Isolation of Esculetin and Umbelliferone from Viola Odorata Cultivated in Iraq

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***Abstract*—*Viola odorata* is a species of the viola family, may be found naturally occurring over *Europe* and *Asia*. This little plant is tough and perennially herbaceous. It's also known as sweet violet and English violet. There are several medicinal uses for this flowering plant, including anticancer, antibacterial, inflammation., antioxidant activity and antipyretic activity. The phytochemical studies of *Viola odorata* different parts resulted in identification and isolation of different chemical constituents such as Coumarins, caffeic acid, methyl salicylate, flavonoids (Quercetin, kaempferol), glycosides (Rutin) and terpenoids (stigma sterol). Coumarins are an important natural phenolic compounds of the family of benzopyrone. Coumarin's basic structure consists of a pyrone ring fused with a benzene ring. Umbelliferone and esculetin are the most common simple coumarins in nature. Coumarins become an attractive backbone drugs with innovative impacts on illnesses and reduced side effects on healthy cells. Anti-inflammatory, anti-carcinogenic, and other biological properties are among those attributed to coumarins derivatives. In this study umbelliferone and esculetin of *Viola odorata* were isolated by PTLC and the structures of isolated umbelliferone and esculetin are elucidated by FTIR, ^{1}H-NMR and ^{13}C-NMR.**

***Keywords*: *Viola; odorata*; Umbelliferone; esculetin; Isolation.**

INTRODUCTION

The fragrant *Viola odorata* is a member of the viola family that may be found growing wild in both *Europe* and *Asia*. This little herbaceous perennial is tough as nails. Several names have been given to this flower, including sweet violet and English violet. [(1)]. The phytochemical studies of *Viola odorata* different parts resulted in the identification and isolation of different chemical constituents such as Coumarins, caffeic acid, methyl salicylate, flavonoids (Quercetin, kaempferol), glycosides (Rutin) [(2)] and terpenoids (stigma sterol) [(3)]. *Viola odorata* has been medicinally used for long time. Expectorant, anti-cancer, anti-inflammatory, antipyretic, antibacterial, diuretic, and laxative are only some of the many medicinal uses for *Viola odorata* [(4)]. Inhalation of the vapors is used to treat bronchitis, coughing, and asthma [(5)]. *Viola odorata* is rich in coumarins. The basic structure of coumarin is shown in Fig (1) Umbelliferone and esculetin are the most common simple coumarins in nature [(6)]. Coumarins become an attractive backbone and Since it has a unique impact on illnesses while being gentler on healthy cells, innovative therapeutic agents are a promising new option for treating these conditions. Anti-inflammatory [(7)], anti-carcinogenic, and other biological properties are among those attributed to coumarin derivatives. [(8)]. Umbelliferone (7-hydroxycoumarin; UMB), the structure of Umbelliferone is shown in Fig (2) is a coumarin that may be found in great quantities in plants like carrots, cilantro, and *Viola odorata*. Several pharmacological effects of umbelliferone have been observed. It has been widely used as a sunscreen agent in cosmetics, and studies suggest it may have antioxidant, anti-inflammatory, antidiabetic, anticancer, and antiepileptic effects [(9,10,11)]. There is evidence that the naturally occurring dihydroxy coumarin esculetin exhibits a broad variety of pharmacological properties [(12)], its structure is shown in Fig (3). For certain diseases, such as cancer [(13)], atherosclerosis [(14),] and other diseases. Umbelliferone and esculetin are simple coumarins generated by the hydroxylation of cinnamic acid to create 4-coumaric acid, which is then manufactured from L-phenylalanine and caffeic acid respectively [(15)].

