ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/316883544

Chemical Composition and Biological Activities of the Essential Oil from Leaves and Flowers of Pulicaria incisa sub. candolleana (Family Asteraceae)

Article · March 2017

CITATIONS
4

READS
267

4 authors:

Esraa Adel Shahat
Modern Sciences and Arts University
3 PUBLICATIONS **9** CITATIONS
SEE PROFILE

Riham Omar Bakr
Modern Sciences and Arts University
31 PUBLICATIONS **101** CITATIONS
SEE PROFILE

Omayma A. Eldahshan
Ain Shams University
92 PUBLICATIONS **518** CITATIONS
SEE PROFILE

Nahla Ayoub
Umm Al-Qura University
82 PUBLICATIONS **679** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Natural Therapeutic Activities of Egyptian Plants and crops against Experimental Cancer Models View project

Lobularia libyca View project

All content following this page was uploaded by Nahla Ayoub on 12 May 2017.

The user has requested enhancement of the downloaded file.

CHEMISTRY & BIODIVERSITY

Chemical Composition and Biological Activities of the Essential Oil from Leaves and Flowers of *Pulicaria incisa* sub. *candolleana* (Family Asteraceae)

Esraa A. Shahat,[a] Riham O. Bakr,[a] Omayma A. Eldahshan,[b] and Nahla A. Ayoub*[b,c]

[a]Pharmacognosy Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt

[b]Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt,
e-mail: naayoub@uqu.edu.sa

[c]Pharmacology Department, Faculty of Medicine, Umm Al-Qura University, Makkah 24382, Saudi Arabia

The composition of the essential oil isolated from leaves and flowers of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin, growing in Egypt, was analysed by GC and GC-MS. Forty-nine and 68 compounds were identified from the oils of the leaves and flowers accounting for 86.69 and 84.29%, respectively of the total detected constituents. Both leaves and flowers oils were characterized by the high content of carvotanacetone with 66.01, 50.87 and chrysanthenone 13.26, 24.3%, respectively. The cytotoxic activity of both essential oils was evaluated against hepatocellular carcinoma cell line HEPG-2, using MTT assay and vinblastine as a reference drug. Leaf oil showed higher activity with IC_{50} 11.4 1g/ml compared with 37.4 1g/ml for flower oil. The antimicrobial activity of both oils was evaluated using agar well diffusion method towards two representatives for each of *Gram* positive and *Gram* negative bacteria as well as four representatives for fungi. The minimum inhibitory concentration of both essential oils against bacterial and fungal strains was obtained in the range of 0.49 – 15.63 1g/ml.

Keywords: Antimicrobial activities, *Pulicaria incisa* sub. *candolleana*, Essential oil, GC-MS Analysis, Cytotoxic activities.

Introduction

Herbs that contain essential oils are commonly utilized worldwide in folk remedies against a variety of complaints.[1] Genus *Pulicaria* (Family Asteraceae, tribe Inuleae) comprises approximately 80 species which are widely distributed from Europe to North Africa and Asia. Various essential oils are isolated from different *Pulicaria* species, analysed by GC-MS and reported to have useful pharmacological activities. *Pulicaria gnaphalodes* essential oil inhibited *Leishmania* major growth in concentrations ranging from 0.1 to 50 1l/ml (parasite culture) in 24 h. Its major components have been reported to be geraniol, 1,8-cineole (eucalyptol), chrysanthenone, *a*-pinene, chrystanthenone, *a*-terpineol, and filifolone.[2] *Pulicaria inuloides* essential oil possesses significant antioxidant and strong antimicrobial activities against *Gram* positive bacteria and *Candida albicans.* The main components identified in this oil were 55.1% of 5-isopropyl-2-methyl-2-cyclohexen-1-one, 20.6% of methylbenzene, and (*Z*)-citral (2.9%).[3] Twenty-seven components were also identified in the essential oil isolated from *Pulicaria. odora* L. roots being thymol (47.8%) and its derivative isobutyrate (30.0%) the main constituents in this oil and it exhibited significant antibacterial activity.[4]

