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Comparative extraction methods, fluorescence, qualitative and quantitative evaluation of *Ammi majus* seed extracts

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Abstract
Ammi majus belong to the family Apiaceae that provide a huge number of compounds with important medicinal activities. The present investigation is aimed to compare the percentage value of *Ammi majus* extracts by using different solvent and extraction methods, to do fluorescence analysis and phytochemical screening (qualitative and quantitative) for the important natural product groups in *Ammi majus* seed. The highest percentage extract was yielded from seed by using ordinary reflex methods and n-hexane 12.74%. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenolic acids, terpenoids, carbohydrate, coumarin and quinone in all fractions and presence of cardioactive glycoside, saponin, amino acid, carbohydrate, quinones in the seed has not been reported before. Presence of flavonoids estimated to be 288.611µg quercetin/gm in seed was determined in high quantity in ethanol 80% extract and tannins content 216.66 µg gallic acid/gm in low quantity. The fluorescent characteristics of seed powder with various chemical reagents were noted under visible and UV light.

Keywords: *Ammi majus*, fluorescence analysis, qualitative, quantitative evaluation

1. Introduction
The natural source of producing huge numbers of phytoconstituents in a most efficient way and with precise selectivity are plants and different bioactive phytoconstituents have been isolated and characterized in the middle of the 19th century, many of these are used as the active ingredients of the modern medicine or as the lead compounds for new drug discovery. Several plant derived medicine are rich in phenolic and other compounds such as those used in protection against the coronary heart diseases and carcinogenesis [1]. *Ammi majus* has different names, the Arabic name khillah, khillah shytani, English name bishops weed, Latin and German name ammi, French name ammi commun, belongs to family Apiaceae. Is an upright annual herb which grows up to 1 meter or more, whitish tap-roots; stem erect, slender, leaves alternate with long petiole, flower whitish actinomorphic or zygomorphic, fruit a cremocarp with slender prominent ribs, *A. majus* is one of the best medicinal plants that was first found in Mediterranean and Egypt. In Europe due to climate conditions the growth of *Ammi majus* is poor, attempts to acclimatize *Ammi majus* in cool climate were not successful, the fruits failed to ripen and plants were highly susceptible to infection [2, 3]. The fruits are valued in indigenous medicine and have been employed for a long time in folklore therapy for the treatment of leucoderma, vitiligo, diuretic, emmenagogue, and abortifacient and blood purifier [4], medicinally used to treat T-cell lymphoma [5], antibacterial activity [6], their effect due to their natural product groups. The main objective of the present study to evaluate the influence of using different extraction methods and solvents on the yield of extracted substances, phytochemical screening for the important natural product groups and determine quantity of most important natural product content in *Ammi majus* seed.

2. Materials and Methods
2.1. Plant material
Ammi majus seeds were collected from orchards during July. The seeds were cleaned, dried under shade for 15 days, coarsely powdered and stored in bottles until used. The identity of the plant was confirmed by the department of Pharmacognosy, College of Pharmacy, Hawler Medical University (Voucher No.2).

2.2. Preparation of plant extracts
2.2.1. Ultrasonic bath
Ten gm of dried finely powdered seed was taken in a beaker extracted first with n-hexane using ultrasonic assisted extractor for 1hr at 40 ^{o}C [7], then the residues were further extracted

with increasing order of polarity like ethyl acetate, ethanol 80% and water. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at -4 °C and yielded quantities of seed extracts in different solvents were obtained recorded.

2.2.2. Ordinary reflex

Ten gm of dried finely powdered seed extracted first with n-hexane using ordinary reflex [8] extractor for 1hr, then the residues were further extracted with increasing order of polarity like ethyl acetate, ethanol 80% and water. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at -4 °C and yielded quantities of seed extracts in different solvents were obtained recorded.

2.3. Phytochemical analysis

2.3.1. Qualitative phytochemical tests

For the identification of alkaloids, cardioactive glycosides, flavonoids, tannins, phenolic acid, saponins, terpenoids, amino acid, carbohydrates, coumarins, quinones and anthraquinones were carried out for n-hexane, ethyl acetate, ethanol and water extracts of seed the by standard methods [9, 10, 11].

2.3.2. Quantitative phytochemical tests

2.3.2.1. Determination of total phenolic content

The concentration of phenolics in ethanol 80% seed extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content with slight modification. The reaction mixture consists of 0.5 ml of ethanol 80% extract and 4.5 ml of distilled water was taken in a volumetric flask (15 ml). 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken well for 5 minutes. After that 5 ml of 7% Sodium carbonate solution was added to the mixture. The volume was made up to 12.5 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 30 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet /Visible spectrophotometer each one was measured in triplicate. Total phenol content was expressed as µg of GAE/gm of extract [12, 13].

