



## Chemical Composition of the *Santolina Pectinata* Lag., Essential Oil from Morocco: Identification of (Z)-heptadeca-10,16-dien-7-one as a New Natural Component



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ESSENTIAL oil of *Santolina pectinata* lag. aerial parts was obtained by hydrodistillation and analyzed, for the first time, by using gas chromatography and gas chromatography-mass spectrometry (GC-MS (EI)). 33 components were identified which a non terpenic oxygenated compound i.e., (Z)-heptadeca-10,16-dien-7-one strongly dominated the oil composition with 28%. The structure of this natural compound was determined for the first time by a Positive electro spray ionization time-of-flight mass spectrometry (ESI (+) -TOF-MS, one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy.

**Keywords:** *Santolina pectinata*, Essential oil, (Z)-heptadeca-10,16-dien-7-one, GC-MS, NMR.

### Introduction

The genus *Santolina* (family *Asteraceae*) from the tribe *Anthemideae* is formed by a taxonomically complex group of species which are aromatic dwarf shrubs bearing essential oils in flowering capitula and leaves. It is represented by more than 10 species, this genus is widely widespread in Mediterranean area, especially in South Europe and North Africa [1]. The phytochemical analysis and biological characteristics of *Santolina* species from various origins have been also widely studied [2]. Previous chemical investigations have shown the presence of terpenoids, particularly eudesmane and germacrane sesquiterpenoids, chrysanthemane monoterpenoids, coumarins and flavonoids and other secondary metabolites [3-6]. Many *Santolina* species have been used in traditional medicine for a long time [2,7-8]. Recently, several studies revealed that *Santolina* species show various biological usages, including antifungal, antibacterial, anti-inflammatory, antiviral, cytotoxic, and hepatoprotective effects [2,9-12]. Botanically, *Santolina pectinata* Lag. is

a synonym: of *Santolina rosmarinifolia* subsp. *pectinata* Lag. Maire. It is an endemic herbaceous medicinal aromatic plant originating from North Mediterranean, mainly found in the Iberian System, North Africa and principally in the eastern Baltic mountains [13]. In Morocco, this species, known locally as “tayrart”, is a spontaneous plant which often grows on calcareous substrates and is frequently associated with *Cedrus atlantica* and *Abie spinsapo* in 1500 m above sea level. On the other hand, it is associated with *Quercus ilex* and *Q. canariensis* at lower levels [14].

*S. pectinata* Lag. is an infrequently studied Iberian–Maghreb endemic and to our knowledge, only one study, published in 1988, has investigated with the chemical composition of essential oil of this plant collected at Pontones (Jakn) from Spain [15]. The main components of this essential oil were  $\beta$ -eudesmol (12.4%),  $\alpha$ -cadinol (9.3%), spathulenol (5.2%),  $\gamma$ -eudesmol (4.6%), and elemol (4.3%) [15]. Therefore, the present work basically aims at studying the chemical composition of the essential oil of the aerial

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parts of *Santolina pectinata* Lag. from Morocco using GC/RI (retention indices) and GC/MS (EI) and reporting the isolation and identification of a previously undescribed natural product by a combination of successive chromatography columns (CC), GC-TOF-MS, 1D ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) and 2D-NMR (heteronuclear multiple-bond correlation spectroscopy (HMBC) and correlation spectroscopy (COSY)).

## Materials and Methods

### *Plant material and isolation of the essential oil*

The aerial parts of *S. pectinata* Lag., were harvested in May and June 2013 (full bloom) from Jorf-Errachidia (Morocco). Voucher specimens (CIM HERB # 147) were deposited in the herbarium of the Faculty of Sciences and Technology of Errachidia. The fresh plant material (100g) was subjected to hydrodistillation (3h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [16] and the essential oil yield was 0.5% which was different the yield of essential oil of this plant collected from Spain (0.3%) [15]. Generally, The yield and essential oil content and composition of aromatic plants could be influenced by harvest time and ecological and climatic conditions [17].

### *Isolation of compound 34*

The aerial parts essential oil of *S. pectinata* Lag. was submitted to column chromatography on silica gel (ICN 200-500  $\mu\text{m}$ , 150 g). A hydrocarbon fraction (HF) and an oxygenated fraction (OF) were obtained by elution with pentane and diethyl ether, respectively. OF was further chromatographed with same apparatus and three polar sub-fractions, OF1, OF2 and OF3 were obtained using an elution of 99/1, 97/3 and 0/100 (pentane/diethyl ether, v/v), respectively. Successive column chromatography on silica gel (ICN 63-200  $\mu\text{m}$ , 50 g) with gradient pentane/diethyl ether mixture of OF2 (**34**: 69.6%) leads to obtaining the compound **34** with a degree of purity of 97% (Figure S1).