The major compounds of the essential oil obtained from the leaves of *P. undulata* Gamal-Eldin from Yemen were the carvotanacetone (91.4%) and 2,5-dimethoxy-*p*-cymene (2.6.%). The oil showed the strongest bactericidal activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus*, as well as *Candida albicans.* The essential oil showed moderate cytotoxic activity against MCF-7 breast tumor cells, with an IC_{50} of 64.6 T 13.7 1g/ml.[5] Another research identified the main chemical components of essential oil from aerial parts of *P. undulata* from Iran as 4-terpineole (20.1%), *a*-terpinene (4.0%), *c*-terpinene (7.0%), *cis*-sabinene hydrate (8.3%), linalool (5.6%), (1*S*)-*cis*-calamenene (13.4%), and junipene (8.7%).[6]

Pulicaria incisa (Lam.) DC. is used by the natives of some upper Egyptian areas in the form of a decoction, sweetened with sugar as a substitute for tea.[7] The plant was also used as a traditional medicine for treating heart diseases by Bedouins.[8][9] The plant has a pleasant aromatic smell and is well known for its essential oil which is partly responsible for its use in preparing the tonic drink. *Pulicaria incisa* (Lam.) DC.

 © 2017 Wiley-VHCA AG, Zurich, Switzerland

was reported to be used as a tonic, antispasmodic, hypoglycemic and as an ingredient of a local perfume in Sudan.[10] Biological studies revealed its hypocholestremic,[3] antimicrobial, antitumor[11] and antioxidant activities.[12] This species was not recorded in any of the Egyptian floras before *Boulos* (1995)[13] who referred that there are two subspecies in Egypt: subspecies *incisa* and subspecies *candolleana* E. Gamal-Eldin. *Pulicaria incisa* sub. *candolleana* also known as *P. desertorum* DC. in Decne.[14] Nothing was traced regarding essential oil study of this species, therefore, the aim of this work is to investigate the oil content of *Pulicaria incisa* sub. *candolleana* growing in Egypt as well its cytotoxic and antimicrobial effects.

Results and Discussion

Essential Oil Yield and Composition

Table 1 shows the compounds identified in the essential oils from leaves and flowers of *Pulicaria incisa* sub. *candolleana* growing wild in Egypt, respectively. The relative contents of the identified volatile compounds, expressed as the percentage of the peak area relative to the total area of all peaks, are also listed in *Table 1*.

Leaf Oil

The oil obtained by hydrodistillation of the fresh leaves is yellow colored with a pleasant aroma; the average yield was 0.66% (*v*/*w*). Forty-nine compounds comprising 86.69% of the leaf oil were identified. The oil was characterized by high content of ketonic components which represented 80.55%. The major identified compounds were, carvotanacetone (66.01%) which was previously reported in *P. jaubertii*[15] and chrysanthenone (13.26%). Among the identified compounds, alcoholic components represented 3.54%, where the main component was linalool (3.14%). Monoterpene hydrocarbons represented 0.32% where the main component was *α*-phellandrene. Phenolic monoterpene represented 0.49% where the major was carvacrol with 0.21% while sesquiterpene represented 0.25%.

Flower Oil

Hydrodistillation of flowers afforded yellow colored oil, with yield 0.33% (*v*/*w*). Sixty-eight components comprising 84.29% of the oil were identified. Ketonic components dominated the oil content (76.84%), where carvotanacetone was the major component (50.87%) followed by chrysanthenone (24.3%). Esters represented 1.54% with verbenyl acetate as the major ester component. Aldehydic component represented 1.37%, while sesquiterpene represented 0.64% with *b*-selinene and *epi*-*α*-cadinol as the major components.

