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## New constituents of *Artemisia monosperma* Del.

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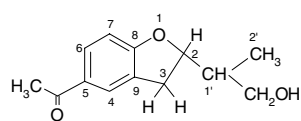
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Phytochemical investigation of air-dried powdered roots of *Artemisia monosperma* growing in Egypt afforded two new compounds; 6-hydroxy-7,8-dimethoxycoumarin (**I**) and 5-acetyl-2-[1'-(hydroxymethyl)ethyl]-2,3-dihydrobenzo[*b*]furan (**IV**), in addition to the known compounds; 6-hydroxy-5,7-dimethoxycoumarin (fraxinol) (**II**), 5-hydroxy-6,7-dimethoxycoumarin (tomentin) (**III**) and methyl- $\beta$ -D-fructofuranoside (**V**), obtained for the first time from the plant. Chemical structures of the isolated compounds were assigned based on different physical, chemical and spectroscopic techniques including UV, IR, MS, 1D- and 2D-NMR spectra. Furthermore, antimicrobial activity of different extracts of roots was carried out.

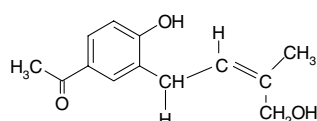
### 1. Introduction

Several secondary metabolites of different chemical nature have been isolated from different *Artemisia* species including sesquiterpenes (Yoshikawa et al. 1996), flavonoids (Saleh et al. 1987; Abu-Niaaj et al. 1993) and coumarins (Al-Hazimi et al. 1992). The isolated compounds or extracts of these plants have been proven to exhibit various pharmacological activities including cytotoxic (Zheng 1994), antiviral (Tan et al. 1998), and hepatoprotective ef-

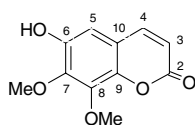
fects (Kiso et al. 1984). In this respect, it was decided to carry out a phytochemical investigation of *Artemisia monosperma* Del.; a well-known wild plant growing in Egypt. This report describes the phytochemical investigation of roots of *A. monosperma* with the aim of finding active compounds that could be of biological interest. The present study led to the isolation and structure elucidation of two new compounds from the roots of *A. monosperma* and were identified as: 6-hydroxy-7,8-dimethoxycoumarin (**I**) and 5-acetyl-2-[1'-(hydroxymethyl)ethyl]-2,3-dihydrobenzo[*b*]furan **IV**, in addition to the known compounds: 6-hydroxy-5,7-dimethoxycoumarin (fraxinol) (**II**), 5-hydroxy-6,7-dimethoxycoumarin (tomentin) (**III**) and methyl- $\beta$ -D-fructofuranoside (**V**). Structure elucidation of the isolated compounds was deduced based on the application of different spectroscopic techniques including UV, IR, EIMS, 1D- and 2D-NMR. Furthermore, antimicrobial activity of different extracts of *A. monosperma* roots was studied.



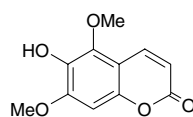
(I)



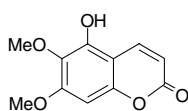
3-(2-hydroxymethyl-2-buten-4-yl)  
4-hydroxyacetophenone



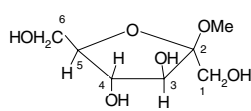
(II)



(III)



(III)



(V)

### 2. Investigations, results and discussion

#### 2.1. Compounds discussion

The bluish green fluorescence in UV light intensified to yellowish green after addition of alkali suggested the presence of a coumarin chromophore in **I–III** (Wagner et al. 1996). Their phenolic nature was confirmed by  $\text{FeCl}_3$  test solution and IR spectra, that illustrated also absorption bands due to C=O groups. Combined spectral data indicated their molecular formulae to be  $\text{C}_{11}\text{H}_{10}\text{O}_5$  ( $m/z$  222,  $[\text{M}]^+$ ).  $^{13}\text{C}$  NMR and DEPT spectra of **I** showed the presence of 11 carbon signals as follow: 2  $\text{CH}_3$ , 3 CH and 6 quaternary carbons of which one is due to a carbonyl carbon observed at  $\delta$  159.69. The  $^1\text{H}$  NMR spectrum revealed the presence of a pair of doublets at  $\delta$  6.21 and 7.88 ( $J = 7.1$  Hz) attributed to protons at positions 3 and 4