### *Gas Chromatography with retention indices (GC-RI) analysis*

The GC analysis were carried out using a Perkin-Elmer Auto system XL GC apparatus equipped with dual flame ionization detection (FID) system and fused-silica capillary columns (60 m $\times$ 0.22 mm I. D., film thickness 0.25  $\mu\text{m}$ ), Rtx-1 (polydimethylsiloxane) and Rtx-wax

(polyethylene glycol). Temperature program was from 60  $^{\circ}\text{C}$  to 230  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$  and then held isothermally at 230  $^{\circ}\text{C}$  for 35 min. Injector and detector temperatures were held at 280  $^{\circ}\text{C}$ . Samples were injected in the split mode (1/50), the carrier gas was  $\text{H}_2$  (1 ml/min); the volume injected was 0.2  $\mu\text{L}$  of pure oil. Retention indices (RIs) of compounds were determined relative to the retention times of series of n-alkanes (C5-C30) with linear interpolation, using the Van den Dool and Kratz equation [18] and software from Perkin-Elmer. Component relative concentrations were calculated based on GC peak areas without employing correction factors.

### *Gas Chromatography–Mass Spectrometry (GC-MS) analysis*

In Electron Impact (EI) Mode, samples were also analyzed using a Perkin-Elmer Turbo mass detector quadrupole, coupled to a Perkin-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. Carrier gas: helium (1 mL/min), ion source temperature: 150  $^{\circ}\text{C}$ , oven temperature programmed from 60  $^{\circ}\text{C}$  to 230  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$  and then held isothermally at 230  $^{\circ}\text{C}$  (35 min), injector temperature: 280  $^{\circ}\text{C}$ , energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35-350 Da, split: 1/80, injection volume : 0.2  $\mu\text{L}$  of pure oil. In GC-TOF-MS, an Agilent 6890 gas chromatograph coupled to a time of Flight (ToF) mass spectrometer was used for fingerprinting ESI-MS analysis. The mass spectrometer was equipped with an ESI source operating in the positive-ion mode. The data were treated with Masslynx software and mass spectra were acquired and accumulated over 60 sec and over the 80 to 1500 m/z range.

### *Nuclear Magnetic Resonance (NMR) analysis*

The structure elucidation of compound **34** was carried out by ESI (+)-MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, DEPT, and 2D-NMR (HMBC, HSQC and COSY). 1D and 2D NMR spectra were measured in  $\text{CDCl}_3$  using a Bruker Avance 300 Fourier Transform spectrometer (Wissembourg, France) operating at 100.13 MHz for  $^{13}\text{C}$  NMR and at 400.52 MHz for  $^1\text{H}$  NMR and equipped with a 5 mm probe. All shifts were referred to the internal standard tetramethylsilane (TMS).  $^{13}\text{C}$  NMR spectra were recorded with the following parameters: pulse width, 4 s (flip angle, 45 $^{\circ}$ ); acquisition time, 2.7s for 128K data table with a spectral width of 25000 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/point. The number

of accumulated scans was 3000-5000 for each sample depending of the amount of product. The  $^1\text{H}$  NMR spectra were recorded with the following parameters: flip angle,  $30^\circ$ ; acquisition time, 2.56 s for 32000 data table with a spectral width of 7000 Hz (17.5 pm). 2D-NMR sequences were recorded using Bruker microprograms.

#### Identification of individual components

Identification of individual components was based on (1) comparison of calculated retention indices (RIs), on polar and apolar columns, with those of authentic compounds or literature data [19]; (2) computer matching with commercial MS-libraries [20] and comparison of mass spectra with those of our own MS-library of authentic compounds or literature data [19,21].