From the above results it is found that carvotanacetone was the major constituent in both oils representing 66.6% of the leaves oil and 50.87% of the flowers oil. This result is similar to the results of other studies which showed that carvotanacetone was also the major component in the essential oils isolated from *Pulicaria jaubertii* leaves from southern Saudi Arabia (98.59%),[15] *Pulicaria jaubertii* flowers from South Yemen (93.5%),[16] leaves of *Pulicaria undulata* from yemen (91.4%),[5] *Pulicaria mauritanica* (87.3%),[17] and *Pulicaria jaubertii* aerial parts from Yemen (64.0%).[18] Also *Pulicaria undulata* oil from Sudan was composed of 55.9% carvotanacetone.[19]

To the best of our knowledge, this work represents the first GC-MS analysis of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin growing in Egypt.

Cytotoxic Activity

The cytotoxic activity of leaf and flower oils against liver cell carcinoma HEPG-2 was determined using MTT assay and vinblastine as a reference drug. Leaf oil showed higher activity with IC_{50} 11.4 μg/ml compared with 37.4 μg/ml for flower oil (*Fig. 1*).

The more potent cytotoxic effect of leaf oil may be attributed to its higher content of carvotanacetone, which was previously reported in *P. jaubertii* to have anticarcinogenic and chemopreventive activity.[15][16]

Antimicrobial Activity

The antimicrobial activity of both leaf and flower oils isolated *Pulicaria. incisa* sub. *candolleana* E. Gamal-Eldin were determined in a comparative study (*Tables 2* and *3*). The *MIC*s values showed by the leaf oil varied between 3.9 and 15.63 μg/ml for the fungi, 1.95 and 3.9 μg/ml for the tested *Gram*-positive bacteria, while 62.45 μg/ml for *Escherichia coli.*

The *MIC*s showed by the flowers oil varied between 62.5 and 125 μg/ml for the fungi, 32.5 and 62.5 μg/ml for the *Gram*-positive bacteria and no activity was observed against *Candida albicans* and the tested *Gram*-negative bacteria.

From the results, the tested *Gram*-positive bacteria (*Streptococcus pneumonia* and *Bacillus subtilis*) and *Geotricum candidum* fungi were more sensitive to the leaf oil than the tested *Gram*-negative bacteria and the other tested fungi. In similar studies, essential oils from *P. inuloides, P. stephanocarpa*, and *P. jaubertii* showed antimicrobial activity against *Bacillis subtilis* while the oil of *Pulicaria inuloides* showed antimicrobial activity against *Streptococcus pneumoniae.*[3][20]

© 2017 Wiley-VHCA AG, Zurich, Switzerland

Table 1. Chemical composition of the Egyptian *Pulicaria incisa* sub. *candolleana* essential oils from leaves and flowers Investigated by GC/MS

