showing khellin standard S, comparing with plant extracts.

TLC qualitative analysis: The separation of khellin from *A. visnaga* extracts by paper chromatography using filter paper by using a different solvent. The best system for separation was Toluene: ethyl acetate: formic acid (6 : 2 : 2), which gave successful separation of khellin from the other constituents of *A. visnaga* and comparing it with a standard of khellin has been achieved on silica gel plates Fig. 4.

Conclusions

In conclusion*, Ammi visnaga* L. that grown in Iraq has a high content of khellin and visnagin compounds and in this study we isolation of khellin using a novel, relatively simple method. Therefore, it is possible to isolate khellin within short period of time. The method has shown good reproducibility of the yield and purity and this method can be used to get a good quantity of khellin, which can be employed for different applications. GC-MS validation result revealed that this method enables rapid, precise, sensitive and for quantification of khellin in the plant source.

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