### Results and Discussion

#### Essential Oil Analysis

The identification of essential oil components of *S. pectinata* Lag. was first carried out by using GC-RI and GC-MS (EI) as described in the Exper. Part. Thus, 33 components were identified in the essential oil (Table 1 and Figure S2) and distributed as 3 hydrocarbon monoterpenes (1.9%), 14 oxygenated monoterpenes (6.8%), 6 hydrocarbon sesquiterpenes (9.6%), 3 oxygenated sesquiterpenes (5.8%), 2 nonterpenic oxygenated compounds (0.7%) and 5 other compounds (6.6%). However, the compound **34**, which is a major constituent of the essential oil with 28%, was not present in GC-RI and GC-MS libraries and remained unidentified. To identify this new natural compound, we investigated its isolation and purification by combining successive CC using normal-phase silica gel (ICN 200-500  $\mu\text{m}$ , 150 g) with a mixture of pentane and diethyl ether.

<sup>a</sup> Order of elution is given on apolar column (Rtx-1)

<sup>b</sup> Normalized % abundances of oil are given on the apolar column

<sup>c</sup> Retention indices from literature on the apolar column

<sup>d</sup> Retention indices on the Rtx-1 apolar column

<sup>e</sup> Retention indices on the Rtx-wax polar column

<sup>f</sup> Percentage composition is given on the apolar column

#### Structure elucidation of new compound **34**

The structure of the compound **34** was determined by combination of ESIMS-(TOF) in positive mode, 1D- and 2D-NMR spectrum. According to mass calculation the most abundant ion, detected at  $m/z$  251.2375, was assigned to the protonated  $[\text{M}+\text{H}]^+$  ion (Figure 1). Thus, the exact mass, measured by GC-TOF-MS, was 251.2375 g/mol, corresponding to the formula  $\text{C}_{17}\text{H}_{30}\text{O}$  (calculated mass = 251.2375 g/mol). This chemical formula was confirmed by the  $^{13}\text{C}$  NMR and DEPT spectrums. In addition, this compound exhibited other mass spectral patterns with characteristic peaks which can attributed to the adduct formed from neutral or protonated molecule and other ions.

In the  $^{13}\text{C}$  NMR and DEPT spectra of the compound (Figures S3 and S4), 17 carbon signals were observed and were assigned to 1 methyl carbon at  $\delta_{\text{C}}$  14.10 ppm, 11 methylene carbons with chemical shifts between  $\delta_{\text{C}}$  43.1 and 22.6 ppm, 1 exomethylene carbon at  $\delta_{\text{C}}$  114.4 ppm, 3 ethylenic methine carbons at  $\delta_{\text{C}}$  139.3, 131.55, and 128.7 ppm, and a quaternary carbon at  $\delta_{\text{C}}$  210.7 ppm characteristic of a carbonyl group (Table 2). Accordingly, the molecular formula of component **34** was deduced as  $\text{C}_{17}\text{H}_{30}\text{O}$ , requiring three degrees of unsaturation, accounted for by two olefins carbons and one carbonyl carbon. The linear carbonyl compound could be attributed to the compound **34** (Figure 2).

The  $^1\text{H}$  NMR spectrum of **34** manifested one triplet ( $\delta_{\text{H}}$  0.82) methyl signal, several multiples between  $\delta_{\text{H}}$  1.22 and 2.40, two downfield signals at  $\delta_{\text{H}}$  5.35 (1H, dt) and 5.36 (1H, dt) and three deshielded ddt signals ( $\delta_{\text{H}}$  4.87, 4.92, 5.74) involved in the three-proton AMX system, suggesting the presence of a terminal allylic moiety (Figure S5). More than that, the absence of an aldehydic proton in the downfield region of the  $^1\text{H}$  NMR spectra indicates that **34** is a ketone.

Finally,  $^1\text{H}$ - $^{13}\text{C}$  HMBC (Heteronuclear Multiple Bond Coherence) and  $^1\text{H}$ - $^1\text{H}$  COSY (Correlation Spectroscopy) spectrums (Figures S6 and S7) confirmed the structure of a diunsaturated linear ketone (Table 2). As shown in Figure 3, the strongly shielded nature of H-6 and H-8, respectively,  $\delta_{\text{H}}$  2.31 (2H, m) and 2.4 (2H, m), and the correlations in the HMBC experiment between C-7/H-6 and C-7/H-8 attested that the ketone group was at the C-7 position. The nonconjugated nature of the carbonyl group was directly proved by observation of cross-peaks in

