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A Study of Chemical Content in Some Species of Tribe Apieae / Apiace

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Abstract

In this study, the flavonoid and alkaloid content in the alcoholic extract of the shoots and flowers were identified in four species of the tribe Apieae / Apiacese *: Ammi majus, Ammi visgana, Anethum graveolens* and *Foeniculum vulgaris*, and the flavonoids that were detected are (Apigenin, Coumarin, Kaempferol and Quercetin). The species *Foeniculum vulgaris* has recorded the highest concentration of total flavonoid content (Shoots and Flowers) among the studied species, reaching 4139.2 µg / ml. The total alkaloids are estimated for these species, and the *Foeniculum vulgaris* has recorded the highest concentration of the total alkaloid content as well.

Keywords: Apiaceae, *Ammi*, Apigenin, Coumarin, Alkaloid.

1. Introduction

The Apiaceae family is considered one of the most important families of flowering plants at the academic and practical levels, as its inflorescences had a great role in diagnosing them long before they are described scientifically for the first time [1] (Heywood,1976). Researchers disagreed about the number of genera and species belonging to this family, as [2] Lawrence (1951) indicated that it includes (200) genera and (2900) species. [3] Judd et. al (1999) 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] (Al-Mousawi, 1987), while Al-Katib [5] (1988) indicated that there are (130) wild species and (9) cultivated species. [6] Ghazanfar and McDaniel (2016) 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] (Trovato et. al, 1996). 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 that 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 [8, 9] (Mutlag , 2007 and Al-Mayah, 2013). This family is considered to be a source of gum and perfumes, and a few of its species are used for decoration [4] (Al-Mousawi, 1987).

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A review of the available scientific sources revealed that the current study is the first local study in which the flavonoid and alkaloid contents of the four studied species are identified.

2. Materials Method

2.1. Collection of Plant Specimens

Fresh plant specimens are collected during the flowering stage and then marked with the necessary herbal information such as (place of collection, collector's name, the 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

Liquid-liquid and solid-liquid extraction are the most commonly used procedures before analysis of Polyphenols and simple phenolic in natural plants. They are still the most widely used techniques, mainly because of their ease of use, efficiency, and wide-ranging applicability. Commonly used extraction solvents are alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate. The first steps of a preparation procedure are milling and homogenization. Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before analysis. It is influenced by their chemical nature, the extraction method employed, sample particle size, as well as the presence of interfering substances. Additional steps may be called for if the removal of unwanted phenolic and non-phenolic substances such as waxes, fats, terpenes, and chlorophylls is of interest. 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 (3) times to obtain an adequate amount before analysis.

2.2.2. HPLC Condition for Analyzed Phenolic and Flavonoid Compounds

Samples are analyzed by high-performance liquid chromatography HPLC model (SYKAM) Germany. Pump model: S 2100 Quaternary Gradient Pump, Autosampler model: S 5200, Detector: UV (S 2340), and Column Oven model: S 4115. The mobile phase was = (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

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

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2.2.4. Qualitative Estimation (Test for Alkaloids)

The presence of alkaloid was confirmed by Dragendroff's method. A part of the extract was dissolved in dilute HCL and 2 drops of Dragon drops are added, a crystalline precipitate indicates the presence of alkaloid. The sample which has shown positive alkaloid is then subjected to further quantitative evaluation.

2.2.5. Separation of Alkaloid

A part of the extract residue is dissolved in 2N HCL and then faltered. 1 ml of this solution is transferred to a reparatory funnel and washed with 10 ml chloroform. The pH of this solution is adjusted to neutral with 0.1 N NaOH. Then 5 ml of Bromocresol Green (BCG) solution and 5 ml of phosphate buffer are added to this solution.

2.2.6. Standard Carve

Accurately measured aliquots (0.4, 0.6, 0.8, 1, and 1.2 ml) of Atropine standard solution were transferred to different reparatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution is taken and the mixture is shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts are then collected in a 10 ml volumetric flask and then have diluted to adjust the solution with chloroform. The absorbance of the complex in chloroform is measured at a spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine.

3. Results and Discussion

The results of the chemical study (**Table 1**) have shown 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 shown that the shoots are free of the flavonoid compound Apigenin.