of the  $\alpha$ -pyrone nucleus, respectively. The presence of one-proton singlet in the aromatic region at  $\delta$  6.09, pointed to the occurrence of a tri-substituted ring A. The  $^1\text{H}$  NMR spectrum revealed also the presence of three singlets, one at  $\delta$  6.09; exchangeable with  $\text{D}_2\text{O}$ ; for a hydroxyl group, and two signals at  $\delta$  3.99, 3.91 assigned for two methoxyl groups. A HMQC experiment correlated these two signals to carbons at  $\delta$  60.67 and 62.46, while HMBC correlated them to carbons at  $\delta$  133.3 (C-7), 143.57 (C-8), respectively. The absence of the long range coupling of H-4 and the aromatic signal indicated a substituted C-8 (Kayser and Kolodziej 1995). In addition, the arrangement of the methoxyl groups on the coumarin nucleus was confirmed from  $^{13}\text{C}$  NMR spectral data (Roitman and James 1985), as their chemical shifts ( $\delta$  60.67, 62.46) established the presence of substituents at the *ortho* positions of each of the methoxyl groups, consistent with the structure depicted for **I** to be: 6-hydroxy-7, 8-dimethoxycoumarin. According to the available literature, **I** appears to be a new coumarin obtained for the first time from a natural source. Compound **I** was only obtained by synthesis before (Wagner and Bladt 1975).

Similar to **I**, the spectral data of **II** and **III** showed the presence of common features characteristic for tri-substituted coumarin derivatives. The  $^1\text{H}$  NMR spectrum of each compound revealed the presence of a typical AB system for H-3 and H-4, and one aromatic singlet, in addition to the presence of two methoxyls and one hydroxyl group. However, a thorough investigation of NMR spectra indicated discriminative differences in the substitution pattern of the hydroxyl and methoxyl groups in ring A. Observation of the long range coupling between H-4 and the aromatic singlet, in **II** and **III**, confirmed that C-8 is unsubstituted in both compounds (Kayser and Kolodziej 1995), and hence, 5,6,7-oxygenation pattern (Wagner and Bladt 1974). Supporting evidence for the arrangement of the methoxyl groups on the coumarin nucleus was available from  $^{13}\text{C}$  NMR spectral data (Roitman and James 1985). The chemical shift of one of the methoxyl group at ( $\delta$  ca 61.3), clearly established the presence of substituents *ortho* positions. On the other hand, the second methoxyl group should be flanked by at least one unsubstituted carbon ( $\delta$  ca 56.2). The HMBC spectrum of **II** showed a strong correlation between the two methoxyl groups at  $\delta$  4.02, 3.83 and the carbon signals at  $\delta$  148.15(C-5), 137.30(C-7), respectively, while the HMBC spectrum of **III** showed a strong correlation between the two methoxyl groups at  $\delta$  3.74, 3.90 and the carbon signals at  $\delta$  131.57(C-6), 155.63(C-7), respectively. Spectral data of **II** were found to be compatible with the occurrence of 6-hydroxy-5,7-dimethoxycoumarin (fraxinol) (Kostova 1992). On the other hand, **III** was identified as 5-hydroxy-6,7-dimethoxycoumarin (tomentin) (Wu et al. 2001).

The chemical structure of **IV** could be depicted to have the molecular formula  $\text{C}_{13}\text{H}_{16}\text{O}_3$  through EIMS ( $[\text{M}]^+$ ,  $m/z$  220) and other spectral analyses. The IR spectrum showed absorption bands corresponding to the presence of  $\text{C}=\text{O}$  ( $1704\text{ cm}^{-1}$ ) and  $\text{OH}$  ( $3322\text{ cm}^{-1}$ ) groups. The  $^{13}\text{C}$  NMR spectrum revealed the presence of 13 well-resolved carbon signals sorted as follows: 6 aromatic carbons, 6 aliphatic carbons and one carbonyl signal. The presence of a ketomethyl moiety, as an integral part of **IV**, was evident from the  $^1\text{H}$  NMR spectrum ( $\delta$  2.53, 3H) and from the  $^{13}\text{C}$  NMR spectrum ( $\delta$  26.42, 196.72). This pointed to a prenylated benzenoid derivative containing an acetyl group. The presence of a dihydrofuran moiety was confirmed by the appearance of the two proton signals at  $\delta$  3.08 (1H,

**Table 1: The most significant long range correlations HMBC of IV**

$\delta\text{H}$ , ppm	HMBC
5.04 (H-2)	10.73 (C-2'), 65.15 ( $\underline{\text{C}}\text{H}_2\text{OH}$ ).
3.08, 3.31 (2H-3)	40.53 (C-1'), 108.69 (C-4), 164.09 (C-8).
7.84 (H-4)	164.09 (C-8), 196.72 ( $\underline{\text{C}}\text{OCH}_3$ ).
7.80 (H-6)	164.09 (C-8), 196.72 ( $\underline{\text{C}}\text{OCH}_3$ ).
6.78 (H-7)	127.67 (C-9), 130.47 (C-5).
2.07 (H-1')	32.38 (C-3).
0.99 (H-2')	65.15 ( $\underline{\text{C}}\text{H}_2\text{OH}$ ), 85.48 (C-2).

dd,  $J = 6.9, 13.5$  Hz) and 3.31 (1H, dd,  $J = 7.5, 13.5$  Hz) with their carbon observed at  $\delta$  32.38 (C-3), in addition to one-proton multiplet at  $\delta$  5.04 along with its carbon resonating at  $\delta$  85.48 (C-2) (Bohlmann et al. 1979; Hansel et al. 1980). The  $^1\text{H}$  NMR spectrum showed the presence of an aromatic ABX system as indicated by the presence of three signals at  $\delta$  6.78 (d,  $J = 7.5$  Hz), 7.80 (dd,  $J = 7.5, 2$  Hz) and 7.84 (d,  $J = 2$  Hz) characteristic for a tri-substituted benzenoid structure. The location of the ketomethyl group was established at position 5 based on biogenetic (Proksch and Rodriguez 1983) and spectroscopic evidence (HMBC, Table 1). The  $^1\text{H}$  NMR spectrum indicated also the presence of a doublet at  $\delta$  0.99 (3H,  $J = 7$  Hz) for a methyl group (H-2') together with two double doublets at  $\delta$  3.70 (1H,  $J = 6.1, 12$  Hz) and 3.77 (1H,  $J = 7, 12$  Hz) for the gem protons of a hydroxymethyl group. In addition, the  $^1\text{H}$  NMR spectrum showed a multiplet at  $\delta$  2.07 due to H-1'. Extensive study of HMQC and HMBC experiments were helpful to assign all the signals. The recorded data are in full agreement with the suggested chemical structure of **IV** to be: 6-acetyl-2-[1'-(hydroxymethyl)ethyl]-2,3-dihydrobenzo[*b*]furan. It is to be mentioned that, this new compound is biogenetically related to the acetophenone derivative (3-(2-hydroxymethyl-2-buten-4-yl)4-hydroxy-acetophenone), previously isolated from the same plant (Hammouda et al. 1978).

The structure of **V** was identified as methyl- $\beta$ -D-fructofuranoside based on extensive study of MS and NMR analyses. Compound **V** gave a positive Fehling's test after acid hydrolysis, indicating its glycosidic nature. MS revealed the presence of a molecular ion peak at  $m/z$  194, in accordance with the molecular formula  $\text{C}_7\text{H}_{14}\text{O}_6$ . The  $^{13}\text{C}$  NMR spectrum was in agreement with the suggested molecular formula, as it showed the presence of 7 well resolved carbon signals. DEPT experiments indicated they are: 1  $\text{CH}_3$ , 2  $\text{CH}_2$ , 3  $\text{CH}$  and one quaternary carbon. The presence of an anomeric carbon at  $\delta$  103.94 and the absence of the correspondence proton indicate the ketonic nature of **V**. The chemical shifts in the  $^{13}\text{C}$  NMR spectrum of **V** pointed to furanose form (Kalinowsti et al. 1984). The HMBC experiment revealed a strong H-C correlation between the methyl protons at  $\delta$  3.25 and the anomeric carbon at  $\delta$  103.94, indicated the location of the  $\text{OCH}_3$  at position 2. The down-shift of C-2 confirmed the above conclusion. Thus, **V** is identified as methyl- $\beta$ -D-fructofuranoside.

## 2.2. Chemotaxonomic significance

The presence of benzofuran derivatives in Asteraceae species is of chemotaxonomic importance as they were reported to be useful as chemotaxonomical markers at the tribal and generic levels with a similar situation to sesquiterpene lactones (Proksch and Rodriguez 1983). The isola-

**Table 2: MIC of the different extracts of roots of *A. monosperma***

Organism	MIC in µg/ml			
	Total alcohol ext.	Chloroform ext.	Ethyl acetate ext.	Butanol ext.
E <sub>22</sub>	1000	1000	1000	1000
E <sub>33</sub>	1000	500	1000	1000
K <sub>36</sub>	500	500	500	500
K <sub>37</sub>	1000	500	1000	1000
Sa <sub>1</sub>	1000	1000	1000	>1000
Se <sub>10</sub>	>1000	1000	>1000	>1000
Ps <sub>79</sub>	1000	500	1000	500
Ps <sub>80</sub>	500	500	500	500
C <sub>1</sub>	500	500	500	500
C <sub>2</sub>	500	500	500	500

– The organisms were clinical isolates from different Egyptian patients of Alexandria University Hospital, Egypt.

– E: *Escherichia coli* K: *Klebsiella pneumoniae*  
 Sa: *Staphylococcus aureus* Se: *Staphylococcus epidermidis*  
 Ps: *Pseudomonas aeruginosa* C: *Candida albicans*

tion of the new compound **IV** could pave the way for further studies in this concern.

### 2.3. Antimicrobial activities

The minimal inhibitory concentration (MIC) of different extracts of *A. monosperma* roots was determined (Table 2). The chloroform extract showed the best results regarding the antimicrobial activity followed by the ethyl acetate extract, in comparison with other examined extracts. Accordingly, chloroform and ethyl acetate extracts need to be reexamined using a narrower concentration range against the same tested organisms, this study is currently underway.

## 3. Experimental

### 3.1. Equipment and methods

UV spectra were recorded on a Perkin Elmer Double Beam Spectrophotometer Model 550S. IR spectra were determined on a Jasco Infrared Spectrophotometer, Model (FT/IR-300E), in KBr pellets. <sup>1</sup>H and <sup>13</sup>C NMR spectra (δ, CDCl<sub>3</sub> [compounds **I, II**] and DMSO [compounds **III-V**]) were recorded on a Bruker instrument at 360 and 90 MHz, respectively. MS spectra were obtained using Finningan Mass Spectrometer, Model SS Q/7000. Silica gel for CC and silica gel GF<sub>254</sub> TLC (Merck) were used. Solvents used in UV measurements were chromatographic grade (BDH, Analar, UK).

### 3.2. Plant material

*Artemisia monosperma* Del. was collected from the Borg El-Arab region, 60 km west to Alexandria, in March 2004. The plant was identified by direct comparison with an authentic sample kept at the Department of Botany and Microbiology, Faculty of Science, University of Alexandria, Egypt.

### 3.3. Extraction and isolation

Six kg air-dried powdered roots of *A. monosperma* were extracted exhaustively with a mixture of petrol-eth.-MeOH (1:1:1) at room temperature. The dried extract (277 g) was fractionated successively with petrol, CHCl<sub>3</sub>, EtOAc and *n*-BuOH. The CHCl<sub>3</sub> extract (25.5 g) was chromatographed on a silica gel column (765 g, 5.5 cm in diameter) using petrol and CHCl<sub>3</sub> mixtures as eluting solvents. Repeated washing with CH<sub>3</sub>OH of the deposit obtained from fractions eluted with 90% CHCl<sub>3</sub> in petrol, afforded 20 mg of white amorphous powder designated as compound **I**. The mother liquor of these fractions was subjected to a pTLC using solvent system (CHCl<sub>3</sub>-MeOH, 9:1) and afforded 6 mg of compound **I** and 7 mg of a yellowish white powder designated as compound **II**. Fractions eluted with 95% CHCl<sub>3</sub> in petrol, afforded 17 mg of a yellow powder designated as compound **III**.

EtOAc extract of the roots (9 g) was chromatographed on a silica gel column (270 g, 3.5 cm in diameter) using CHCl<sub>3</sub> and EtOAc mixtures as elut-

ing solvents. Fractions eluted with 50% EtOAc in CHCl<sub>3</sub>, afforded 13 mg of a pale orange powder, designated as compound **IV**. Fractions eluted with 70% EtOAc in CHCl<sub>3</sub>, afforded 14 mg of a white powder, designated as compound **V**.

### 3.4. Compounds characterization

Compound **I** was obtained as a white amorphous powder. UV λ<sub>max</sub>, nm (abs.) MeOH: 260 (0.50), 325 (0.60). IR ν<sub>max</sub> cm<sup>-1</sup>: 3435 (O–H), 1726 (C=O), 1641 (C=C), 1053 (C–O). <sup>1</sup>H NMR: δ 6.21 (1H, d, J = 7.1 Hz, H-3), 7.88 (1H, d, J = 7.1 Hz, H-4), 6.09 (1H, s, H-5), 6.09 (1H, s, C-6-OH), 3.99 (3H, s, C-7-OCH<sub>3</sub>), 3.91 (3H, s, C-8-OCH<sub>3</sub>). <sup>13</sup>C NMR: δ 159.69 (C-2), 113.25 (C-3), 139.14 (C-4), 103.13 (C-5), 138.88 (C-6), 133.3 (C-7), 60.67 (C-7-OCH<sub>3</sub>), 143.57 (C-8), 62.46 (C-8-OCH<sub>3</sub>), 149.29 (C-9), 108.63 (C-10). EI/MS m/z (rel. int.): 222 (50), 149 (18), 119 (10), 109 (20), 77 (17).

Compound **II** was obtained as yellowish white powder. UV λ<sub>max</sub>, nm (abs.) MeOH: 270 (0.35), 330 (0.48). IR ν<sub>max</sub> cm<sup>-1</sup>: 3430 (O–H), 1723 (C=O), 1635 (C=C), 1065 (C–O). <sup>1</sup>H NMR: δ 6.25 (1H, d, J = 7.1 Hz, H-3), 7.93 (1H, d, J = 7.1 Hz, H-4), 4.02 (3H, s, C-5-OH), 6.09 (1H, s, C-6-OH) 3.83 (3H, s, C-7-OCH<sub>3</sub>), 6.62 (1H, s, H-8). <sup>13</sup>C NMR: δ 158.50 (C-2), 112.98 (C-3), 138.32 (C-4), 148.15 (C-5), 61.24 (C-5-OCH<sub>3</sub>), 130.69 (C-6), 137.30 (C-7), 56.33 (C-7-OCH<sub>3</sub>), 95.52 (C-8), 151.50 (C-9), 107.20 (C-10). EI/MS m/z (rel. int.): 222 (50), 149 (18), 119 (10), 109 (20), 77 (17).

Compound **III** was obtained as yellow powder. UV λ<sub>max</sub>, nm (abs.) MeOH: 230 (1.50), 322 (1.55). IR ν<sub>max</sub> cm<sup>-1</sup>: 3322 (O–H), 1704 (C=O), 1666 (C=C) 1022 (C–O). <sup>1</sup>H NMR: δ 6.12 (1H, d, J = 9.5 Hz, H-3), 7.97 (1H, d, J = 9.5 Hz, H-4), 9.8 (1H, s, C-5-OH), 3.74 (3H, s, C-6-OCH<sub>3</sub>), 3.90 (3H, s, C-7-OCH<sub>3</sub>), 6.45 (1H, s, H-8). <sup>13</sup>C NMR: δ 161.48 (C-2), 111.76 (C-3), 138.62 (C-4), 145.68 (C-5), 131.57 (C-6), 61.39 (C-6-OCH<sub>3</sub>), 155.63 (C-7), 56.23 (C-7-OCH<sub>3</sub>), 92.37 (C-8), 151.78 (C-9), 102.56 (C-10). EI/MS m/z (rel. int.): 222 (45), 149 (19), 135 (18), 109 (18).

Compound **IV** was obtained as pale orange powder. UV λ<sub>max</sub>, nm (abs.) MeOH: 212 (2.96), 276 (1.01), 300 (sh.). IR ν<sub>max</sub> cm<sup>-1</sup>: 3322 (O–H), 1704 (C=O), 1621 (C=C). <sup>1</sup>H NMR: δ 2.07 (1H, m, H-1'), 0.99 (1H, d, J = 7.0, H-2'), 2.53 (3H, s, COCH<sub>3</sub>), 3.70 (1H, dd, J = 6.1, 12 Hz, CH<sub>2</sub>OH), 3.77 (1H, dd, J = 7, 12 Hz, CH<sub>2</sub>OH), 5.04 (1H, m, H-2), 3.08 (1H, dd, J = 6.9, 13.5 Hz, H-3α), 3.31 (1H, dd, J = 7.5, 13.5 Hz, H-3β), 7.84 (1H, d, J = 2 Hz, H-4), 7.80 (1H, dd, J = 7.5, 2 Hz, H-6), 6.78 (1H, d, J = 7.5 Hz, H-7). <sup>13</sup>C NMR: δ 40.53 (C-1'), 10.73 (C-2'), 26.42 (COCH<sub>3</sub>), 196.72 (C=O), 65.15 (CH<sub>2</sub>OH), 85.48 (C-2), 32.38 (C-3), 108.69 (C-4), 130.47 (C-5), 130.64 (C-6), 125.47 (C-7), 164.09 (C-8), 127.67 (C-9), EI/MS m/z (rel. int.): 220(5), 167(45), 113(20), 71(43), 55(75).

Compound **V** was obtained as a white powder. <sup>1</sup>H NMR: δ 3.42 (2H, m, H-1), 4.05 (1H, d, J = 5 Hz, H-3), 3.85 (1H, t, H-4), 3.62 (1H, m, H-5), 3.48 (2H, m, H-6), 3.25 (3H, s, OCH<sub>3</sub>). <sup>13</sup>C NMR: δ 60.95 (C-1), 103.94 (C-2), 76.65 (C-3), 75.26 (C-4), 81.92 (C-5), 62.60 (C-6), 48.61 (OCH<sub>3</sub>). EI/MS m/z (rel. int.): 194 (10), 180 (5), 149 (18), 73 (35), 56 (100).

### 3.5. Antimicrobial activity

The prepared sterile herbal extract was serially twofold diluted with sterile distilled water in sterile test tubes to a final volume of 5 ml. Each dilution of the herbal extract was aseptically well mixed with 5 ml double strength sterile molten agar which was then poured in sterile Petri dishes (90 mm in diameter). A control plate was carried out side by side with the experiment by replacing the 5 ml of the extract with sterile distilled water. The plates were allowed to set for complete solidification of the medium.

The microorganisms under test were inoculated in sterile nutrient broth and incubated at 37 °C for 18 h. The microbial culture was 10-fold diluted using sterile saline, and aliquots of 10 µl of the dilution were then dropped onto the surface of the dried plates using a micropipette fitted with sterile disposable plastic tips. The inoculated plates were allowed to stand at room temperature until the drops were absorbed and incubated in an inverted position at 37 °C for 24 h.

The plates were then visually inspected for microbial growth and the MIC was taken as the lowest concentration of the herbal extract resulting in a complete inhibition of the growth (Jennifer 2001).

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## References

- Abu-Niaaj L, Abu-Zarga M, Sabri S, Abdalla S (1993) Isolation and biological effects of 7-O-methylerythrodityol, a flavanone isolated from *Artemisia monosperma* on rat isolated smooth muscles. *Planta Med* 59: 42–45.

- Al-Hazimi HMG, Basha RM, Al-Zoman MM (1992) A *p*-coumaric acid and flavonoids from *Artemisia monosperma*. *Oriental J Chem* 8: 203–207.
- Bohlmann F, Suwita A, Robinson H, King RM (1979) Ein neues Toxol-Derivat aus *Austrobrickellia patens*. *Phytochemistry* 18: 2055.
- Hammouda FM, Rizk AM, Ismail SI, Hassan NM (1978) Isolation of an acetophenone derivative and coumarins from *Artemisia monosperma* Del. *Fitoterapia* 49: 53–55.
- Hansel R, Cybulski E-M, Cubukcu B, Mericli AH, Bohlmann F, Zdero, C (1980) Neue Pyron-Derivate aus *Helichrysum*-Arten. *Phytochemistry* 19: 639–644.
- Jennifer MA (2001) Determination of MIC. *Journal of antimicrobial Chemotherapy* 48, Suppl. 51, 5–16.
- Kalinowski HO, Burger S, Braun S (1984) <sup>13</sup>C Spektroskopie; George Thieme Verlag, Stuttgart, New York.
- Kayser O, Kolodziej H (1995) Highly oxygenated coumarins from *Pelargonium sidoides*. *Phytochemistry* 39: 1181–1185.
- Kiso Y, Ogasawara S, Hirota K, Watanabe N, Oshima Y, Konno C, Hikino H (1984) Antihepatotoxic principles of *Artemisia capillaris* Buds. *Planta Med* 50: 81–85.
- Kostova I (1992) Hydroxycoumarins from *Fraxinus ornus* bark. *Planta Med* 58: 484.
- Proksch P, Rodríguez E (1983) Chromones and benzofurans of the Asteraceae, their chemistry and biological significance. *Phytochemistry* 22: 2335–2348.
- Roitman JN, James LF (1985) Chemistry of toxic range plants highly oxygenated flavonol methyl ethers from *Gutierrezia microcephala*. *Phytochemistry* 24: 835–848.
- Saleh NAM, El-Negoumy SI, Abou-Zaid MM (1987) Flavonoids of *Artemisia judaica*, *A. monosperma* and *A. herba-alba*. *Phytochemistry* 26: 3059–3064.
- Tan RX, Zheng WF, Tang HQ (1998) Biologically active substances from the genus *Artemisia*. *Planta Med* 64: 295–302.
- Wagner H, Bladt S (1974) New coumarin from *Pelargonium reniforme*. *Tetrahedron Lett*: 3807–3808.
- Wagner H, Bladt S (1975) Coumarine aus Südafrikanischen *Pelargonium*-Arten. *Phytochemistry* 14: 2061–2064.
- Wagner H, Bladt S, Zgainsk EM (1996) *Plant Drug Analysis, A Thin Layer Chromatography Atlas*. 2<sup>nd</sup> ed., Translated by Scott, Th., A., Springer-Verlag, Berlin, Heidelberg and New York.
- Wu T-S, Tsang Z-J, Wu P-L, Lin F-W, Li C-Y, Teng C-M, Lee K-H (2001) New constituents and antplatelet aggregation and anti-HIV principles of *Artemisia capillaries*. *Med Chem* 9: 77–83.
- Yoshikawa M, Shimada H, Matsuda H, Yamahara J, Murakami N (1996) Bioactive constituents of Chinese natural medicines. I. New sesquiterpene ketones with vasorelaxant effect from Chinese Moxa, the processed leaves of *Artemisia argyi* Levl. et Vant.: Moxartenone and Moxartenolide. *Chem Pharm Bull* 44: 1656–1662.
- Zheng G-Q (1994) Cytotoxic terpenoids and flavonoids from *Artemisia annua*. *Planta Med* 60: 54–57.