

## FLAVONOIDS AND COUMARINS OF *Thymus capitatus*

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### ABSTRACT

Chromatographic investigation of phenolic, flavonoid and coumarin contents of *Thymus capitatus* revealed the presence of 5 compounds of flavonoids, one phenolic acid and 3 compounds of coumarins, identified through  $R_f$  values, complete and partial acid hydrolysis, UV spectral data, mass spectral data;  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR analysis, where the following flavonoids were proved to be present in *Thymus capitatus*: kaempferol-3-rutinoside, kaempferol-3-O-rhamnoside, 4-O-glucoside, rutin, apigenin-7-O-rhamnoglucoside, quercetin and P-coumaric acid. Meanwhile the coumarins were scopoletin, 6, 7 dimethoxycoumarin and herniarin. The percentage of total flavonoids reached 0.30%.

### INTRODUCTION

Flavonoids are important dietary compounds, having a capacity to inhibit DNA damage and lipid peroxidation, and to quench free radicals. They also have anticarcinogenic and antiproliferative effects (Romanova and Vachalkova, 1999).

In plants, most species have a wealth of phenolic constituents. Some phenolic compounds possess antifungal and antibacterial activities. There are some coloured flavonoids which contribute to plant pigmentation, but the majority of the flavonoids are colourless in daylight, which have a role in providing plants with protection from damaging UV radiation (Micheal, 1997).

Adzet and Martinez (1978), isolated luteolin from *Thymus capitatus*.

*Thymus capitatus* are dried powdered and subjected to bioassay tests to determine the efficiency against *Biomphalaria alexandrina*, snails, *Schistosoma mansoni*, cercariae and miracidium. The plant sample was also subjected to phytochemical screening for the presence of carbohydrates and/or glycosides, flavonoids, saponins, tannins and alkaloids. Results showed that *Thymus capitatus* has great effect against the snails and *S. mansoni* and miracidia. Phytochemical screening revealed that flavonoids were present in *Thymus capitatus* (Mansour et al. 2001).

### MATERIALS AND METHODS

#### 1. Materials:

##### 1.1. Plant material:

*Thymus capitatus* grow widely at Matruh (North western coast). The plant obtained from this region was identified and subjected prepared for chemical analysis.

##### 1.2. Solvents:

Ethyl alcohol (80,96%), n-butanol, acetic acid, petroleum ether (40-60°C), ether, chloroform and ethyl acetate.

##### 1.3. Reagents:

Sodium methoxide solution (2.5 gm NaOH was added cautiously to 100 ml dry methanol), sodium acetate, boric acid, hydrochloric acid (50% 1:2, v/v) and aluminum chloride /ethanol solution (9.6%, w/v).

**1.4. Solvent systems:**

- 1- n- Butanol - Acetic - Water (4: 1 : 5, v/v/v) = BAW
2. Acetic acid- Water (15: 85 ,v/v)

**2. Methods:**

**2.1. Isolation of the flavonoid constituents:**

*Thymus capitatus* was cleaned, air dried, ground to fine powder and extracted with 80% aqueous ethanol. The ethanolic extract was evaporated under reduced pressure and low temperature. The latter was extracted with chloroform. The obtained residue treated with excess of ethanol and filtered to remove inorganic salts and nonphenolic compounds.

**2.2. Chromatography:**

Alcoholic extract of plant was chromatographed on Whatman No.1. Paper chromatography using the solvent system n-butanol: acetic acid: water (4: 1: 5, v/v/v) for the first way and the solvent system acetic acid: water (15: 85) for the second way. The developed chromatograms were air dried, and examined under ultraviolet (UV) light.

The concentrated ethanolic extract of *Thymus capitatus* was applied on the top of a cellulose column. Elution was started with water followed by a mixture of water/ methanol and finally pure methanol were used. The received fractions were evaporated and similar fractions were collected together, evaporated and subjected to paper chromatography (Liu *et al.*, 1989). Preparative paper chromatography was applied on Whatman No. 3 paper chromatography using the solvent system BAW. The separated flavonoid compounds were purified on a sephadex LH - 20 column using methanol/ water system.

Carboxylic acids, hydroxyl flavonoids and sugars were detected by spraying the air dried chromatograms with the following reagents.

**2.3.Reagent for carboxylic acids :**

**Aniline/ glucose (Smith, 1960).**

Two grams glucose were dissolved in 2 ml water.Two ml aniline was dissolved in 20 ml ethanol. The two solutions were mixed and completed to 100 ml by n- butanol. The chromatogram was sprayed with the reagent and heated for 5- 10 min. at 105°C.Brown to red spots were appeared.

**2.4.Reagents for hydroxy- flavonoids:**

a) Aluminum chloride:

The chromatograms were sprayed with  $AlCl_3$  (1 % methanolic solution), then heated at 110°C for 10 minutes and observed under UV light (Markham, 1982).

b) Tetra phenyl diboroeroxie Ethanol Amino Complex (Markham, 1982,) 1% Ethanolic solution.

**2.5.Reagents for phenolics:**

1. Ferric chloride (Smith, 1960).

1% ethanolic solution.

2. Glibb's reagents (Neish, 1960).

a) A freshly prepared N-2 ,trichloro-P-benzoquinone-4- ammonium(0.5% methanolic solution).

b) Saturated aqueous sodium bicarbonate solution.

**2.6. Reagent for sugars:**

Aniline / hydrogen phthalate (Stahl, 1969).

Aniline (0.9g) and O-phthalic acid anhydride (1.6 g) were dissolved in 100 ml n-butanol saturated with water, the chromatogram was sprayed and heated in an oven at 100- 105°C for 10 minutes. The developed colour ranged from brown/, yellowish brown to red.

**2.3. Physical tests:**

**A. Ultraviolet Spectrophotometric Analysis:**

Chromatographically pure materials were dissolved in pure methanol and subjected to U.V. spectrophotometric investigation using Shimadzu UV-visible spectrophotometric UV- 240. In case of flavonoids, AlCl<sub>3</sub>/ HCl, NaOAc/H<sub>3</sub>BO<sub>3</sub> and NaOMe are used.

**B. <sup>1</sup>H - and <sup>13</sup>C Nuclear Magnetic Resonance Analysis (NMR):**

The NMR measurements were carried out on Bruker AMX- 500 ml, Varian Inova- 500 and/ or JEOL EX- 270 NMR spectrometer apparatus as described by Mabry *et al.*, (1970).

**C. Mass Spectrometric Analysis (MS):**

The mass spectra were conducted using mass spectrometer Varian Mat 711, Finnegan SSQ 7000 and MM 7070 E according to the method of (Mabry *et al.*, 1970).

**2.4. Chemical Reactions:**

**A. Controlled (mild) Acid Hydrolysis:**

The pure material was hydrolyzed using 0.1 N HCl under reflux for one hour. The resultants were traced chromatographically every 5 min. using comparative paper chromatography as stated by (Harborne *et al.* 1975).

**B. Complete (normal ) Acid Hydrolysis:**

The pure material was hydrolysed using 2N HCl under reflux for one hour. The released aglycone and sugar were subjected to comparative paper chromatography using authentic samples (Harborne *et al.*, 1975).

**2.5. Total Flavonoids:**

Estimation of the total flavonoids in the ethanolic extract of the *Thymus capitatus* was colourimetrically determined according to the method of ( Karawya and Aboutabl, 1982).

**2.6. Calibration curve : (Karawya and Aboutabl, 1982).**

Different aliquots of ethanolic solution of kaempferol equivalent to 5-200 µg were separately introduced into test tube, evaporated to dryness on a hot water bath (40- 50°C), 5 ml of 0.1 M aluminum chloride reagent were added. The absorbance of the colour developed was measured at 445 nm (wave length of maximum absorbance) against a blank. Three determinations for each concentration of standard solution were carried out.

**2.7. Estimation of Flavonoid Content:**

The powdered air- dried plant material (2 g) of *Thymus capitatus* was defatted with petroleum ether and extracted with 96% ethanol until exhaustion. The ethanolic extract was adjusted to 50 ml. Five ml aliquots were, separately, introduced into test tubes, evaporated to dryness on a water bath. Five ml of 0.1 M aluminum chloride were added. The absorbance

of the colour developed was measured at 445 nm. Three determinations for each concentration of standard solution were carried out.

### **3. Investigation of coumarins:**

#### **Extraction:**

750 gm of *Thymus capitatus* were defatted by petroleum ether (b.p. 40- 60°C) then extracted with ether until exhaustion in a soxhlet apparatus. The marc were left then dried until free from the solvent .The ether extract was dissolved in methanol and subjected to TLC analysis using precoated silica gel plates (Silica gel 60 GF<sub>254</sub>, Machery Nagel) using the following developing systems

S1. Chloroform : ethyl acetate (9 : 1, v/v)

S2. Chloroform : methanol (9 : 1 ,v/v)

S3. Hexane : ethyl acetate (9 : 1 , v/v)

S4. Benzen : ethyl acetate (8 : 2 ,v/v)

The chromatograms were examined in UV light before and after exposure to ammonia vapour.

## **RESULTS AND DISCUSSION**

### **1. Investigation of flavonoids:**

When the concentrated ethanolic extract of *Thymus capitatus* was applied on the top of cellulose column using water/ methanol system, column of *Thymus capitatus* gave six fractions . Each fraction was subjected to preparative paper chromatography using Whatman No. 3 , B:A:W system and finally purified on sephadex LH - 20 using methanol/ water system.

#### **1.1. Isolation and identification of compound (1):**

Compound (1) was obtained as yellow crystals, soluble in ethyl acetate and methanol, its R<sub>f</sub> values and colour reactions are recorded in table (1):

#### **UV spectrum:**

UV spectral data ,  $\lambda_{max}$  in MeOH and different shift reagents are recorded in table (2). Data showed that:

Band I in MeOH at (353 nm) and band II at (267 nm), indicates its flavonoid type with substitution at position 3. The remaining UV spectral data were found to be similar to that of kaempferol type compounds (Mabry *et al.* 1970).

#### **Acid hydrolysis analysis :**

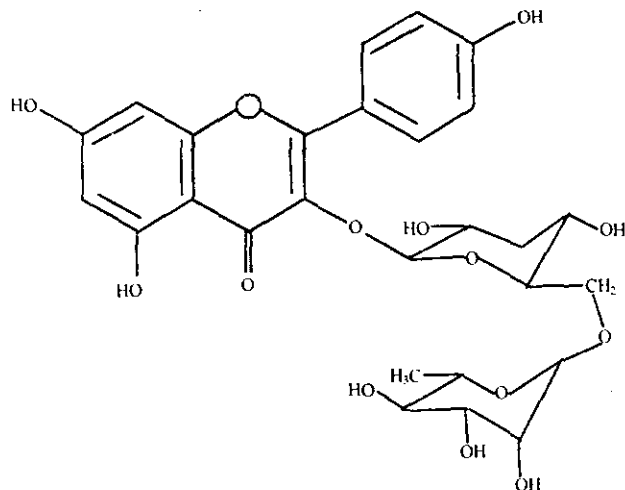
Compound (1) was subjected to acid hydrolysis using 0.1 N HCl(Harborne *et al.* 1975)., Kaempferol was detected as the aglycone and the sugar moiety was identified as glucose and rhamnose using TLC and solvent system ethyl acetate: MeOH : AcOH: water (65: 15: 15: 10) along with the authentic sugar reference.

#### **<sup>1</sup>H-NMR**

$\delta$  (ppm) at 8.1 (2 H, d, J = 8.7 Hz, H-2' and H-6' ) ,  $\delta$  6.8 (2 H, d, J = 8.7 Hz, H-3' and H-5' ) ,  $\delta$  6.4 (1 H, d, J = 1.5 Hz, H-8)  $\delta$  6.2 (1 H, d, J = 1.5 Hz, H-6) ,  $\delta$  4.4 (1 H, d, J = 2 Hz, H1'' rhamnose),  $\delta$  5.4. (1H, anomeric

proton, d, J = 7 Hz, H-1'' glucose),  $\delta$  3-4 (m, remaining sugar protons) and  $\delta$  0.9 (3 H, d, J = 6 Hz, CH<sub>3</sub> rhamnose).

UV spectrum showed kaempferol type with substitution at position 3, <sup>1</sup>H NMR gave signals similar to that of kaempferol besides the signals characteristic for rutinose sugar (Mabry *et al.* 1970) at 4.4 H<sup>1''</sup> rhamnose, 5.4. H-1'' glucose and doublet at 0.9 indicating the presence of methyl group on an aliphatic chain confirming the presence of rhamnose moiety. Also acid hydrolysis gave kaempferol as the aglycone and glucose and rhamnose as the sugar moieties, this also confirms that compound (1) is kaempferol-3-rutinoside.



**Kaempferol-3- rutinoside**

### 1.2. Isolation and identification of compound (2)

Elution of band (2) was performed, purified and subjected to TLC where one major spot of flavonoid nature was detected and the identification was illustrated with the following data; R<sub>f</sub> values and colour reactions are outlined in table (1).

#### Ultraviolet spectral analysis:

The flavonoid substance (compound 2), when subjected to UV spectral analysis in methanol and with shift reagents according to (Mabry *et al.* 1970), where the following results were obtained and summarized in table (2).

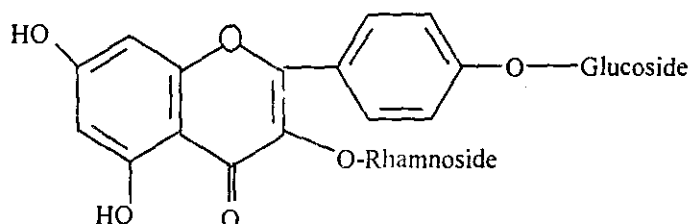
In methanol the band I at 328 nm and band II at 265 nm showed that the compound may be flavone or flavonol with a substituted at 3-hydroxyl group compared with the result of (Mabry *et al.* 1970). The bathochromic shift in band I with NaOMe (+62 nm) with an increase of intensity which indicated the presence of free OH group at 7 or 4' positions. The bathochromic shift in band I with addition of NaOAc (+57 nm) indicated the presence of free OH group at position 7, which was confirmed by the bathochromic shift of band II (+7 nm). With addition of NaOAc/ H<sub>3</sub>BO<sub>3</sub> no bathochromic shift in band I, which indicated the absence of ortho-dihydroxy groups at B- ring especially at 3' and 4' positions. The bathochromic shift in band I with addition of AlCl<sub>3</sub> (+47

nm) indicated the presence of free OH group at position 5 and/or orthodihydroxy group. After the addition of HCl no hypthochromic shift in band I in  $AlCl_3$  spectrum, which indicated the absence of ortho dihydroxy groups.

**$^1H$ -NMR spectral analysis:**

The  $^1H$ -NMR in DMSO showed that following signals at  $\delta$ ppm 6.2(1H,d,J=1-5Hz,H-6),  $\delta$ 6.5(1H-d,J=1.5Hz,H-8)  $\delta$ 6.8(2H,d,J=8-7Hz,H-3' and H-5'),  $\delta$ 7.95(2H,d,J=8-7Hz,H-2',H-6'),  $\delta$ 4.9(1H,d,J=2.5Hz,H-1'' glucose),  $\delta$ 4-1(1H,broad,J=2.5Hz,H 1'' rhamnose), CH<sub>3</sub> rhamnose (d, broad 0.8) and 3.5 (m, remaining sugar protons).

From the  $R_f$  value, colour reaction, UV spectral data and  $^1H$ -NMR spectral analysis, the compound (2) was confirmed as Kaempferol-3-O-rhamnoside-4'-O-glucoside



**Kaempferol-3-O-rhamnoside-4'-O-glucoside**

**1.3 Isolation and identification of compound (3)**

It was obtained as yellow crystals, its  $R_f$  values and colour reactions are recorded in table (1)

**UV spectrum:**

UV spectral data of compound (3) were recorded in table (2) .

The absorption maxima in methanol, band I at 350 nm, indicates that compound (3) is a flavonol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of quercetin type compounds.

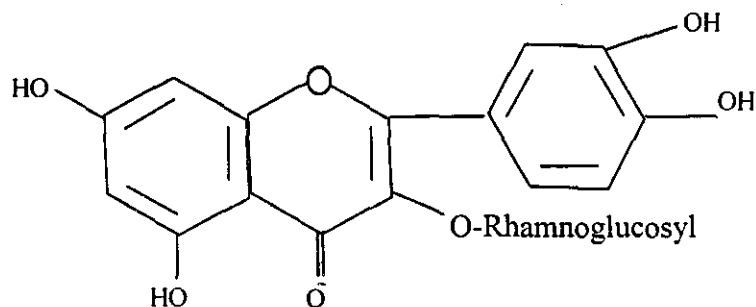
**$^1H$ -NMR spectrum:**

$^1H$ -NMR spectrum was, give the following signals :  $\delta$  (ppm) 7.6 (1 H, d, J = 2.5 Hz, H-2'),  $\delta$  7.5 (1 H, dd, J = 8.5, 2.5 Hz, H- $\delta$ ),  $\delta$  6.8 (1 H, d, J = 8 Hz, H-5')  $\delta$  6.4 (1 H, d, J = 1.5 Hz, H-8),  $\delta$  6.2 (1 H, d, J = 1.5 Hz, H-6),  $\delta$  5.3 (1 H, d, J = 8 Hz, H-1'' glucose),  $\delta$  4.5 (1 H, d, J = 2.5 Hz, H-1''-rhamnose),  $\delta$  3.4 (m, remaining sugar protons) and  $\delta$  0.8 ( 3 H, d , J= 6 Hz, CH<sub>3</sub> rhamnose).

**$^{13}C$ - NMR spectrum:**

$^{13}C$ - NMR spectrum declared the presence of substitution on C-3 beside the disaccharide nature of compound (3) and the CH<sub>3</sub> carbon of rhamnose was shown at 18.1 ppm, C-6 of glucose at 62.7 ppm so the linkage is (1 $\rightarrow$ 6) (Harborne *et al.* 1975)

$R_f$  values, colour reactions, UV,  $^1\text{H}$ - NMR and  $^{13}\text{C}$ - spectral data proved that compound(3) was rutin [quercetin 3-O- $\alpha$ -L-rhamnoside(1 $\rightarrow$ 6) $\beta$ -D-glucoside].



Rutin

#### 1.4 Isolation and identification of compound (4):

Elution of band No. (4) was performed, purified and subjected to TLC where one major spot of flavonoid nature was detected and the structure of the compound was confirmed by  $R_f$  values and colour reactions outlined in table (1).

#### (2) Ultraviolet spectral analysis:

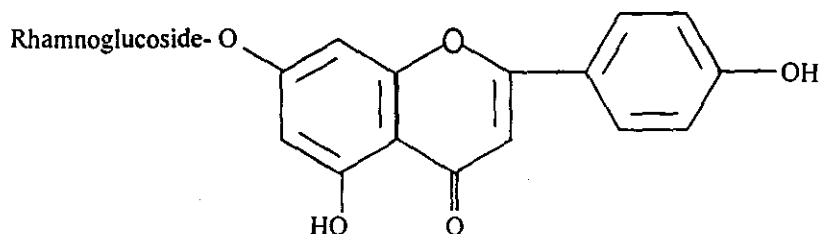
The flavonoid substance( compound 4 )was subjected to UV spectral analysis in methanol and with shift reagents according to (Mabry *et al.*, 1970), where the obtained results were summarized in table (2).

The band I at 330 nm and band II at 267 nm declared that the compound may be flavone or flavonol with a substituted 3- hydroxyl group as compared with the results of (Mabry *et al.* 1970). The bathochromic shift in band I with NaOMe (+66 nm) with an increase of intensity indicated the presence of free OH group at 7 or 4' positions. The bathochromic shift in band I with addition of NaOAc (+55 nm), while band II exhibit no shift, which indicated the presence of free OH group at position 4'. With addition of NaOAc/ $\text{H}_3\text{BO}_3$  no bathochromic shift in band II or I, which indicated the absence of orthodihydroxy groups at B-ring especially at 3' and 4' positions. The bathochromic shift in band I with addition of  $\text{AlCl}_3$  (+15 nm) indicated the presence of free OH group at position 5 and/or orthodihydroxy group. After the addition of HCl no hypthochromic shift in band I in  $\text{AlCl}_3$  spectrum, which indicated the absence of orthodihydroxy groups. From the colour reaction and UV spectral data this compound may be confirmed as apigenin derivative.

#### $^1\text{H}$ - NMR spectral analysis:

The  $^1\text{H}$ -NMR in DMSO showed the following signals:  $\delta$  6.7(1H,s,H-3) ,  $\delta$ 6.6( 1H,d,H-6) ,  $\delta$  6.8 (1H,d,J=8Hz,H-8) ,  $\delta$  7.7( 2H,d,J=2.5Hz,H-3' and H-5' ) ,  $\delta$  8.0(1H,d,J=2.5,H-6' ) ,  $\delta$ 0.8(3H,d,J=6Hz, $\text{CH}_3$  - rhaminose) ,  $\delta$ 5.2(1H,d,J=8Hz,H-1'glucoside) ,  $\delta$  4.2(d,H-1''rhaminose) and  $\delta$ 3.2-4(m, remaining sugar and proton).

From the  $R_f$  value, colour reaction, UV spectral data and  $^1\text{H-NMR}$  spectral analysis, the compound (4) was confirmed as apigenin-7-O-rhamanoglucoside.



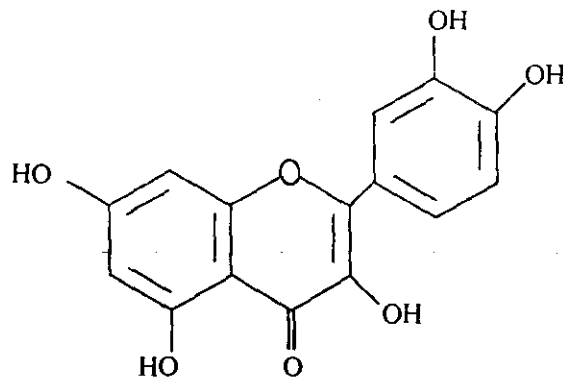
Apigenin-7-O-rhamanoglucoside

#### 1.5. Isolation and Identification of compound (5):

It was detected in the plant where,  $R_f$  values and its bright yellow colour reactions are outlined in table (1) which gave yellow colour under light (366 nm) and bright yellow colour under  $\text{NH}_3 + \text{UV}$ . Partial and complete acid hydrolysis of compound (5) revealed the presence of quercetin as aglycone and absence of any sugar moiety.

UV spectral data of compound (5) showed two major UV absorption bands in MeOH, band I at (370 nm) and band II at (255 nm), indicating that it is a flavonol with free hydroxyl group at 3-position. The addition of NaOMe resulted in a formation of new band at (321 nm) indicating the presence of free hydroxyl group at 7-position. A bathochromic shift in band I (+ 18 nm) by the addition of NaOAc/  $\text{H}_3\text{BO}_3$  relative to MeOH spectrum indicated the presence of hydroxyl group at 3'- and 4'-positions in  $\beta$ -ring. Addition of  $\text{AlCl}_3/\text{HCl}$  caused a hypochromic shift in band I (-30nm) relative to  $\text{AlCl}_3$  spectrum confirmed the presence of hydroxyl group at 3' and 4'- positions in  $\beta$ -ring.

$^1\text{H-NMR}$  spectral data of compound (5) showed singals at  $\delta$  7.6 (1H, d,  $J = 8.5$  Hz, H-2'),  $\delta$  7.5 (1H, d,  $J = 8.5$  Hz, H-6'),  $\delta$  6.89 (1H, d,  $J = 8.5$  Hz, H-5') and  $\delta$  6.4 (1H, d,  $J = 2.5$  Hz, H-6) and  $\delta$  6.2 (1H, d,  $J = 2.5$  Hz, H-8).



Quercetin

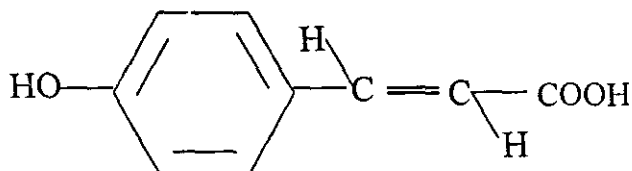


From  $R_f$  values, UV and  $^1\text{H-NMR}$  spectral data, the isolated compound (5) was identified as quercetin.

#### 1.6. Isolation and Identification of compound (6)

Compound (6) was obtained as white powder, soluble in methanol and acetone. The  $R_f$  values and colour reaction of compound (6) were outlined in table (1) indicated that it has a phenolic nature. The compound was directly compared along with an authentic sample of *p*- coumaric acid in two solvent systems using paper chromatography. From the  $R_f$  values and colour reaction the isolated compound (6) can be identified as *p*- coumaric acid.

Compound (6) was obtained as white powder, soluble in methanol and acetone



*P-coumaric acid*

## 2. Isolation and identification of coumarin:

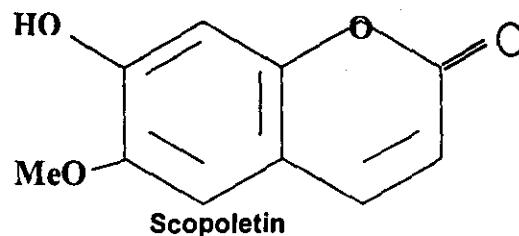
The ether extract was subjected to TLC, yielded 3 main spots using solvent system  $S_2$  and  $S_4$ . Using preparative TLC afforded fractions (1-3). Similar fractions were pooled together and combined to give three major collective fractions (1-3). The eluted solvent, was evaporated under reduced pressure. The individual residues, were separately dissolved in methanol and re- examined by TLC.

### 1. Identification of Coumarin(1):

Compound (1) soluble in chloroform and methanol, m.p. 204- 206 °C,  $R_f$  and colour reaction in solvent system  $S_2$  (Table 3) .was purified on several sephadex columns using methanol. UV spectrum showed  $\lambda_{\text{max}}$  in MeOH at 254 sh, 296 and 348. Addition of NaOAc gave 276 sh and 388 nm are similar to those reported by Murray *et al.*, ( 1982) for 7 hydroxy- 6-methoxycoumarin compound with free OH at position 7.  $^1\text{H-NMR}$  spectrum  $\delta$  ppm (DM SO-  $d_6$ ) at  $\delta$  8 ( 1H, d, J = 9 Hz, H-4),  $\delta$  7.2 (1H, s, H-5),  $\delta$  6.75 (1H,s,H-8),  $\delta$  6.2. (1H, d, J = 9 Hz, H-3) and  $\delta$  3.8 (3 H,s, OCH<sub>3</sub>). The presence of pair of doublets at  $\delta$ 8 and  $\delta$  6.2 ppm indicates that C1 is unsubstituted coumarin (Pyranone ring).

Mass spectrum : m/z (rel. int %) peak at 192 (100%) [ $M^+$ ], other peaks at 177 (59%) [ $M^+ - \text{CH}_3$ ], 164 (32%) [ $M^+ - \text{CO}$ ], 149 (61%), 121 (35%) [ $149 - \text{CO}$ ], 79 (35.3%), 69 (60%).

From the previous data comparing with (Paris *et al.*, 1975) and the (Merck index, 2001) coumarin compound (1) could be identified as 7-hydroxy - 6 - methoxycoumarin (Scopoletin).



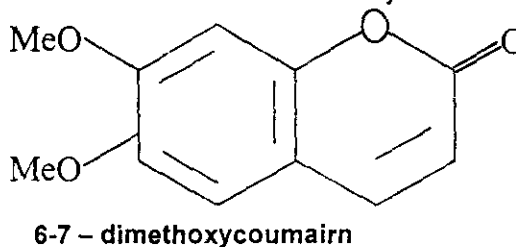
## 2. Identification of coumarin compound (2):

From Table 3, it can be suggested that Compound (2) soluble in methanol.  $R_f$  value 0.5 using solvent  $S_4$  (Murray *et al.*, 1982).

UV  $\lambda_{max}$  nm in MeOH at : 344, 295, 260 (sh), 252 (sh), 228.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) spectrum showed the following signals at  $\delta$  ppm at  $\delta$  6.2 (1H, d,  $J = 9.5$  Hz, H-3)  $\delta$  7.8 (1H, d,  $J = 9.5$  Hz, H-4),  $\delta$  7.2 (1H, s, H-4),  $\delta$  6.9 (1H, s, H-8),  $\delta$  3.9 (6 H, s, methyl group) and 3.8 (7 H, s, methyl group).

El/ Mass:  $m/z$  (rel. int%), molecular ion peak, base peak at 206 (100%), 191(28%), 163 (27%), 178 (12%) and 135 (12%)

From the previous data and comparing with the (Merck Index, 2001), the coumarin compound (2) could be identified as 6-7- dimethoxycoumarin .



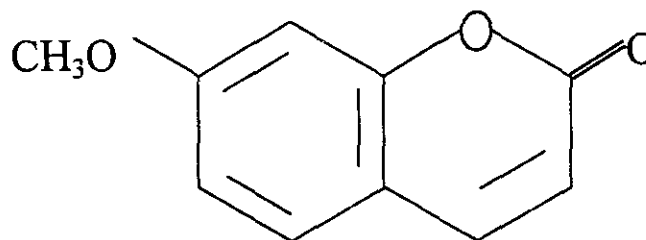
## Identification of coumarin compound (3):

Compound 3, soluble in methanol, chloroform, ether but insoluble in water.  $R_f = 0.78$  in solvent system  $S_4$  and colour. (Table 3) (Murray *et al.*, 1982). m.p. 117- 118°C.

UV spectrum  $\lambda_{max}$  nm in MeOH at : 216, 242 and 320 which is characteristic for simple coumarin and not affected by addition of alkali.

Mass spectra  $m/z$  (rel. int %), 176 (71.3%) [ $M^{+1}$ , 148 (76%)] [ $M^{+}-\text{CO}$ ], 133 (100%) [148- $\text{CH}_3$ ], 105 (18%) [133-CO], 102 (22%) [133- $\text{OCH}_3$ ] 77 (88.5%) and 51 (61%).

From the previous data, comparison with the published data (Murray *et al.*, 1982) and Paris *et al.*, 1975) it was suggested that compound 3 may be herniarin.



Herniarin

### 3. Estimation of total flavonoids :

The percentage of total flavonoids reached 0.30% in *Thymus capitatus*.

Table (1):  $R_f$  values and color reactions of the isolated flavonoid compounds.

Compounds	$R_f$ values		Color reactions	
	BAW	15% HOAc	U.V	UV. + NH <sub>3</sub>
Kaempferol- 3 - rutinoside	0.34	36	Brown	Yellow
kaempferol-3-O-rhamnoside4-O-glucoside	0.35	0.65	Deep purple	Yellow
Rutin	0.55	0.62	Purple	Yellow
Apigenin-7-O-rnamano glucoside	0.63	0.20	Deep purple	Yellowish Green
Quercetin	0.71	0.30	Yellow	Bright Yellow
P-coumaric acid	0.92	0.65	Pale blue	Mauve

Table (2): UV- spectral data ( $\lambda_{max}$  nm) of the isolated flavonoid compounds.

Compounds	Reagents					
	MeOH	NaOMe	NaOAc	NaOAc+ H <sub>3</sub> BO <sub>3</sub>	AlCl <sub>3</sub>	AlCl <sub>3</sub> +HCl
kaempferol-3-rutinoside	267, 320, 353	272, 320, 400	270, 310, 382	260, 370	266, 355, 422	266, 298, 422
kaempferol-3-o-rhamnoside-4-O-glucoside	328,265	390,265	385,272	328,272	375,295	375,295
Rutin	257(sh), 267, 295, 350	272, 315, 405	268, 300, 365	268, 300, 365	272, 302, 335, 405	272, 302, 345, 400
Apigenin-7-O-rhamnoglucoسدie	330, 267	396, 273	385, 270	337, 270	345, 276	345, 275
Quercetin	255, 269 (sh), 301(sh), 370	247 (sh), 321	257 (sh), 274, 329, 390	261, 306 (sh), 388	272, 304(sh), 333, 458	265, 301 (sh), 359, 428

sh =Shoulder

Table (3): Results of TLC investigation of the ether extract of *Thymus capitatus*

Coumarin Compounds	R <sub>f</sub>		Colour	
	S <sub>2</sub>	S <sub>4</sub>	UV	UV+ NH <sub>3</sub>
1	0.72		Blue	Blue
2		0.5	Blue	Bluish violet
3		0.78	Fluorescence violet	Intensified

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فلافونيدات وكومارينات نبات ثيمس كابيتاتوز

حنونه سامى يعقوب - ناهد عبد الرؤوف السيد سالم

مركز بحوث الصحراء - المطرية- القاهرة

تم فصل وتنقية ستة مركبات فلافونيدية وثلاث مركبات من الكومارينات بنبات ثيمس كابيتاتوز. المركبات الفلافونيدية هي كامفيرول -3- ريتنوزيد وكامفيرول -3- رامنوزيد -0-3- رامنوزيد -0-4- جلوكوزيد وريوتين وبيجينين -0-7- رامنوجلوكونيد وكوارستين وكوماريك.

بينما مركبات الكومارين هي سكوپولتين و٦، ٧ داى ميثوكسى كومارين وهيريتيرين وقد تم التعرف على هذه المركبات من خلال طرق التحليل الكيمائى المختلفة وتحليل أطيف الأشعة فوق بنفسجية ودراسة طيف الرنين المغناطيس لانونية الهيدروجين وانونية الكربون وقياس طيف الكتلة وكانت نسبة الفلافونيدات الكلية فى النبات ٠,٣٠%.