The highest concentration of Coumarin in the shoots is 632.7 µg / ml in *F.vulgare*, and the lowest concentration of this compound reached 402.0 µg/ ml in *A. visgana*. As for the compound Catchine, the highest concentration reached 145.0 g/ ml in the type *A. majus*, while the species *A.visgana* recorded the lowest concentration of this compound, which is 105.4 µg / ml. It should be noted that the concentrations of Kaempferol are similar in the four studied species, and its highest concentration is 39.7 g/ml in species *A.visgana*, while the lowest concentration is 37.7 µg / ml in species *A.majus*, and the rest of the species has ranged between these values mentioned (**Table 1**). Regarding Quercetin, the highest concentration is 156.2 µg/ ml in *F. vulgare*, and the lowest concentration is 51.7 µg/ ml in *A.visgana* .

It is found from this study also 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 *F.vulgare* and reached 944.8 µg / ml, while the lowest value for the total number of flavonoids is 598.8 \g / ml in the type *A.visgana* (**Table 1**).

Concerning the total content of flavonoids in the alcoholic extract of flowering inflorescences in the studied species (**Table 2**), the highest value of this total is recorded in *F.vulgare* as it is 3194.4 µg / ml, while the lowest total value is 418.6 µg / ml in *A.visgana*. It is revealed through this study that only Apigenin and Coumarin are present in the alcoholic extract of the inflorescences (**Table 2**).

The species *F.vulgare* have shown the highest concentration of these two compounds

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(395.7 and 2798.7) µg / ml, respectively. The flavonoid compound Apigenin is absent from the flowers of *A. majus*. The lowest concentration of Coumarin is 313.2 µg / ml in *A.visgana*.

It should be noted that the species *F.vulgare* has recorded the highest concentration of compounds Apigenin, Catchine, Coumarin, and Quercetin for the whole plant (both the vegetative and flowering parts). Also, the species *F.vulgare* has the highest concentration of flavonoids in the vegetative and flowering parts, as it reaches 4139.2 g / ml, while *A.visgana* has the lowest concentration and reaches 1017.4 µg / ml (**Table 3**).

It is worth noting that coumarins are the most abundant flavonoids in the studied species, and this is in agreement with [3] (Judd *et.al*, 1999) who have indicated that coumarins are among the compounds most present in the plants of the Apiaceae family.

Table 1. Flavonoid concentrations measured in µg / ml in the shoot parts of the studied species.

no.	Flavonoides Sp.	Apigenin	Coumarin	Catchine	Kaempferol	Quercetin	total
1	*A.majus*	0	568.2	145.0	37.7	114.8	865.7
2	*A.visgana*	0	402.0	105.4	39.7	51.7	598.8
3	*A.graveolens*	0	468.4	130.5	38.2	98.7	735.8
4	*F. vulgare*	0	632.7	116.5	39.4	156.2	944.8
Total		0	2071.3	497.4	155	421.4	3145.1

Table 2. Flavonoid concentrations measured in µg / ml in the flowering parts of the studied species.

no.	Flavonoides Sp.	Apigenin	Coumarin	Catchine	Kaempferol	Quercetin	total
1	*A.majus*	0	527.7	0	0	0	527.7
2	*A.visgana*	105.4	313.2	0	0	0	418.6
3	*A.graveolens*	328.6	1482.7	0	0	0	1811.3
4	*F. vulgare*	395.7	2798.7	0	0	0	3194.4
total		829.7	5122.3	0	0	0	5952

Table 3. Total group of flavonoids (vegetative and flowering parts) measured in µg / ml in each of the studied species.

no.	Flavonoides Sp.	Apigenin	Coumarine	Catchine	Kaempferol	Quercetin	total
1	*A.majus*	0	1095.9	145	37.7	114.8	1393.4
2	*A.visgana*	105.4	715.2	105.4	39.7	51.7	1017.4
3	*A.graveolens*	328.6	1951.1	130.5	38.2	98.7	2547.1
4	*F. vulgare*	395.7	3431.4	116.5	39.4	156.2	4139.2
total		829.7	7193.6	497.4	155	421.4	9097.1

Regarding the total alkaloids, it is found from this study that the highest percentage of alkaloids in the shoot system is 7.2% in *A.visgana*, while *A. majus* recorded the lowest percentage, reaching 5.6% (**Table 4**).

The highest percentage of alkaliod content recorded in the flowering system is in *F.vulgare* and is 16.2%, while the lowest percentage recorded is 13.4% in *A.visgana*. It is evident from the observation of **Table (4)** that the species *F.vulgare* possesses the highest percentage of total alkaloid in the vegetative and flowering systems and of the studied species, which reaches 22.4%, while the two species *A.majus* and *A.visgana* recorded the lowest total percentage, which is 20.6% for each.

The flowering system is higher in its total alkaloid content than the shoots of the species

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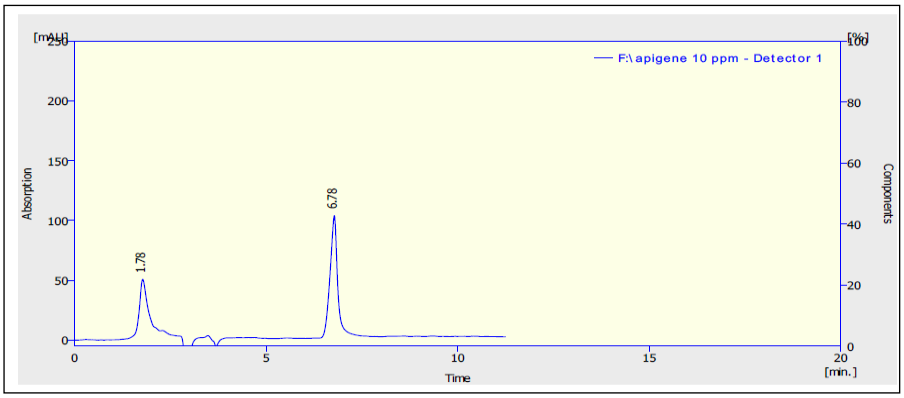
under study, and the percentages are close in the studied species, which are few percentages and do not exceed 30% in one species, and perhaps these few percentages are the reason for the lack of interest of researchers in studying these important chemical compounds.

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

No	Sp.	Shoots %	Inflorescents %	Total %
1	*A.majus*	5.6	15	20.6
2	*A.visgana*	7.2	13.4	20.6
3	*A.graveolens*	5.7	16	21.7
4	*F. vulgare*	6.2	16.2	22.4
Total		24.7	60.6	85.3



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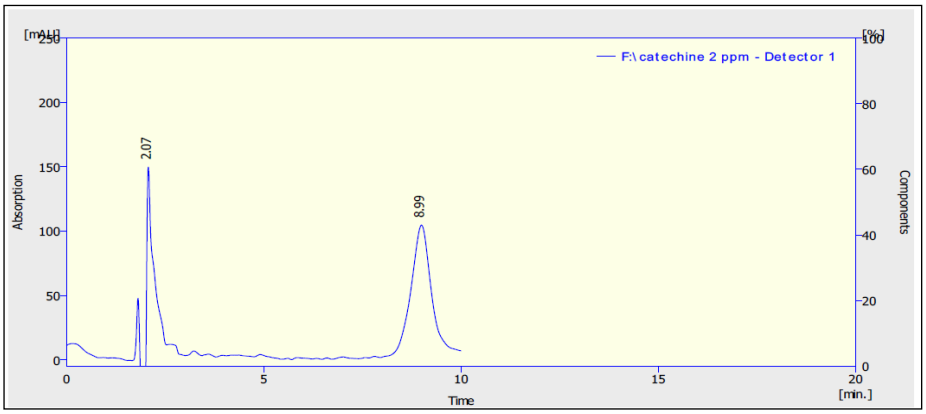

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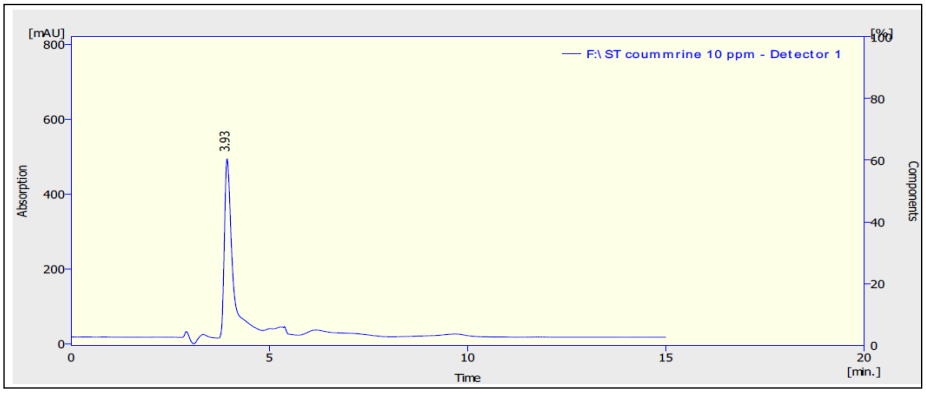

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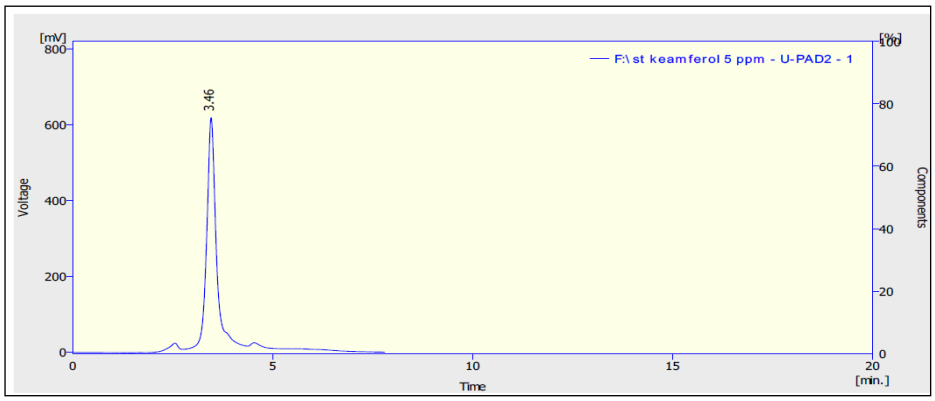

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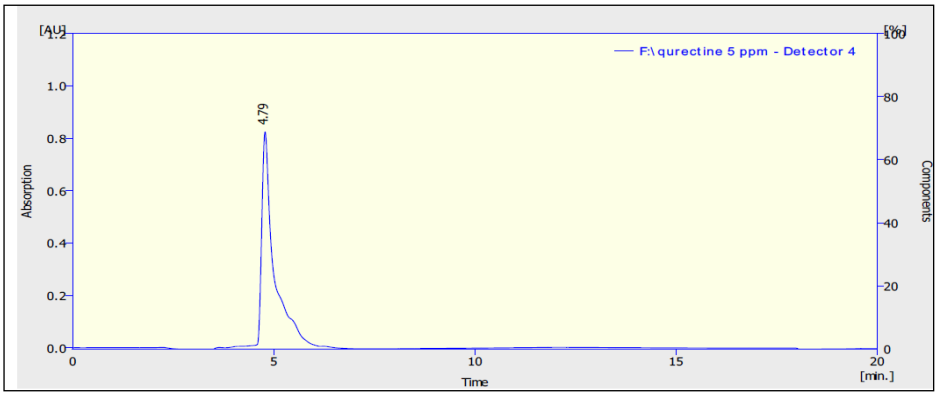

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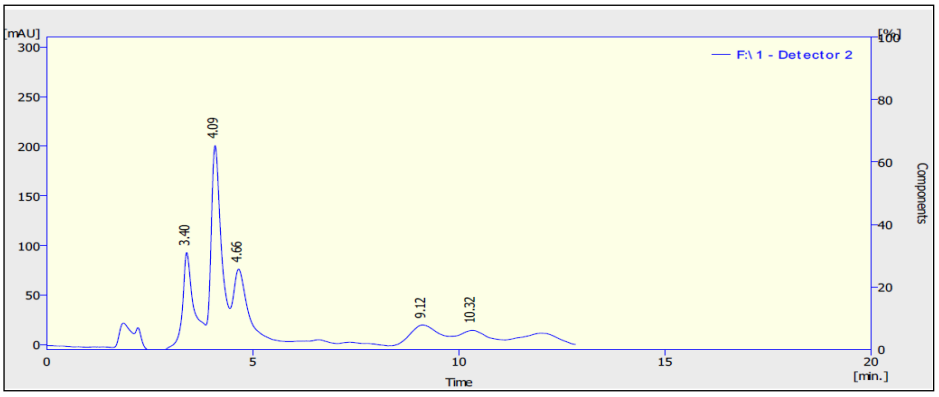

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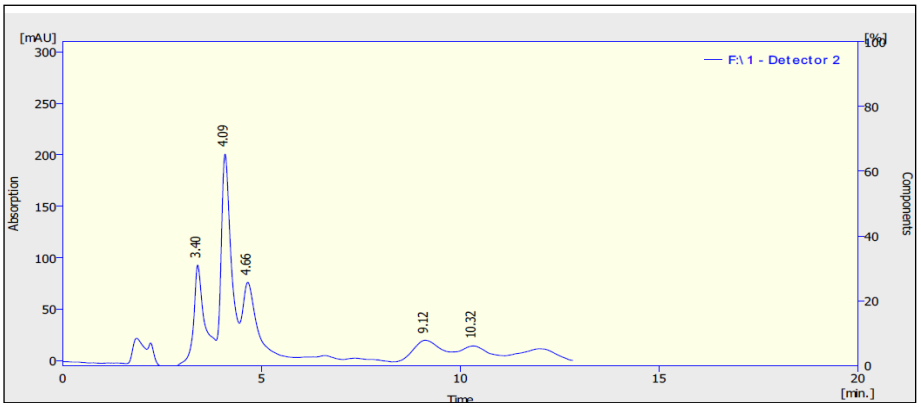

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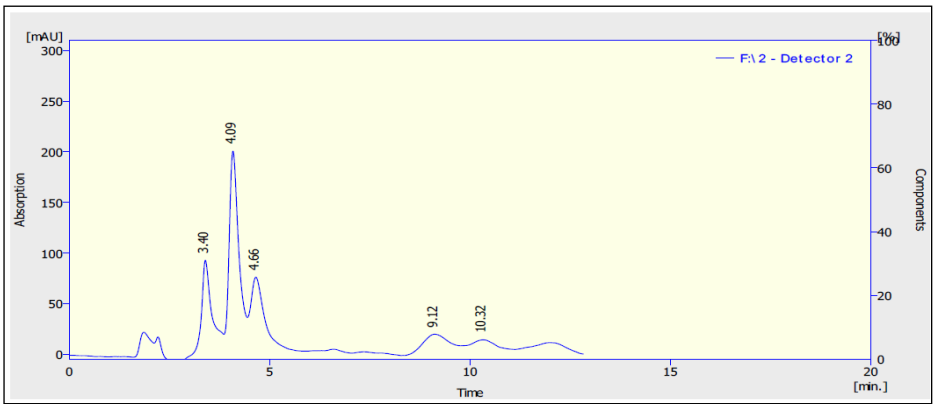

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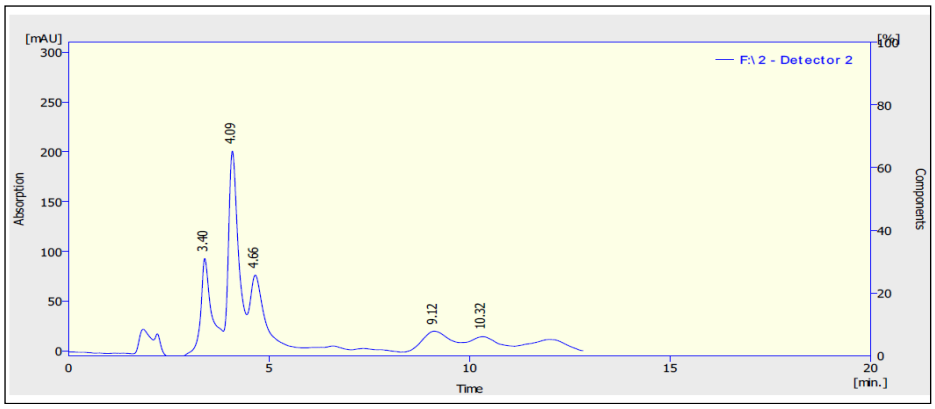

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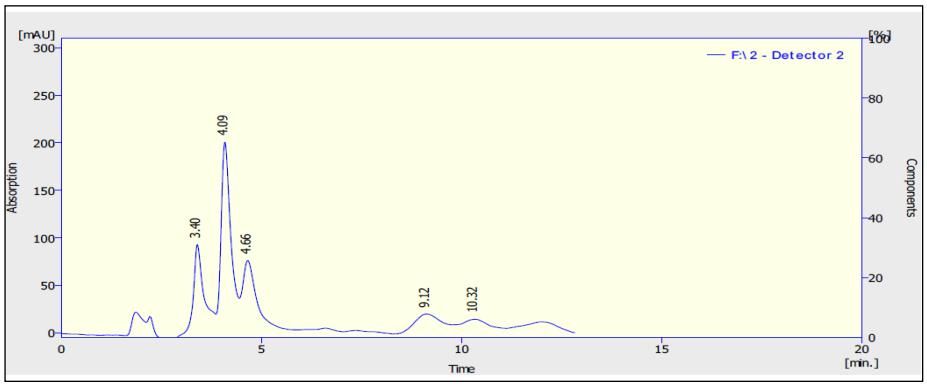

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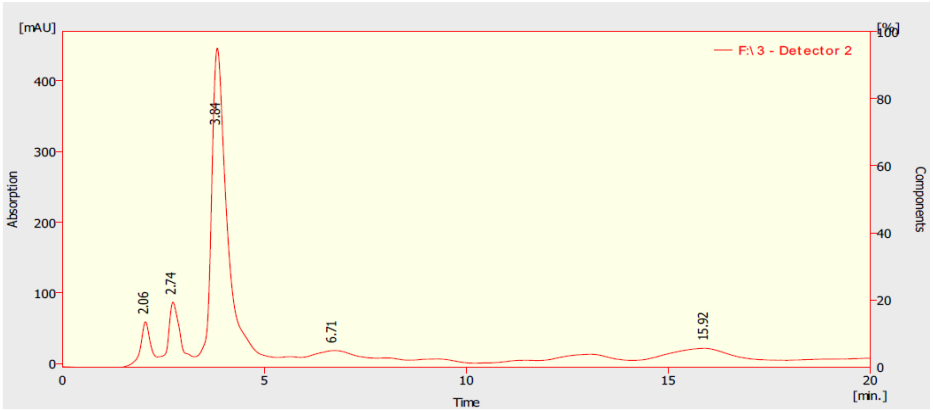

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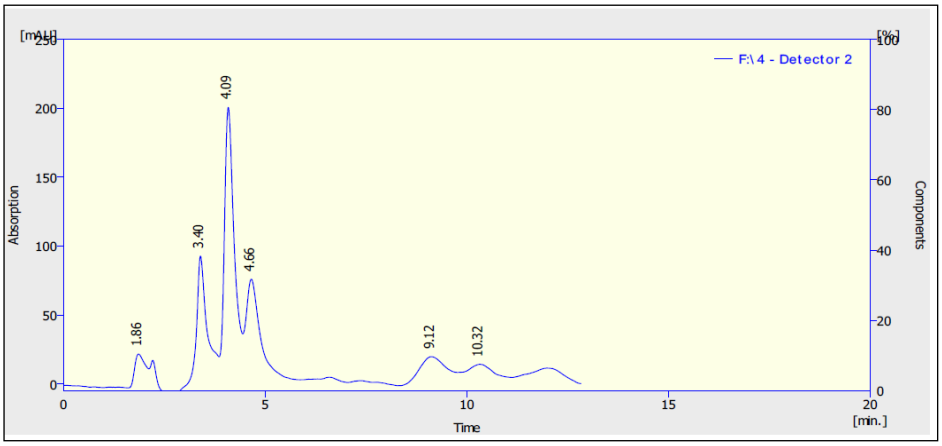

