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Abstract

The essential oil (EO) obtained by hydrodistillation of aerial parts of Lebanese *Achillea santolinoides* subsp *wilhelmsii* (K. Koch) Greuter was analysed by GC and GC-MS. The identity of the second major compound was uncertain. Its structure was elucidated using 1D and 2D NMR spectroscopy as well as MS data. It appeared to be a new sesquiterpene with a 1-oxa-bicyclo[5,4,0]-undecane skeleton which was named santolinoidol. The EO revealed interesting antimicrobial potential particularly against *Trichophyton* species, *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values ranging from 16 to 128 µg/ml.

Keywords:

Achillea santolinoides subsp *wilhelmsii*; Antimicrobial activity; Santolinoidol; Structural determination; Essential oil

Antimicrobial resistance is a global problem and a major public health threat. This applies to both developed and developing countries. In fact, according to the United States Centers for Disease Control and prevention (CDC), there are more than 23000 deaths per year in the US as a direct result of antibiotic-resistant infections.[1] Finding alternative complementary antibiotics to be used for non-lethal conditions can contribute to drive back infectious diseases and pathogen resistance.

The genus Achillea comprises many species known for their therapeutic benefits.[2] It was named after Achilles the hero of Iliad who, according to the legend, used the leaves of *Achillea millefolium* known commonly as Yarrow to treat his wounded soldiers.[3] *Achillea santolinoides* subsp *wilhelmsii* is used in Pakistan as blood purifier and as cure for stomach-ache, gastric troubles and fever.[4,5] It is also used in Iran and Turkey for bleeding and wound healing.[6,7] The main purpose of this study was to characterize the essential oil (EO) of Lebanese *A. santolinoides* subsp *wilhelmsii* and assess its antibacterial and antifungal activities. Although the EO of *A. santolinoides* subsp *wilhelmsii* has been described elsewhere,[8-11] no new metabolites have ever been detected in these oils. In the literature, the major compounds were camphor (48.2 %),[8] linalool (24.2 %) and 1,8-cineole (15.5 %),[9] (3*Z*)-cemberene A (22.6 %) and linalool (14.8 %),[9] carvacrol (25.1%) and linalool (11.0%),[10] or carvacrol (22.5 %), dihydrocarvone (13.2 %), and linalool (12 %).[11]

A. santolinoides subsp *wilhelmsii* aerial part EO was light green and was obtained with a 0.58% yield (v/w). GC and GC-MS analyses led to the identification and quantification of 35 components accounting for 84.3% of the EO (Table 1). However, the comparison with MS databases and kovats index (KI) wasn't enough for the identification of the second most abundant compound (**1**) which accounted for 7.1% of the EO and was eventually characterized by purification and NMR. The other main compounds were eucalyptol (8.0%), terpinen-4-ol (6.9%), α-pinene (6.0%), *and trans*-pinocarveol (3.5%).

Table 1

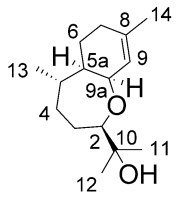
Partial chemical composition of *Achillea santolinoides* subsp *wilhelmsii* essential oil, including compounds accounting for more than 6 % of the oil[a]

RI[b]	RI[c]	Compounds	(%)
938	1076	α-Pinene	6.0
1034	1213	Eucalyptol	8.0
1176	1611	Terpinen-4-ol	6.9
1640	2188	T-Cadinol	2.6
1651	2160	Santolinoidol (**1**)	7.1

[a]) Full table is given in supplementary material.
[b]) Retention index on a DB-5MS column.
[c]) Retention index on an HP Innowax column.