	Component	*KI*	Relative content [%][a]	
			Leaf oil	Flower oil
1	Hexanal	801.7	0.02	0.19
2	(2*E*)-Hexenal	847	0.06	0.28
3	(*Z*)-Hex-3-en-1-ol	851	n.d	0.03
4	(2*E*)-Hexenol	861	n.d	0.03
5	*n*-Hexanol	863	n.d	0.07
6	Tricyclene	915	0.01	0.01
7	*a*-Thujene	921	0.03	0.03
8	*a*-Pinene	929	0.05	0.06
9	Camphene	945	0.03	0.03
10	Sabinene	972	0.04	0.07
11	*b*-Pinene	976	0.01	0.01
12	Oct-1-en-3-ol	980	n.d	0.01
13	*a*-Phellandrene	1006	0.08	0.09
14	(*E*,*E*)-2,4-Heptadienal	1013	n.d	0.02
15	*a*-Terpinene	1018	0.01	0.02
16	1,2,4-Trimethylbenzene	1025	0.03	0.01
17	*o*-Cymene	1026	0.16	0.11
18	Sylvestrene	1031	0.06	0.05
19	Eucalyptol (1,8-cineole)	1034	0.12	0.07
20	Benzene acetaldehyde	1047	0.05	0.03
21	*c*-Terpinene	1054	0.03	0.05
22	*cis*-Linalool oxide (furanoid)	1076	0.39	0.22
23	Linalool	1105	3.14	0.27
24	Chrysanthenone	1136	13.26[b]	24.3
25	*trans*-*p*-Menth-2-en-1-ol	1145	0.03	0.03
26	Eucarvone	1150	n.d	0.24
27	(*E*)-2-Nonenal	1163	n.d	0.05
28	(*Z*)-Isocitral	1169	n.d	0.68
29	Terpinen-4-ol	1183	0.08	0.15
30	Decanal	1209	n.d	0.12
31	*cis*-Carveol	1212	n.d	0.04
32	(*Z*)-Anethole	1240	0.09	n.d
33	(*S*)-Carvotanacetone (5-isopropyl-2-methyl-2-cyclohexen-1-one)	1267	66.01	50.87
34	*trans*-Verbenyl acetate	1271	n.d	0.98
35	*trans*-Linalool oxide acetate (pyranoid)	1295	0.19	0.12
36	Thymol	1302	0.2	0.21
37	Carvacrol	1312	0.21	0.21
38	6-Hydroxycarvotanacetone	1309	0.01	n.d
39	*trans*-Carvyl acetate	1345	0.01	0.02
40	Eugenol	1366	0.07	0.05
41	*a*-Copaene	1384	0.01	0.04
42	*b*-Bourbonene	1394	n.d	0.03
43	(*E*)-Jasmone	1399	0.26	n.d
44	(*Z*)-Jasmone	1409	0.98	1.38
45	Dodecanal	1413	n.d	0.03
46	2,5-Dimethoxy-*p*-cymene	1431	0.23	n.d
47	Geranyl acetone	1460	0.03	0.05
48	*c*-Curcumene	1489	n.d	0.04
49	*b*-Selinene	1500	n.d	0.15
50	*b*-Curcumene	1521	0.05	0.1
51	*d*-Cadinene (bicyclic sesquiterpene)	1535	0.03	0.08

© 2017 Wiley-VHCA AG, Zurich, Switzerland

Table 1. (cont.)

	Component	*KI*	Relative content [%][a]	
			Leaf oil	Flower oil
52	*cis*-Sesquisabinene hydrate	1553	0.01	n.d.
53	Thymohydroquinone	1566	0.01	0.01
54	Dodecanoic acid	1571	n.d	0.11
55	(3*Z*)-Hexenylbenzoate	1580	n.d	0.01
56	Hexyl benzoate	1586	n.d	0.01
57	2-Phenylethyl tiglate	1595	n.d	0.05
58	Fokienol	1604	n.d	0.03
59	*epi-a*-Cadinol (sesquiterpenoid alcohol)	1656	0.1	0.15
60	Methyl (*Z*)-jasmonate	1660	0.06	0.09
61	*epi-b*-Bisabolol	1684	0.05	0.05
62	1-*n*-hexadecanol	1887	0.02	0.1
63	Methyl Hexadecanoate methyl palmitate	1932	n.d	0.05
64	Ethyl hexadecanoate	1998	0.04	0.17
65	Isopropyl palmitate	2030	0.03	0.04
66	(*E*,*E*)-Geranyllinalool	2041	n.d	0.02
67	*n*-Heneicosane	2108	0.02	0.03
68	Ethyl octadecanoate	2207	n.d	0.05
69	Tricosane	2308	0.03	0.29
70	Tetracosane	2402	0.01	0.07
71	Pentacosane	2508	0.13	0.7
72	Heptacosane	2708	0.11	0.5
73	Octacosane		n.d	0.03
	Total identified compounds		86.69	84.29
	Functional group			
	Monoterpene		0.32	0.4
	Monoterpene alcohol		0.2	0.26
	Monoterpene phenol		0.49	0.27
	Sesquiterpenes		0.25	0.64
	Alcohols		3.54	0.78
	Aldehydes		0.08	1.37
	Esters		0.33	1.54
	Ketones		80.55	76.84
	Aromatics		0.47	0.2
	Hydrocarbon Alkanes		0.17	0.92
	Acids		n.d	0.11

[a] Values are expressed as relative area percentage. n.d: Not detected. [b] The major components are highlighted in bold.