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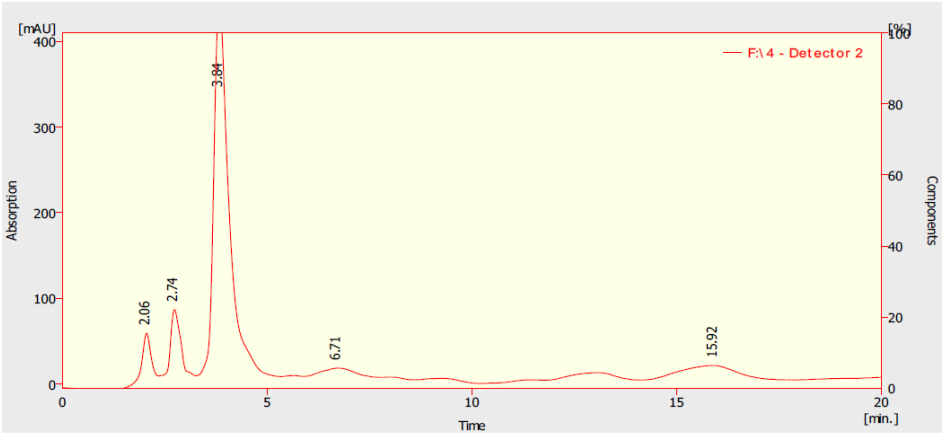
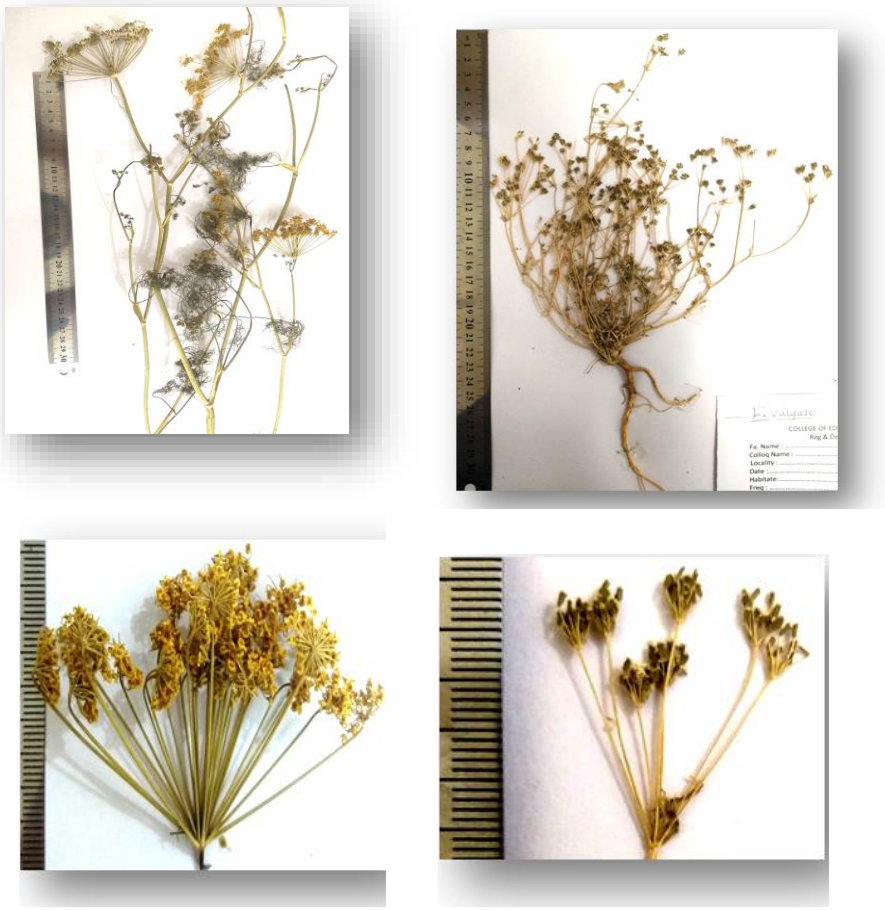


9b

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. *A.majus*, 7. *A.visgana*, 8. *A.graveolens*, 9. *F.vulgare*).

1 **2**



3 4

Figure 2. Studied species with their flowers: (1. *A.majus* , 2. *A.visgana* , 3.*A.graveolens*, 4.*F.vulgare*).

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