2.3.2.2. Determination of total flavonoid Content

Total flavonoid content was measured by the aluminium chloride colorimetric assay with slight modification. A volume of 5 mL of the ethanol 80% extract and 5 mL of aluminium chloride 2% were placed in vials and allowed to stand for 1hr at room temperature. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 415 nm with an UV/Visible spectrophotometer each one was measured in triplicate. The total flavonoid content was expressed as µg of QE/g of extract [14].

2.3.2.3. Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method with slight modification. About 0.1 ml of the ethanol 80% extract was added to a volumetric flask (15 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent after 5 min, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer each one was measured in triplicate. The tannin content was expressed in terms of µg of GAE /g of extract [15, 16].

2.3.3. Fluorescent study of seed powder

From seed powder 0.5gms of was taken into clean and dried test tubes. To each tube 10ml of different organic solvents like methanol, 1% glacial acetic acid, 10% sodium hydroxide, dilute ammonia, nitric acid, 5% ferric chloride, acetone + methanol, 10% iodine, 50% sulphuric acid and 50% hydrochloric acid were added separately. Then all the tubes were shaken and they were allowed to stand for about 10 min. The solutions obtained were observed under the visible light and UV light for their characteristic colour [17].

3. Results and Discussion

The percentage of extraction yield, obtained from different extraction techniques are presented in (Table 1**)**. The extraction yield depends on variety of factors such as solvents, time, temperature, as well as the chemical nature of the sample [18]. Here, we found that the extraction procedure and solvent highly influenced the extractive yield of the seed. The yield of ordinary reflex extract was found to be the highest and may be due to the heating effect followed by ultrasonic extract. Though, it is noteworthy that the extraction yield was directly correlated with the heat applied during the extraction procedure. Also, it should be noted that, because of polarity differences between solvents, the solubility of the solute into the solvent is expected to be different. The yield of the extract was found to be more in n-hexane which is 9.5% and 12.74% for ultrasonic extract and ordinary reflex respectively, compare to the rest of the solvent and this is indicated that the seed of *Ammi majus* contain high percentage lipid and other non polar constituents.

Table 1: Percentage yield and appearance of different extracts of *Ammi majus* seed

Extraction methods	Ultrasonic bath			Ordinary reflex		
Solvents	**Yields**	**Color**	**Consistency**	**Yields**	**Color**	**Consistency**
n-hexane	9.5%	Yellow	Oily	12.74%	Yellow	Oily
Ethyl acetate	4.07%	Yellowish brown	Oily	8.19%	Yellowish brown	Oily
Ethanol	7.94%	Yellowish brown	Dry powder	9.28%	Yellowish brown	Dry powder
Water	5.95%	Dark brown	Dry powder	6.08%	Dark brown	Dry powder

The phytochemical substances are the major important compounds which are responsible for the medicinal activities of the herbs. In a qualitative analysis of various extracts of *Ammi majus* seed in (Table 2) showed the presence of most important phytoconstituents such as alkaloids, flavonoids, tannins, phenolic acids, terpenoids, carbohydrate, coumarin and quinone in all fractions and cardioactive glycoside present in all fractions except n-hexane. While saponins present only

in water fraction and amino acid in ethanol fraction, anthraquinones absent in all fractions. As a results polar solvent contain more constituents than nonpolar solvents. The presence of alkaloids, flavonoids, tannins, phenolic acids, terpenoids and coumarin were in agreement with that previously recorded data [6, 19, 20, 21, 22], while presence of cardioactive glycoside, saponin, amino acid, carbohydrate, quinones in the seed has not been reported before.

Table 2: Qualitative analysis of selected phytochemicals in *Ammi majus* seed

Test \ Extracts	n-hexane	Ethyl acetate	Ethanol	Water
Alkaloids	+Ve	+Ve	+Ve	+Ve
Cardioactive glycosides	-Ve	+Ve	+Ve	+Ve
Flavonoids	+Ve	+Ve	+Ve	+Ve
Tannins	+Ve	+Ve	+Ve	+Ve
Phenolic acid	+Ve	+Ve	+Ve	+Ve
Saponins	-Ve	-Ve	-Ve	+Ve
Terpenoids	+Ve	+Ve	+Ve	+Ve
Amino acids	-Ve	-Ve	+Ve	-Ve
Carbohydrates	+Ve	+Ve	+Ve	+Ve
Coumarin	+Ve	+Ve	+Ve	+Ve
Quinones	+Ve	+Ve	+Ve	+Ve
Anthraquinones	-Ve	-Ve	-Ve	-Ve

(+Ve) Present, (-Ve) Absent

In quantitative analysis were total phenol, flavonoid and tannin content of ethanol 80% measured in seed extracts the results are shown in (Figure 1-3). The total phenolic and tannins were examined in seed extracts expressed in term of gallic acid equivalent (the standard curve equation: Y = 0.006X – 0.095, R^2 = 0.994 and Y = 0.003X – 0.172, R^2 = 0.985 respectively), they were estimated to be 243.33 µg for phenol and 216.66 µg for tannin, gallic acid equivalents/gram of sample extract. The total flavonoid content in seed extracts is also expressed in terms of quercetin equivalent (the standard curve equation: y = 0.018x + 0.498, R_2 = 0.999), estimated to be 288.611 µg quercetin equivalents/gram of sample extract. The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities such as anticarcinogenic, antiatherosclerotic and anti-inflammatory activities [23].