Conclusion

The leaf oil of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin appeared as promising candidate for cytotoxic researches with powerful effect against hepatocellular carcinoma cell line HEPG-2 (IC_{50} 11.4 **1**g/ml). Also the leaf oil showed a stronger antimicrobial effect than that isolated from flowers in comparison with the reference drugs.

The stronger antimicrobial and cytotoxic activities of the leaf oil may be due to its higher concentration of carvotanacetone which was previously reported to have anticarcinogenic and chemopreventive activity.

The tested *Gram*-positive bacteria (*Streptococcus pneumonia* and *Bacillus subtilis*) and *Geotricum candidum* fungi were more sensitive to the leaf oil than the tested *Gram*-negative bacteria and the other tested fungi. Hence the essential oil of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin leaves may be effective in the treatment of diseases caused by these organisms such as pneumonia, ear infections, sinus infections, meningitis, and bacteremia, geotrichosis.

Experimental Section

Plant Material

Aerial parts of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin was collected during the flowering stage in February 2015 from km 67, Cairo-Suez road, Egypt. The plant was collected and identified by Prof. *Abdel-Haleem*

 © 2017 Wiley-VHCA AG, Zurich, Switzerland

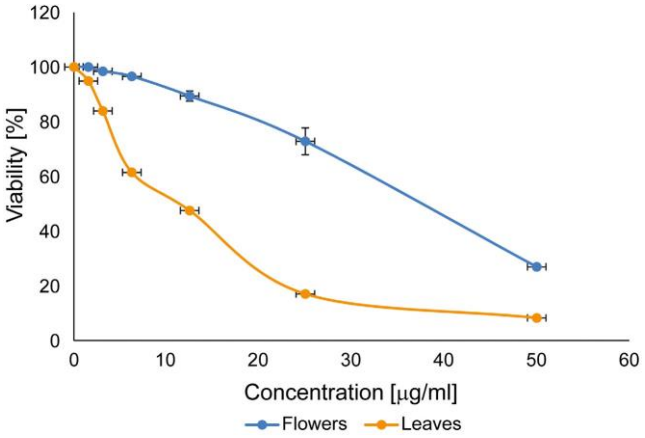

Figure 1. Cytotoxic activity of *Pulicaria incisa* sub. *candolleana* oils against HEPG2.

Abdel-Mogaly, Department of Plant Taxonomy, Herbarium of Horticultural Research Institute, Agricultural Research Centre, Dokki, Cairo, Egypt. Voucher specimen (PHG-P-PI-193) was deposited in Herbarium of Faculty of Pharmacy at Ain Shams University.

Extraction and Isolation of Essential Oil

Fresh leaves and flowers were dried and hydrodistilled separately using a *Clevenger*-type apparatus for 5 h. The oils were collected, dried over anhydrous sodium sulfate and stored at 4 °C until analyzed.

Gas-Chromatography-Mass Spectrometry (GC-MS) Analysis. The GC analyzes of *Pulicaria incisa* sub. *candolleana* E. Gamal-Eldin essential oils from both

Table 2. Antimicrobial activity of the leaves oil expressed as inhibition zone diameter [mm][a,b,c,d]

Tested microorganisms	Leaves oil	Standard
Fungi		Amphotericin B
Aspergillus fumigatus (RCMB 02568)	17.2 T 0.4	23.7 + 0.1
Syncephalastrum racemosum (RCMB 05922)	16.3 T 0.2	19.7 + 0.2
Geotricum candidum (RCMB 05097)	20.1 T 0.6	28.7 + 0.2
Candida albicans (RCMB 05036)	NA[e]	25.4 + 0.1
Gram Positive Bacteria		Ampicillin
Streptococcus pneumoniae (RCMB 010010)	19.3 T 0.4	23.8 + 0.2
Bacillis subtilis (RCMB 010067)	21.4 T 0.6	32.4 + 0.3
Gram Negative Bacteria		Gentamicin
Pseudomonas aeruginosa (RCMB 010043)	NA	17.3 + 0.1
Escherichia coli (RCMB 010052)	13.1 T 0.3	19.9 + 0.3

[a] Mean zone of inhibition in mm T standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. [b] The test was done using the diffusion agar technique. Well diameter: 6.0 mm. [c] Data are expressed in the form of mean T SD. [d] The conc. of oil was 200 1g/ml. [e] NA: no activity. RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms.