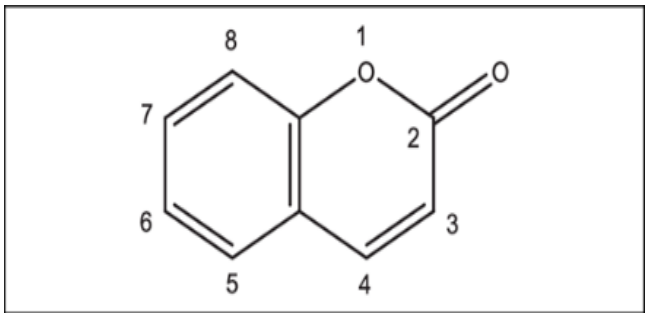

Fig 1. The basic structure of coumarin

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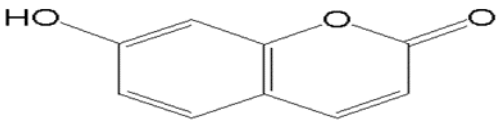


Fig 2. The basic structure of Umbelliferone

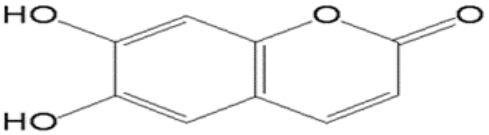


Fig 3. The basic structure of Esculetin

Material & Methods

Plant Material

Viola odorata aerial parts were collected from nurseries in *Baghdad*. The collected plant was authenticated at the University of *Baghdad*'s / Faculty of Science herbarium by Dr. *Israa Abdul-Razaq*. The plant's aerial parts were collected in *March* and *April* and air-dried in the shade. Finally, they were finely powdered and measured.

Preparation Of Ethyl Acetate Extract Of Viola Odorata Aerial Parts

Viola odorata powdered aerial parts (100) gram extracted with 1000 mL of 70% ethanol after defatting with hexane in a soxhlet system. Filtering the extract before rotating and evaporating it to dryness was the next step. The ethyl acetate and water extract were partitioned three times (50mL). Fractions A and B were separated by collecting the lower aqueous-ethanolic layer and the top ethyl acetate layer, respectively.

Isolation Of Umbellefirone And Esculetin By PLC

Concentrated ethyl acetate extract of aerial parts was subjected to preparative TLC (PLC) performed by silica gel GF-254 precoated plate (0.25 mm thickness), then developed in mobile phase7: (Toluene: ethyl acetate: formic acid) (5:4:1) [16]. The detected bands as compared with the standard, were visualized under UV light at 254nm and 366nm, and scrapped off and extracted eluted not extracted with acetone, Solvents were evaporated to collect the final product [17].

Structure Elucidation Of Isolated Umbellefirone And Esculetin

- Fourier Transform Infrared Spectroscopy (FTIR):

The isolated compounds were analyzed using FTIR spectrophotometer. It was performed by using the SHIMADZU apparatus at the College of Pharmacy/Mustansiriyah University.

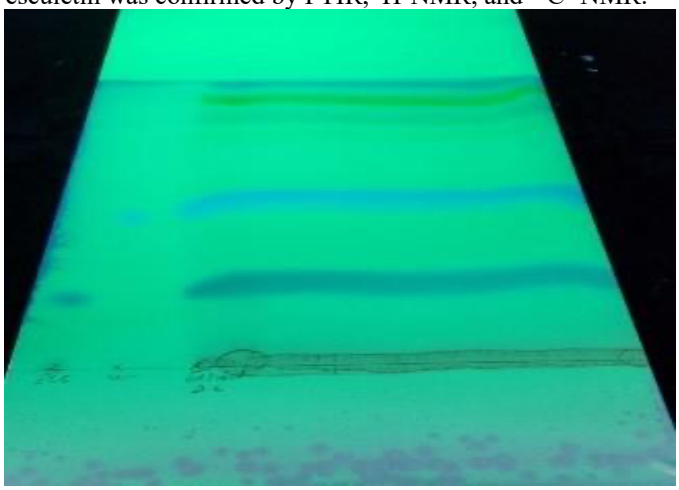
- ^{1}H- NMR and ^{13}C- NMR:

The NMR spectra were performed at the Pharmaceutical research center/*Jordan* University of Science and technology Instrument Model: Bruker 400 MHz-Avanc III. chemical shift expressed as part per million (ppm).