TABLE 1. Chemical composition of *S. pectinata* Lag. aerial parts essential oil

N <sup>a</sup>	Components <sup>b</sup>	Ir Lit <sup>c</sup>	Ir apo <sup>d</sup>	Ir pol <sup>e</sup>	f %
1	Cineole 1,8	1024	1019	1206	0.1
2	Limonene	1024	1021	1199	0.2
3	(Z)-b-Ocimene	1029	1025	1231	1.6
4	(E)-b-Ocimene	1041	1036	1247	0.1
5	Nonanal	1076	1081	1394	0.1
6	Linalool	1086	1083	1544	0.2
7	Cis Sabinene hydrate	1083	1097	1553	0.4
8	$\beta$ -Thujone	1103	1107	1422	0.4
9	Camphor	1123	1121	1519	0.5
10	Lyratol	1150	1138	1779	0.4
11	Borneol	1150	1149	1689	0.3
12	$\alpha$ -Terpineol	1176	1172	1684	0.2
13	Estragole	1175	1175	1661	0.5
14	Cuminaldehyde	1217	1212	1779	0.9
15	p-Anisaldehyde p	1215	1214	2028	0.6
16	Peryllaldehyde	1248	1248	1768	1.6
17	Lyratyl acetate	1270	1257	1634	0.2
18	E-Anethole	1262	1263	1813	4.1
19	Bornyl acetate	1270	1269	1573	0.8
20	Thymol	1266	1272	2190	0.2
21	Undecan-2-one	1273	1276	1586	0.6
22	Carvacrol	1278	1278	2214	0.6
23	$\alpha$ -Copaene	1379	1371	1489	0.6
24	Methyleugenol	1369	1374	2000	0.2
25	Isocaryophyllene	1409	1403	1570	0.2
26	E-Caryophyllene	1421	1416	1593	6
27	E- $\beta$ -Farnesene	1446	1447	1661	1.2
28	$\alpha$ -Curcumene	1473	1470	1765	0.3
29	Germacrene D	1479	1475	1701	1.3
30	Spathulenol	1572	1564	2105	1.1
31	Caryophyllene oxyde	1578	1570	1967	3.4
32	Dillapiole	1590	1591	2338	1.2
33	Tau cadinol	1633	1625	2154	1.3
34	NI		1834	2242	28
				Total identified	59.4
				Hydrocarbon monoterpenes	1.9
				Oxygenated monoterpenes	6.8
				Hydrocarbon sesquiterpenes	9.6
				Oxygenated sesquiterpens	5.8
				Nonterpenic oxygenated compounds	0.7
				Others	6.6
				Non identified (34)	28

*a* Order of elution is given on apolar column (Rtx-1). *b* Normalized % abundances of oil are given on the apolar column. *c* Retention indices from literature on the apolar column. *d* Retention indices on the Rtx-1 apolar column. *e* Retention indices on the Rtx-wax polar column. *f* Percentage composition is given on the apolar column.

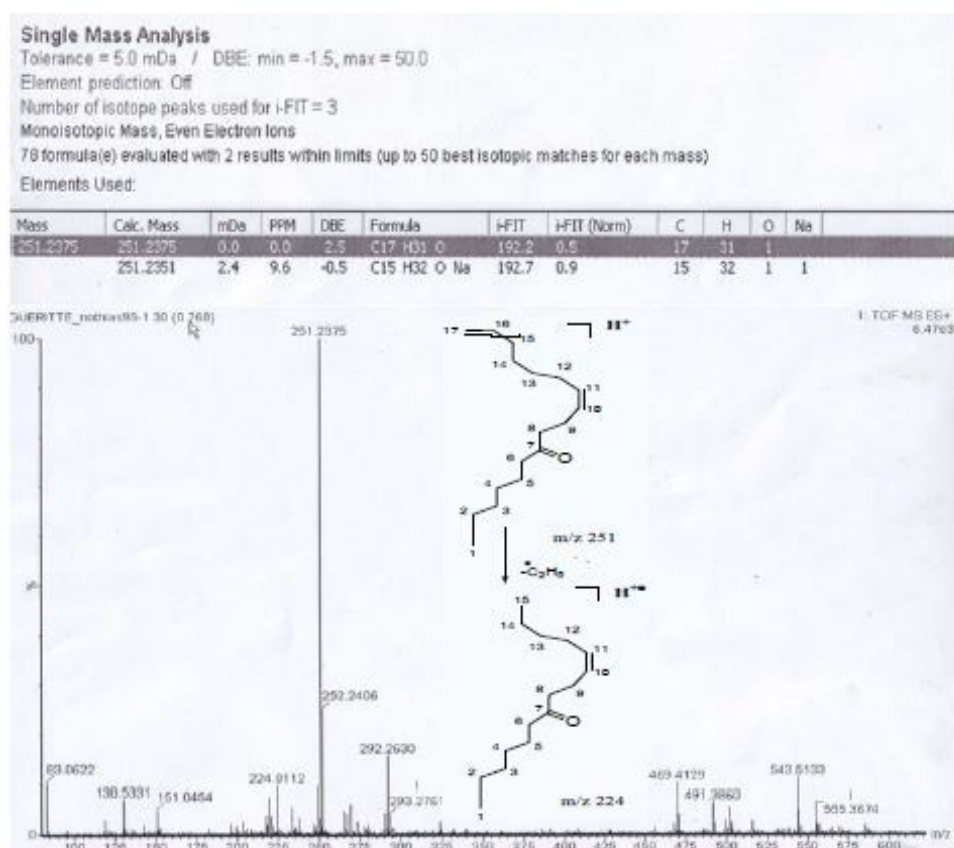


Fig. 1. ESIMS spectrum in positive ion mode (TOF) of (Z)-heptadeca-10,16-dien-7-one

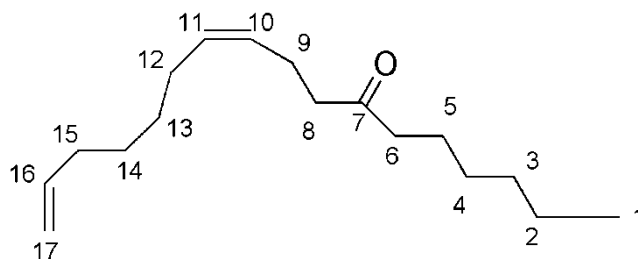


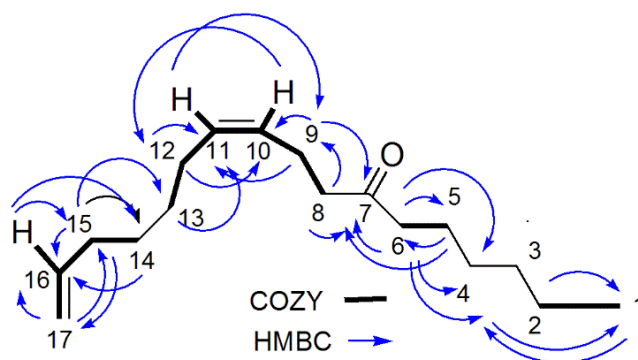
Fig. 2. Structure of (Z)-heptadeca-10,16-dien-7-one (**34**).

**TABLE 2. NMR Data of (Z)-Heptadeca-10,16-dien-7-one (34)**

C	<sup>13</sup> C NMR <sup>a</sup>	<sup>1</sup> H NMR <sup>b</sup>	HMBC <sup>c</sup> H-C	<sup>1</sup> H- <sup>1</sup> H COSY
1	14.10 (CH <sub>3</sub> )	0.82 (t, J <sub>1,2</sub> =6.6)	2,3	2
2	22.6 (CH <sub>2</sub> )	1.22 (m)	1,3,4	1,3
3	31.8 (CH <sub>2</sub> )	1.22 (m)	1,2,4,5	2,4
4	29 (CH <sub>2</sub> )	1.22 (m)	6,5,3,2	5,3
5	23.9 (CH <sub>2</sub> )	1.51 (m)	7,6,3,4	6,4
6	43.1 (CH <sub>2</sub> )	2.31 (m)	7,8,5,4,3	5
7	210.7 (C)		6, 8, 9	
8	42.7 (CH <sub>2</sub> )	2.40 (m)	11,10,9,7,6	9
9	27 (CH <sub>2</sub> )	2.20 (m)	7,8,11,10	8,10,11
10	128.7 (CH)	5.36 (dt, J <sub>10,11</sub> =11.0, J <sub>10,9</sub> =6.4, J <sub>10,12</sub> =2.3)	12,9	9,11
11	131.5 (CH)	5.35 (dt, J <sub>11,10</sub> =11.0, J <sub>11,12</sub> =6.4, J <sub>11,9</sub> =2.3)	9,12,13	10,12
12	32.5 (CH <sub>2</sub> )	1.92 (m)	10,11,13	11,13
13	29 (CH <sub>2</sub> )	1.31 (m)	12,14,11	12,14
14	28.5 (CH <sub>2</sub> )	1.31 (m)	15,16,13,12	13,15
15	33.7 (CH <sub>2</sub> )	1.98 (m)	17a,17b,16,14,13	16,14
16	139.3 (CH)	5.74 (ddt, J <sub>16,17a</sub> =10.4, J <sub>16,17b</sub> =17.3, J <sub>16,15</sub> =6.7)	15, 14	17a,17b,15
17	114.4 (CH <sub>2</sub> )	a 4.87 (dd, J <sub>17a,16</sub> =10.4, J <sub>17a,17b</sub> =2.0) b 4.92 (d, J <sub>17b,16</sub> =17.3, J <sub>17b,17a</sub> =2.0)	15	16

<sup>a</sup>δ<sub>C</sub>, multiplicity given by DEPT is in parentheses. <sup>b</sup>δ<sub>H</sub>, multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; coupling constants are reported as numerical values in hertz.

<sup>c</sup>Signal correlating with <sup>1</sup>H resonance.



**Fig. 3. Key COSY and HMBC correlations of (Z)-heptadeca-10,16-dien-7-one (34).**



the HMBC spectrum between signals at  $\delta_C$  210.7 (s, C-7) and  $\delta_H$  2.2 (2H, m, H-9), which had COSY correlations with the two ethylenic protons of the double bond 5.36 (1H, dtt, H-10) and 5.35 (1H, dtt, H-11) as depicted in **Figure 3**. However, in case of conjugation, the chemical shift of the carbonyl is broadly less than 200 ppm.

The relative configuration of the  $\Delta^{10,11}$  double bond, localized on the linear chain, was also pinpointed by analysis of  $^1H$  NMR coupling patterns and the steric effect observed in  $^{13}C$  NMR spectra. The *Z* configuration of the  $\Delta^{10,11}$  double bond was identified by the medium coupling constant ( $^3J_{H_{10}, H_{11}} = 11.0$  Hz). This configuration could be justified by the shielded methylene signals at  $\delta_C$  32.5 (C-12) and 27 (C-9), which are aspects of a  $\gamma$  steric effect between two carbons relative to compounds with *Z* configuration [22]. Search in the literature confirmed that the spectral data of our compound fitted perfectly with those reported in the literature. Indeed, another *Z* isomer ((*Z*)-heptadeca-9,16-dien-7-one) was then identified by Darriet et al (2012) in Corsican and Sardinian *Chamaemelum mixtum* essential oils [23]. Both ketones show very similar data; however, the only difference is the position of the double bond.

### Conclusions

In conclusion, the combined use of CC, GC-RI, GC-MS, ESI (+)-MS, 1D and 2D-NMR analyses of aerial parts essential oil of *S. pectinata* allowed identifying 34 components which accounted to 59.4% of the total amount. The essential oil was dominated by oxygenated compounds (47.9 %) while hydrocarbon compounds accounted for 11.5 % of the oil. A non terpenic oxygenated compound *i.e.*, (*Z*)-heptadeca-10,16-dien-7-one was the major component with 28 % of the oil. It should be noted that this new natural product was identified for the first time and previously undescribed in *Santolina* species essential oils. So, it was probably related, biosynthetically, to fatty acids and / or carotenoid biosynthetic pathway. In short, this study has given the originality and specificity of essential oil of *S. pectinata* from Morocco.

### Supplementary Material

See attached file: **Figures S1-S7**

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### التركيب الكيميائي للزيت العطري لنبته *Santolina pectinata* من المغرب: تحديد كمركب طبيعي جديد (Z)-heptadeca-10,16-dien-7-one

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تمت دراسة التركيب الكيميائي للزيت العطري للأجزاء الهوائية لنبته *Santolina pectinata*، من الجنوب الشرقي للمغرب باستخدام التحليل الكروماتوغرافي في المرحلة الغازية مقرون بمطياف الكتلة (GC-MS). يتميز زيت الأجزاء الهوائية بالمحتوى العالي من المركب (Z) heptadeca-10,16-dien-7-one كمبرك أساسي، تم تحديد بنية هذا المركب الطبيعي لأول مرة بواسطة مطياف زمن الطيران موصول بمنبع أيونات ذو تأين بالإرذاذ الإلكتروني مقرون بمطياف الكتلة ESI-TOF-MS (+)، التحليل بالرنين النووي المغناطيسي احادي الابعاد 1D RMN وثنائي الابعاد 2D RMN.