Table 3. Antimicrobial activity as *MIC* [1l/ml] of the essential oil from leaves and flowers, respectively

Tested microorganisms	Leaves oil	Flowers oil	Standard
Fungi			Amphotericin B
Aspergillus fumigatus (RCMB 02568)	15.6	125	0.98
Syncephalastrum racemosum (RCMB 05922)	32.3	125	3.9
Geotricum candidum (RCMB 05097)	3.9	62.5	0.49
Candida albicans (RCMB 05036)	NA[a]	NA	0.49
Gram Positive Bacteria			Ampicillin
Streptococcus pneumoniae (RCMB 010010)	3.9	62.5	0.98
Bacillis subtilis (RCMB 010067)	2	32.3	0.49
Gram Negative Bacteria			Gentamicin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	15.63
Escherichia coli (RCMB 010052)	62.5	NA	3.9

[a] NA: no activity.

 © 2017 Wiley-VHCA AG, Zurich, Switzerland

leaves and flowers were carried out on a *Shimadzu GC MS-QP2010* equipped with *Rtx-5MS* column (30 m 9 0.25 mm 9 0.25 1m; *Restek*, USA). He was used as the carrier gas with a flow rate of 2 ml/min). The column temp. was programmed from 45 to 300 °C at a rate of 5 °C/min. The injector and detector temps. were programmed at 250 and 300 °C, resp. GC/MS Analysis of the oils were performed by split mode with a split ratio 1:15. *PeakSimple q2000* chromatography data system (*SRI Instruments*, Torrance, USA) was used for recording and integrating of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used for calculating the% composition of each component. A homologous series of *n*-alkanes (C_8 – C_{18}; *Aldrich* chemical company, USA) was injected under the same conditions described.

Identification of Essential-Oil Components

The essential oil components were identified by comparing their mass spectra with the available spectra in the equipment database. Additionally, the calculated *Kovats* retention indices (KIs) were compared with those in the literature and *Adams* library.[21] The relative contents of individual components expressed as a percentage were calculated based on the GC-peak areas without any correction factors.

Cytotoxicity Assay

The viability of control and treated cells were evaluated at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University using the MTT assay in triplicate. Briefly, liver carcinoma cell line (HepG2), was seeded in 96-well plates containing 100 1l of the growth medium. Cells were permitted to adhere for 24 h till confluence, washed with PBS, and then treated with the tested compound. Untreated cells used as negative control. Vinblastine sulfate was used as the positive control drug. Serial two-fold dilutions of the tested compound and reference compounds were added into a 96-well tissue culture plate using a multichannel pipette (*Eppendorf*, Germany). After treatment (24 h), the culture supernatant was replaced by fresh medium. Then the cells in each well were incubated at 37 °C with 100 1l of MTT solution (5 mg/ml) for 4 h. After the end of incubation the MTT soln. was removed, then 100 1l of DMSO was added to each well. The absorbance was detected at 570 nm using a micro plate ELISA reader (*Sun Rise TECAN, Inc*., USA).The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments.[22]

Data Analysis

The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows:

$$\% \text{ Cell viability} = (\text{Mean Abs control} - \text{Mean Abs test metabolite} \times 100) \div \text{Mean Abs control}$$

Where: Abs: absorbance at 590 nm.

The 50% inhibitory concentration (IC_{50}), the concentration required to kill or cause visible changes in 50% of intact tumor cells, was estimated from graphic plots. STATA statistical analysis package was used for the dose response curve drawing in order to calculate IC_{50}.