A. santolinoides subsp *wilhelmsii* EO was fractionated by HPLC. The fractions were searched by GC-MS in order to point out the one containing the unknown compound **1**, which according to GC accounted for 77% of fraction 20. This fraction was analyzed by high resolution MS and NMR. Compound **1** had a pseudomolecular ion peak at *m/z* 239.2011 in the positive ionization mode corresponding to the molecular formula $C_{15}H_{27}O_2$. Its planar structure was deduced by analysis of ^{1}H, ^{13}C, COSY, HSQC and HMBC spectral data (Table 2). COSY spectrum made it possible to follow the sequence of protons H-2 to H-13, and a group of protons between H-5a and H-9a forming a cyclohexene ring. Chemical shifts and COSY correlation between H-14 and H-9 indicated that H-14 was linked to a double bond bearing the vinyl proton H-9. ^{1}H and ^{13}C chemical shifts indicated that carbon 9a was linked to an oxygen. Two singlet methyl groups at δ_H 1.13 and 1.18, and δ_C 23.7 and 26.3 were not correlated in COSY. HMBC correlations indicated that these two methyl groups were linked to the same quaternary carbon at δ 72.7. This carbon was linked to an oxygen and HMBC correlations between both H-11 and H-12 with C-2 ascertained the position of this isopropenol moiety which was linked to position 2. Long range proton carbon correlations between H-5 and C-5a and between H-13 and C-5a confirmed the existence of the C-

5–C-5a bond. The HMBC correlations between H-9a and C-2 and, conversely, between H-2 and C-9a made it obvious that C-2 and C-9a were linked through an oxygen atom. Overall, the compound was a sesquiterpene with an unusual 1-oxa-bicyclo[5,4,0]-undecane skeleton (Fig. 1). The relative stereochemistry was deduced from $^{1}H–^{1}H$ vicinal couplings and NOESY spectral data. Selected NOESY correlations are reported in Figure 2. NOE correlation between H-9a and H-2, and H-9a and H-5a, indicated that these protons are on the same side of the molecule. The stereochemistry at C-5 was doubtful with NOE; however H-5a coupling figure indicated that there were two axial coupling, which could only be explained with an anti-periplanar arrangement of the C-5–H-5 and C-5a–H-5a bonds. Hence, the unknown compound was identified as the 2-(5,8-dimetyl-2,3,4,5,5a,6,7,9a octahydrobenzo[b]oxepin-2-yl)propan-2-ol and was given the trivial name santolinoidol. It can be postulated that the biosynthesis of compound **1** occurs via the epoxidation of a C-2–C-10 double bond followed by the cyclisation of a hydroxyl in C-9a on the epoxide to form a 7-membered ring. The β-sesquiphellandrene, which accounts for 2 % of the EO, may be a precursor of santolinoidol.



Santolinoidol (**1**)

Fig. 1. Santolinoidol (**1**) from *A. santolinoides* subsp *wilhelmsii*

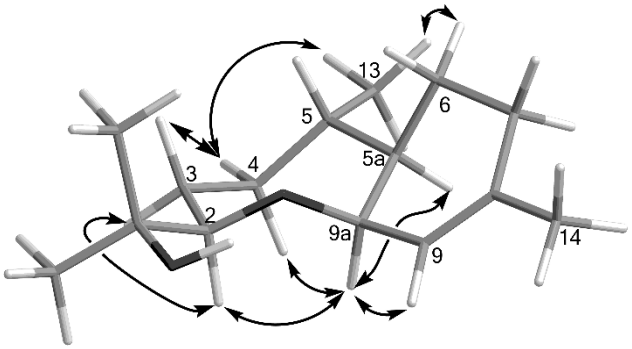


Fig. 2. Selected NOESY correlations in **1**

Table 2

Spectral data for santolinoidol (**1**) in $CDCl_3$

Position	δ_C, type	δ_H, (*J* in Hz)	COSY	HMBC	NOESY
2	90.5, CH	3.24, dd (11.7, 2.9)	3x, 3y	3, 9a, 10, 11	3x, 9a, 12
3	32.0, CH_2	x: 1.78, m	2, 3y, 4		2, 3y, 12
		y: 1.45, ddd (16.1, 12.5, 2.9)	2, 3x, 4		3x, 4
4	32.3, CH_2	1.54-1.64, m	3x, 3y, 5	2, 3, 5, 5a, 13	3y, 9a, 13
5	38.3, CH	1.37, m	4, 5a, 13	5a	
5a	46.3, CH	1.15 ddd (16.9, 12.5, 4.0)	5, 6y, 9a		9a
6	25.1, CH_2	x: 1.68, m	6y, 7x, 7y		6y, 13
		y: 1.38, m	5a, 6x, 7x, 7y		6x
7	31.0, CH_2	x: 1.96, br dd (17.6, 4.4)	6x, 6y, 7y	5a, 6, 8, 9	9
		y: 1.87, m	6x, 6y, 7x, 8	5a, 9	
8	139.0				
9	121.5, CH	5.49, br d (3.7)	7y, 9a, 14	7	7x, 9a, 14
9a	76.4, CH	3.93, br t (4.0)	5a, 8, 14	2, 6, 8, 9	2, 4, 5a, 9
10	72.1				
11	23.7, CH_3	1.13, s		2, 10, 12	
12	26.3, CH_3	1.18, s		2, 10, 11	2, 3x
13	22.6, CH_3	1.03, d (6.6)	5	4, 5, 5a	4, 6x
14	23.6, CH_3	1.72, br s	8, 9a	7, 8, 9	9

Minimum inhibitory concentrations (MICs) of *A. santolinoides* subsp *wilhelmsii* EO are presented in Table 3. EOs with MICs of 128 µg/ml and below are generally considered useful as antimicrobial agents.[12,13]

Table 3

Antimicrobial activity (MIC in µg/ml) of *A. santolinoides* subsp *wilhelmsii* EO

Strain tested[a]	*A. santolinoides* EO	Reference compound
S. aureus ATCC 29213	128	1[b]
S. aureus ATCC 33591	128	1[c]
C. albicans ATCC 10231	256	1[d]
P. aeruginosa CIP 82118	>512	0.25[e]
T. rubrum SNB-TR1	32	2[d]
T. mentagrophytes SNB-TM1	32	1[f]
T. soudanense SNB-TS1	16	2[d]
T. violaceum SNB-TV1	16	2[d]
T. tonsurans SNB-TT1	16	4[d]

[a] *Staphylococcus aureus* ATCC 29213; Methicillin-resistant *Staphylococcus aureus* ATCC 33591; *Candida albicans* ATCC 10231; *Pseudomonas aeruginosa* CIP 82118; *Trichophyton rubrum* SNB-TR1; *Trichophyton mentagrophytes* SNB-TM1; *Trichophyton soudanense* SNB-TS1; *Trichophyton violaceum* SNB-TV1; *Trichophyton tonsurans* SNB-TT1

[b] Oxacillin; [c] Vancomycin; [d] Fluconazole; [e] Gentamicin; [f] Itraconazole

A. santolinoides EO was most active on dermatophytes (MIC values 16-32 µg/ml). It also exhibited a significant activity against both strains of *Staphylococcus aureus* (MIC 128 µg/ml). *Candida albicans* was moderately sensitive (MIC 256 µg/ml), whereas *Pseudomonas aeruginosa* was resistant (MIC > 512 µg/ml). The origin of the antimicrobial activity remains uncertain. The oil is composed of many compounds and none of them really dominates. However, the antimicrobial activity of *Melaleuca alternifolia* (tea tree) oil is principally attributed to its terpinen-4-ol content.[14] Hence, this compound may have had an important contribution to the whole activity. Unfortunately, the fraction enriched in santolinoidol was obtained in very small amount and could not be tested alone.

In conclusion, this letter reports the first description of santolinoidol, an unusual 1-oxa-bicyclo[5,4,0]-undecane sesquiterpene isolated in the essential oil of *Achillea santolinoides* subsp *wilhelmsii*.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/.... These data include experimental procedure and all NMR data of compound **1**.

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