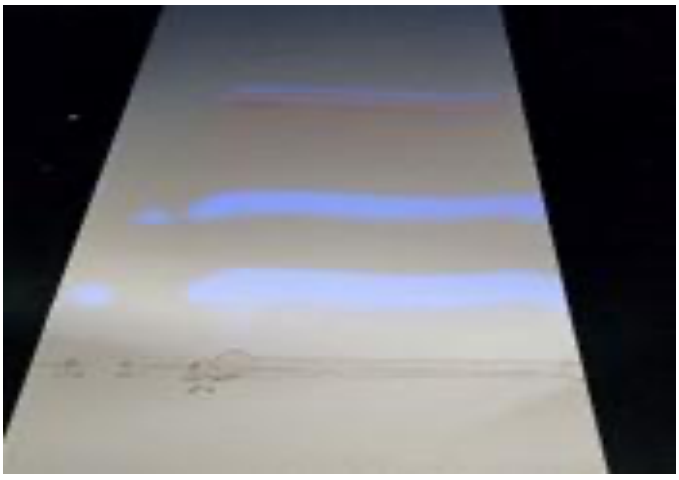
Results and Discussion

Isolation of umbelliferone and esculetin from ethyl acetate extract of *Viola odorata* aerial parts

Umbelliferone and esculetin were isolated by PLC as shown in Fig (4). Structure elucidation of isolated umbelliferone and esculetin was confirmed by FTIR, ^{1}H-NMR, and ^{13}C- NMR.



A



B

Fig 4. Isolation of esculetin and umbelliferone by PLC (A) at 254nm (B) at 366nm

Structural elucidation of umbelliferone and esculetin from ethyl acetate extract of Viola odorata aerial parts

1. Structure elucidation of isolated umbelliferone by FTIR.

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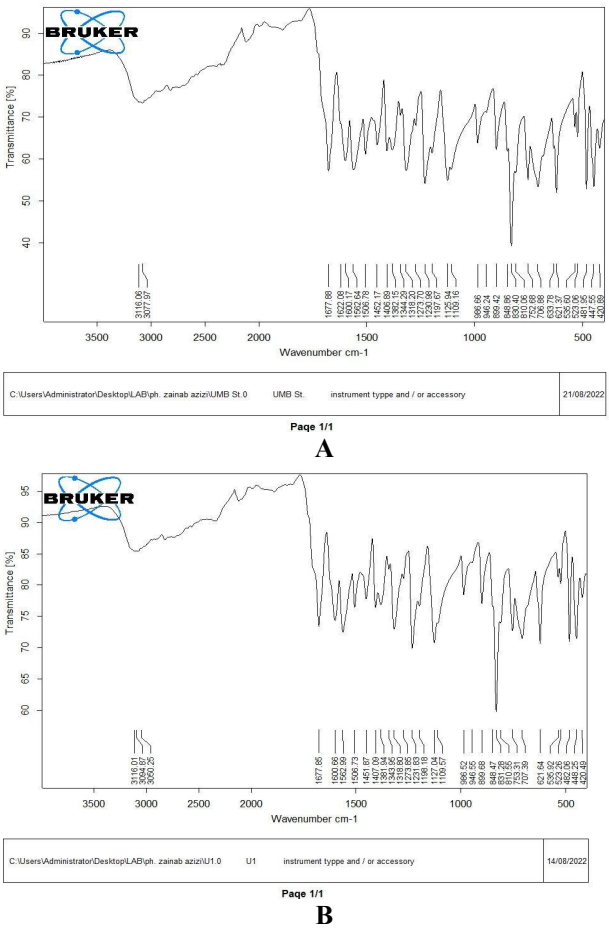
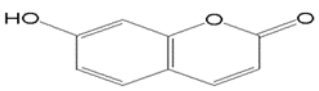