Determination of the Minimum Inhibitory Concentrations

This work was performed in Regional Center for Mycology and Biotechnology Antimicrobial unit, Al-Azhar University, Nasr City, Cairo, Egypt. The *MIC* was determined by the broth micro dilution method using 96-well micro-plates.[23] The inoculate of the microbial strains was prepared from 24 h broth cultures and suspensions were adjusted to 0.5 *McFarland* standard turbidity. Each sample (1.0 mg) was dissolved in DMSO (1 ml) to obtain 1000 1g/ml stock soln. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 1l) were added to the well from row B to H. The stock solutions of samples (100 1l) were added to the wells in rows A and B. Then, the mixture of samples and sterile broth (100 1l) in row B was transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 500, 250, 125, 62.5, 31.25, 15.6 and 7.81, 3.9, 1.95, 0.98 and 0.49, 0.24, 0.12 1g/ml). The inoculums (100 1l) were added to each well and a final volume 200 1l was obtained in each well. Plates were incubated at 37 °C for 24 h in case of antibacterial activity and 48 h at 25 °C for antifungal activity. Microbial growth was indicated by the presence of turbidity of the well. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (*MIC*).

References

[1] C. Navarro, A. Zarzuelo, J. Jim´enez, J. Duarte, J. Quevedo, *Fitoterapia* 1989, *60*, 277.
[2] G. Asghari, F. Zahabi, A. Eskandarian, H. Yousefi, M. Asghari, *Res. J. Pharmacogn.* 2014, *1*, 27.
[3] N. Q. M. Al-Hajj, H. X. Wang, C. Ma, Z. Lou, M. Bashari, R. Thabit, *Trop. J. Pharm. Res.* 2014, *13*, 1287.

 © 2017 Wiley-VHCA AG, Zurich, Switzerland

[4] F. E. Hanbali, M. Akssira, A. Ezoubeiri, C. E. A. Gadhi, F. Mellouki, A. Benherraf, A. M. Blazquez, H. Boira, *J. Ethnopharmacol.* 2005, *99*, 399.
[5] N. A. Ali, F. S. Sharopov, M. Alhaj, G. M. Hill, A. Porzel, N. Arnold, W. N. Setzer, J. Schmidt, L. Wessjohann, *Nat. Prod. Commun.* 2012, *7*, 257.
[6] M. Ravandeh, J. Valizadeh, M. Noroozifar, M. Khorasani-Motlagh, *J. Med. Plants Res.* 2011, *5*, 2035.
[7] M. M. A. Amer, M. F. Ramadan, W. Abd El-Gleel, *Deut. Lebensm.-Rundsch.* 2007, *103*, 320.
[8] R. M. A. Mansour, A. A. Ahmed, F. R. Melek, N. A. M. Saleh, *Fitoterapia* 1990, *61*, 186.
[9] N. A. M. Saleh, *Phytochemistry* 2003, *63*, 239.
[10] E. I. Karim, K. E. Ishag, A. A. B. Elegami, E. N. Mahmoud, I. Abu-Al-Futuh, *Fitoterapia* 1992, *63*, 281.
[11] E. A. Ewais, M. M. Abd El-Maboud, M. I. Haggag, *Rep Opin.* 2014, *6*, 27.
[12] M. F. Ramadan, M. M. A. Amer, H. T. Mansour, K. M. Wahdan, R. M. El-Sayed, S. El-Sanhoty, W. A. El-Gleel, *J. Verbrauchersch. Lebensmittelsich.* 2009, *4*, 239.
[13] L. Boulos, Flora of Egypt Checklist, Al-Hadara Publishing, Cairo, 1995.
[14] E. Gamal-Eldin, *Phanerog. Monogr.* 1981, *14*, 166.
[15] G. A. Fawzy, H. Y. Al Ati, A. A. El Gamal, *Pharmacogn. Mag.* 2013, *9*, 28.
[16] M. Al-Fatimi, N. A. A. Ali, M. Wurster, S. S. Al-Sokari, U. Lindequist, W. N. Setzer, *World J. Pharm. Res.* 2015, *4*, 1.
[17] G. Cristofari, M. Znini, L. Majidi, A. Bouyanzer, S. S. Al-Deyab, J. Paolini, B. Hammouti, J. Costa, *Int. J. Electrochem. Sci.* 2011, *6*, 6699.
[18] M. N. Algabr, S. Ameddah, A. