Study of chemical content in *Pimpinella armena* and *Pimpinella kotschyana*: tribe Pimpinelleae /Apiaceae

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Abstract. The flavonoid and alkaloid content in the alcoholic extract of the shoots and flowers were identified in two species of the tribe Pimpinelleae / Apiaceae: *Pimpinella armena* and *Pimpinella kotschyana*, and the flavonoids that were detected in this study are: (Apigenin, Coumarin, Kaempferol and Quercetin). The total alkaloids are estimated for these two species. It has been noticed that the species *Pimpinella armena* is a new record on the Iraqi flora, as it was identified through the genetic analysis of nucleotide sequences and registered in the National Center of Biotechnology (NCBI).

Keywords. Apiaceae, *Pimpinella*, flavonoids, alkaloid.

1. Introduction

The Apiaceae family is considered one of the most important families of flowering plants at the functional level, as its inflorescences had a great role in diagnosing them long before they are described scientifically for the first time [1]. Researchers disagreed about the number of genera and species belonging to this family, as [2] indicated that it includes (200) genera and (2900) species. [3] indicated that this family is widespread and spreads in tropical to temperate regions and that it includes (400) genera and (4250) species. In Iraq, this family is represented wildly by about (60) genera and (143) species [4]. The largest genera by the number of species *Pimpinella* genus, which includes about 15 species, while [5] indicated that there are (130) wild species and (9) cultivated species. [6] also mentioned that this family is the fifth largest plant family in Iraq and that it is represented by approximately (67) genera and (155) species. The plants of this family contain many important chemical compounds that have contributed to strengthening their role as medicinal plants and a source of treatment for diseases, and the most important of these compounds are flavonoids [7, 8]. This family is considered to be one of the most economically important families, as many of its species are used as food or flavorings such as the *Foeniculum vulgare* Mill., and *Anethum graveolens* L. In addition, many of the species are medicinal plants, such as *Pimpinella*. *Pimpinella* plants have been used in the treatment of several medical conditions due to their containment of very important effective compounds such as essential oils, volatile oils, flavonoids and alkaloids [9, 10, 11]. A review of the available scientific references revealed that the current study is the first local study in which the
flavonoid and alkaloid contents of the two studied species are identified. The species *Pimpinella armena* is a new record on the Iraqi flora, as it is identified through the genetic analysis of nucleotide sequences and registered in the National Center of Biotechnology (NCBI).

2. Materials and Methods

2.1. Collection of Plant Specimens

Fresh plant specimens are collected during the flowering time and then labelled with the necessary herbal information such as (place of collection, collector's name, common name of the plant, and date of collection). These samples are dried and pressed with the aforementioned herbal information recorded. The taxonomic keys and the Iraqi flora / fifth volume are used for the diagnosis of the samples, and it is confirmed by comparing the collected samples with the herbal samples kept in the Kurdistan Botanical Foundation herbarium in Sulaymaniyah and the Iraqi national herbarium in Abu Ghraib.

2.2. Chemical Study

2.2.1. Extraction of plant samples

The most common procedures used to analyse polyphenols' and simple phenolic in natural plants are Liquid-liquid and solid-liquid extraction. The main reasons behind this widely usage are their ease of use, effectiveness, and wide ranging applicability. Commonly used extraction solvents are alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate. Milling and homogenization are the first steps in preparation process. Extraction is the major step for recovering and isolating the bioactive phytochemicals from plant materials, before analysis. The main factors that influence extractions are their chemical nature, the extraction method employed, sample particle size, as well as the availability of interfering substances. Additional steps would be of such importance if the removal of unwanted phenolic and non-phenolic substances just like waxes, fats, terpenes, and chlorophylls. Thirty grams of plant powdered was extracted using 15 ml chloroform with constant stirring for 24 hours at the ambient temperature. The extract was placed in an ultrasonic device for 15 minutes. Then 100 ml of butanol was added and then transferred to the separation funnel. The polar organic layer (butanol) is collected and transferred to the rotary evaporator device to obtain a dry extractor. The operation is repeated three times to gain an adequate amount prior to analysis.

2.2.2. Condition of HPLC for analysed phenolic and flavonoid components

Samples are analysed by using high performance liquid chromatography HPLC model (SYKAM) Germany. Pump model: S 2100 Quaternary Gradient Pump, Auto sampler model : S 5200, Detector: UV (S 2340 ) and Column Oven model : S 4115. The mobile phase is = (Methanol : D.W : acetic acid) (85 : 13 : 2), the column is C18-ODS (25 cm * 4.6 mm) and detector UV – 360 nm at flow rate 1ml/min.

2.2.3. Total alkaloid content

Extraction: The 20 gm of each plant material was ground and then extracted with methanol for 24 hours in a continuous extraction (Soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C until dryness.

2.2.4. Qualitative estimation (Test for alkaloids)
Presence of alkaloid was confirmed by Dragendorff’s method. A part of extract was dissolved in diluted HCL and 2 drops of Dragon drops are added, presence of alkaloid is indicated by the presence of crystalline precipitate. The sample which has showed positive alkaloid is then subjected to further quantitative evaluation.

2.2.5. Separation of alkaloid

A part of extract residue is dissolved in 2N HCL and then faltered. 1 ml of this solution is transferred to separatory funnel and washed with 10 ml chloroform. The pH of this solution is modulated to neutral with 0.1 N NaOH. This step is followed by adding 5 ml of Bromocresol Green (BCG) solution and 5 ml of phosphate buffer to the solution.

2.2.6. Standard curve

Carefully-measured aliquots of Atropine standard solution (0.4, 0.6, 0.8, 1 and 1.2 ml) is altered into various separatory funnels. After that 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution are taken, then the outcome mixture is shaken with extract of 1, 2, 3, and 4 ml of chloroform. These extracts have been collected later in 10 ml volumetric flask and then have mixed to adjust solution with chloroform. The absorbance of the complex in chloroform is measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank that is prepared according to the method mentioned above but without Atropine.

3. Results and Discussion

The results of the chemical study (Table 1) have showed the presence of four types of flavonoid compounds in the alcoholic extract of the shoots. These compounds are: Coumarin, Catchine, Kaempferol and Quercetin. The results of the chemical analysis have showed that the shoots are free of the flavonoid compound Apigenin. The concentration of Coumarin in the shoots is 361.4µg. ml⁻¹ in P.armena, and the concentration of this compound reached 404.1µg / ml in P.kotschyana. As for the compound Catchine, the concentration reached 71.9 µg/ml in the species P.armena, while the species P.kotschyana recorded higher concentration of this compound, which is 121.8µg/ml. It should be noted that the concentrations of Kaempferol is 39.6 µg / ml in species P.armena =, while the concentration is lowest in species P.kotschyana which is 28.64µg. ml⁻¹ (Table1). Regarding Quercetin, the highest concentration is 55.14µg. ml⁻¹ in species P.armena, and the lowest concentration is 51.2 4µg. ml⁻¹ in P.kotschyana. It is also found from this study that the flavonoid compound Coumarin is the highest concentration compound in the alcoholic extract of the shoot of all the studied species (Table 1). As for the total content of flavonoids in the alcoholic extract of the shoots in the studied species, the highest value of it is in the P.kotschyana and reached 606.6 µg ml⁻¹, while the lowest value for the total content of flavonoids is 528 µg. ml⁻¹ in P.armena (Table 1). Concerning the total content of flavonoids in the alcoholic extract of flowering inflorescences in the studied species (Table 2), the highest value is recorded in P.kotschyana as it is 2625.4 µg ml⁻¹, while the lowest total value is 418.6 µg. ml⁻¹ in P.armena. It is revealed through this study that only Apigenin and Coumarin are present in the alcoholic extract of the flowers of P.kotschyana which have showed the highest concentration of these two compounds (120.8 and 2504.6) µg.ml⁻¹, respectively. The flavonoid compound Apigenin is absent from the flowers of P.armena. The lowest concentration of Coumarin is 261.4 µg. ml⁻¹ in A.visgana (Table 2). It should be noted that the species P.kotschyana has recorded the highest concentration of compounds Apigenin, Catchine and Coumarin for the whole plant (both the vegetative and flowering parts). While the species P.armena has the highest concentration of Kaempferol and Quercetin in the vegetative and flowering (Table 3). It is worth noting that coumarins are the most abundant flavonoids in the studied species, and this is in agreement with [3] who has indicated that coumarins are among the compounds most present in the plants of the Apiaceae family.
Table 1. Flavonoid concentrations measured in µg. ml\(^{-1}\) in the shoot parts of the studied species.

| no. | Flavonoides | Sp.       | Apigenin | Coumarin | Catchine | Kaempferol | Quercetin | total |
|-----|-------------|-----------|----------|-----------|----------|------------|-----------|-------|
| 1   | P.armena    | 0         | 361.4    | 71.9      | 39.6     | 55.1       | 528       |
| 2   | P.kotschyana| 0         | 404.1    | 121.8     | 28.6     | 51.2       | 606.6     |
| Total|             | 0         | 765.5    | 193.7     | 68.2     | 106.3      | 1134.6    |

Table 2. Flavonoid concentrations measured in µg. ml\(^{-1}\) in the flowering parts of the studied species.

| no. | Flavonoides | Sp.       | Apigenin | Coumarin | Catchine | Kaempferol | Quercetin | total |
|-----|-------------|-----------|----------|-----------|----------|------------|-----------|-------|
| 1   | P.armena    | 120.8     | 261.4    | 0         | 0        | 0          | 261.4     |
| 2   | P.kotschyana| 120.8     | 2504.6   | 0         | 0        | 0          | 2625.4    |
| Total|             | 120.8     | 2766.0   | 0         | 0        | 0          | 2886.8    |

Table 3. Total group of flavonoids (vegetative and flowering parts) measured in µg. ml\(^{-1}\) in each of the studied species.

| no. | Flavonoides | Sp.       | Apigenin | Coumarine | Catchine | Kaempferol | Quercetin | total |
|-----|-------------|-----------|----------|-----------|----------|------------|-----------|-------|
| 1   | P.armena    | 0         | 622.8    | 71.9      | 39.6     | 55.1       | 789.4     |
| 2   | P.kotschyana| 120.8     | 2908.7   | 121.8     | 28.6     | 52.1       | 3232      |
| Total|             | 120.8     | 3531.5   | 193.7     | 68.2     | 106.3      | 4021.4    |

Regarding to the total alkaloids, it is found from this study that the highest percentage of alkaloids in the shoot system is 6.3% in P.armena, while P.kotschyana recorded the lowest percentage, reaching 5.3% (Table 4). The highest percentage of alkaloid content recorded in the Inflorescences is in P.armena and is 16.5%, while the lowest percentage recorded is 12.5% in P.kotschyana. It is evident from the observation of Table (4) that the species P.armena possesses the highest percentage of total alkaloid in the vegetative and flowering systems and of the studied species, which reaches 22.8%. Whilst, the species P.kotschyana recorded the lowest total percentage, which is 17.8% (Table 4). The flowering system is higher in its total alkaloid content than the shoots of the species under study, and the percentages are close in the studied species, which are sort of a few percentages and do not exceed 30% in each species. Perhaps these few percentages are the reason for the little attention of researchers in studying these important chemical compounds.

Table 4. Percentages of total alkaloids in shoots and Inflorescences of the studied species.

| No | Sp.       | Shoots % | Inflorescent % | Total % |
|----|-----------|----------|----------------|---------|
| 1  | P.armena  | 6.3      | 16.5           | 22.8    |
| 2  | P.kotschyana | 5.3    | 12.5           | 17.8    |
| Total |           | 11.6    | 29.0           | 40.6    |
Figure 1. Standard curves of shoots and flowers extract of the studied species. (Standards: 1: Apigenin, 2: Catchine, 3: coumarin, 4: Kaempferol, 5: Quercetine) (Species: a. shoot, b. flowers 6. P.armena, 7. P.kotschyana).
4. References

[1] Heywood VH 1976 Plant Taxonomy. 2nd Ed. Edward Arnold, London.
[2] Lawrence GHM 1951 Taxonomy of vascular plants The Macmillan Company New York. 823.
[3] Judd, WS, Campbell, CS, Kellogg EA and Stevens PF 1999 Plant systematics Sinauer associates, Inc., Sunderland, Massachusetts, U. S. A. 464 pp.
[4] Al-Mousawi AH 1987 Plant Taxonomy Books House for Printing and Publishing. University of Al Mosul 379.
[5] Al-Katib YM 1988 Classification of seed plants Baghdad University Ministry of Higher Education and Scientific Research, Iraq 243.
[6] Ghazanfar SA and McDaniel T 2016 Flora of the middle east: quantitative analysis biogeography of the flora of Iraq Edinburgh J. Bot. 73 1.
[7] Askari F, Sefidkon F and Teimouri M 2011 Chemical Composition and Antimicrobial Activity of Pimpinella kotschyanu Boiss. Oil in Iran J. Essential Oil Bear. Plants (Jebop) 14 124.
[8] Trovato A, Monforte MT, Rossito A and Forestieri AM 1996 In vitro cytotoxic effect of some medicinal plants containing flavonoids Boll. Chim. Farm. 135 263.
[9] Amiri MS and Joharchi MR 2016 Ethnobotanical knowledge of Apiaceae family in Iran: A review Avicenna J. Phytomed. 1.
[10] Özdemir E and Alpmar K 2015 An ethnobotanical survey of medicinal plants in western part of central Taurus Mountains: Aladaglar (Nigde-Turkey) J. Ethnopharmacol. 166 53.
[11] Al-Mayah AA 2013 Medicinal plants and herbal remedies Al-Basaer Press Beirut, Lebanon 358.