Fig 5. FTIR spectra of umbelliferone (A) = umbelliferone standard, (B) = isolated umbelliferone

TABLE 1: CHARACTERISTIC FTIR ABSORPTION BANDS OF THE ISOLATED UMBELLIFERONE



Bands	Interpretation
OH stretching of phenol	3116.15 cm^{-1}
C-H stretching aromatic ring	3015.12&3077.94 cm^{-1}
C=O carbonyl stretching	1677.91 cm^{-1}
C=C stretching of the aromatic ring	1600.20&1622.34 cm^{-1}

2. Structure elucidation of isolated umbelliferone by ^{1}H-NMR and ^{13}C-NMR.

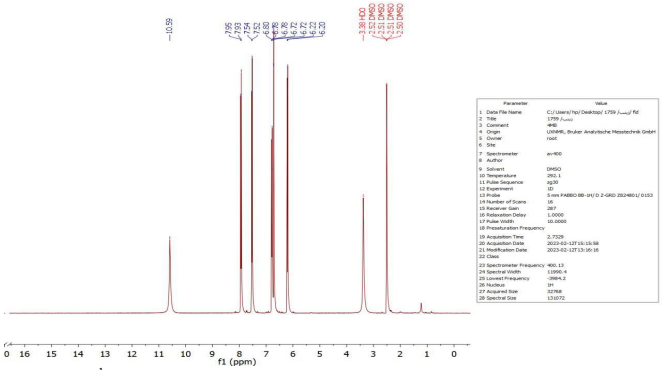
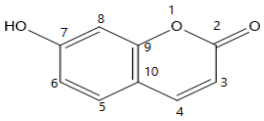


Fig 6. The ^{1}H-NMR spectrum of isolated umbelliferon

TABLE 2. ^{1}H -NMR DATA AND THEIR INTERPRETATION OF ISOLATED UMBELLIFERON



Position of H	Chemical shift ppm	No. of H	Interpretation
C5-H	6.21	1	Duplet
C3-H	6.21	1	Duplet
C8-H	6.72	1	Singlet
C6-H	6.79	1	Duplet
C4-H	7.53	1	Duplet
OH- H	10.59	1	Singlet

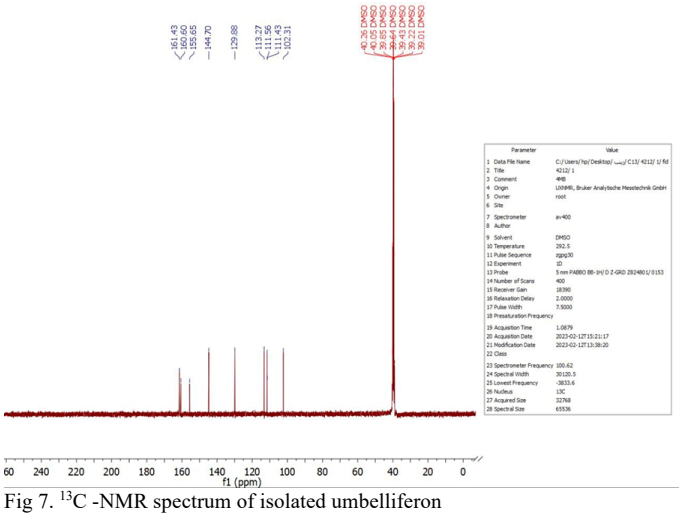


Fig 7. ^{13}C -NMR spectrum of isolated umbelliferon

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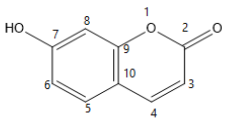
TABLE 3. ^{13}C-NMR DATA AND THEIR INTERPRETATION OF ISOLATED UMBELLIFERON



Position of C	Chemical shift ppm	Interpretation
C8	102.32	For aromatic carbon C8 of ring A
C6	111.45	For aromatic carbon C6 of ring A
C10	111.58	For aromatic carbon C10 of ring A
C3	113.28	For carbon C3 of ring B
C5	129.89	For aromatic carbon C5 of ring A
C4	144.71	For carbon C4 of ring B
C9	155.66	For aromatic carbon C9 of ring A
C7	160.62	For aromatic carbon C7 of ring A
C2	161.45	For carbon C2 of ring B

3. Structure elucidation of isolated esculetin by FTIR.

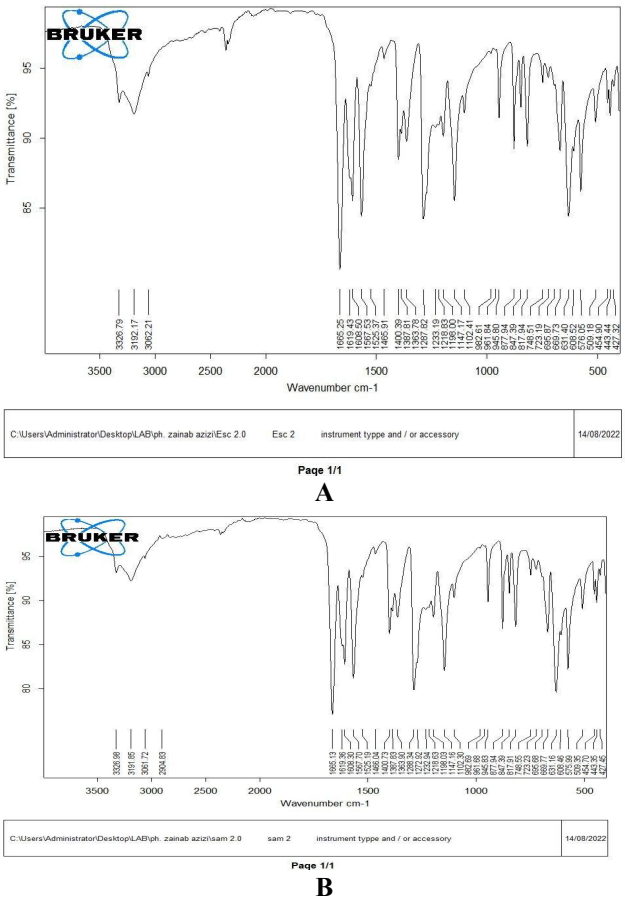
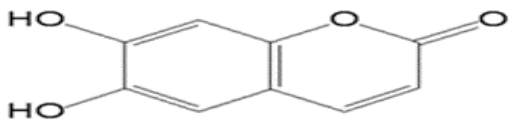


Fig 8. FTIR spectra of esculetin (A) = esculetin standard, (B) = isolated esculetin

TABLE 4. CHARACTERISTIC FTIR ABSORPTION BANDS OF THE ISOLATED ESCULETIN



Bands	Interpretation
OH stretching of phenol	3326.79-3192.17
C-H stretching of the aromatic ring	3062.21
C=O carbonyl stretching	1665.25
C=C stretching of the aromatic ring	1619.34-1608.50