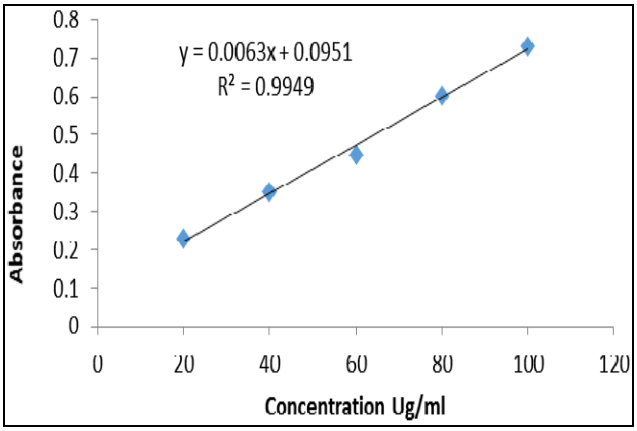

Fig 1: Calibration graph for total phenolic content

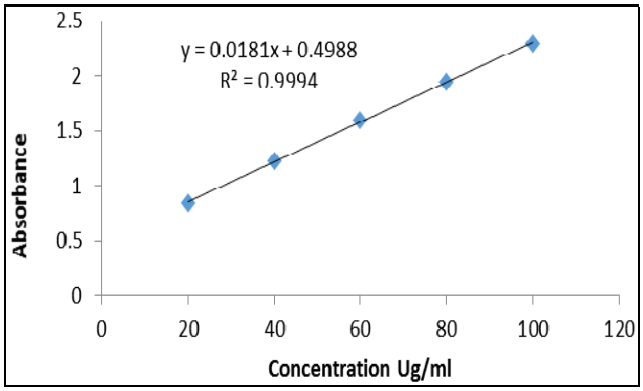

Fig 2: Calibration graph for total flavonoid content

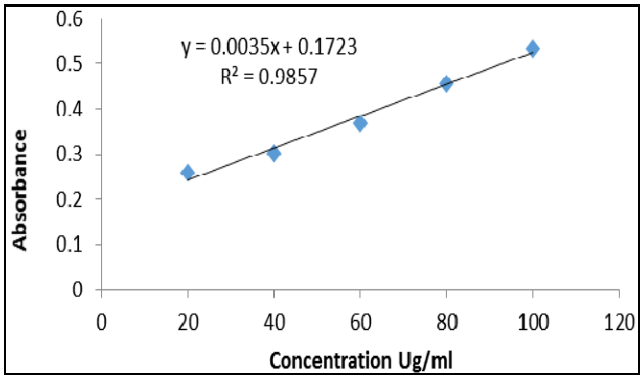

Fig 3: Calibration graph for tannin content

Many of plants fluorescence when their powder is exposed to ultra violet radiation. The results of fluorescent studies of seed powder with different chemical reagents are given in (Table 3 and Figure 4). Under visible and UV light the powdered seed was treated with various chemical reagents among the different solvents tested acetone + methanol did not show any fluorescence in seed powder, whereas methanol, glacial acetic acid, sodium hydroxide, dilute ammonia, nitric acid, ferric chloride, iodine, sulphuric acid and hydrochloric acid, showed characteristic colouration and is an important parameter of qualitative pharmacognostical evaluation.

Table 3: Fluorescent analysis of seed powder of *Ammi majus*

Powdered drug	Visible/day light	UV 254 nm (Short)	UV 366 nm (Long)
Powder drug as such	Brown	Green	Light green
Powder + Methanol	Light yellow	Violet	Light gray
Powder + 1% Glacial acetic acid	Light yellow	Light green	Violet
Powder + 10% NaOH	Yellowish brown	Dark yellowish brown	Green
Powder + Ammonia	Brown	Green	Olive green
Powder + Nitric acid	Orangish brown	Green	White
Powder + 5% Ferric chloride	Dark olive	Dark green	Dark blue
Powder + Acetone + Methanol	Light yellow	Colour less	Colour less
Powder + 10% Iodine	Yellowish brown	Light green	Violet
Powder + 50% Sulfuric acid	Reddish brown	Dark green	Blue violet
Powder + 50% Hydrochloric acid	Yellow green	Light green	Green

A

B

Fig 4: Fluorescent analysis of seed powder of *Ammi majus* A-Day light B-UV light

4. Conclusion

From the comparison study on different extraction methods used for seed of *Ammi majus* it was concluded that the highest percentage extract was yielded by using ordinary reflex method and non-polar solvents, a number of important phytoconstituent natural product groups were detected on qualitative screening in seed extract which are responsible on medicinal activity. Ethanol 80% extract has the highest flavonoid content followed by phenol and tannins.

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