4. Structure elucidation of isolated esculetin by ^{1}H-NMR and ^{13}C-NMR.

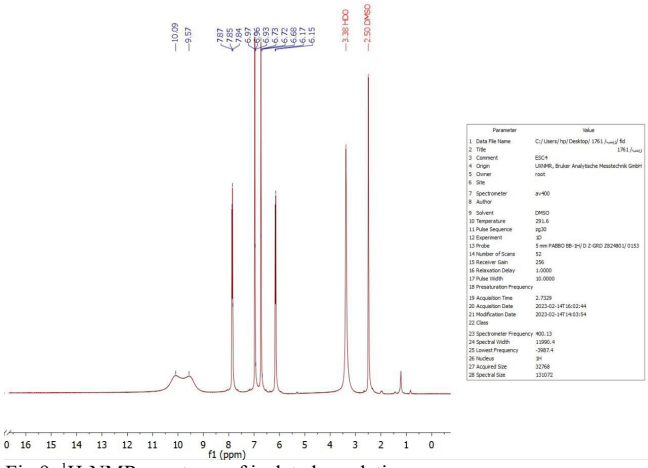
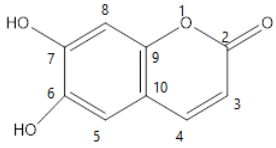


Fig 9. ^{1}H-NMR spectrum of isolated esculetin

TABLE 5. ^{1}H -NMR DATA AND THEIR INTERPRETATION OF ISOLATED ESCULETIN



Position of H	Chemical shift ppm	No. of H	Interpretation
C8- H	6.73	1	Singlet
C3- H	6.96	1	Duplet
C5–H	6.96	1	Singlet
C4- H	7.86	1	Duplet
OH at C6	9.57	1	Singlet
OH at C7	10.09	1	Singlet

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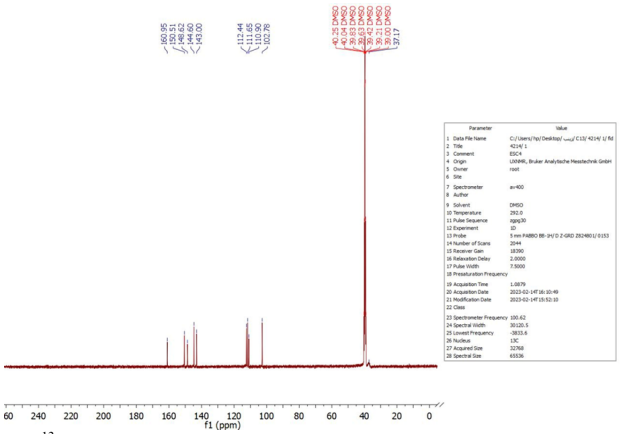
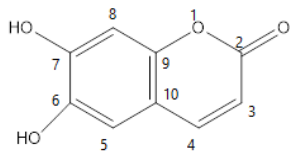


Fig 10. ^{13}C -NMR spectrum of isolated esculetin

TABLE 6. ^{13}C-NMR DATA AND THEIR INTERPRETATION OF ISOLATED ESCULETIN



Position of C	Chemical shift ppm	Interpretation
C8	102.79	For aromatic carbon C8 of ring A
C10	110.91	For aromatic carbon C10 of ring A
C3	111.66	For carbon C3 of ring B
C5	112.45	For aromatic carbon C5 of ring A
C6	143.02	For aromatic carbon C6 of ring A
C4	144.62	For carbon C4 of ring B
C7	148.63	For aromatic carbon C7 of ring A
C9	150.52	For aromatic carbon C9 of ring A
C2	160.97	For carbon C2 of ring B

CONCLUSION

Coumarins become an attractive backbone and new therapeutic medicines because they have innovative impacts on illnesses and do less harm to healthy cells. Umbelliferone (UMB), has many pharmacological activities including antioxidant, anti-inflammatory, anti-diabetic, and anticancer activities [9], and antiepileptic activity [10] in addition to its widespread cosmetic application as a sunscreen agent [11]. Esculetin which is a natural dihydroxy coumarin has displayed a variety of pharmacological effects [12]. Cancer [13], atherosclerosis [14], and other diseases. Umbellefirone and esculetin were isolated from *Iraqi Viola odorata* ethyl acetate extract of aerial parts by PLC and the structures of isolated umbelliferone and esculetin are elucidated by FTIR, ^{1}H-NMR and ^{13}C-NMR.

Iraqi plants are rich in active constituents therefore more research is required to learn about the plant's phytochemical components and the bioactivity of these compounds.

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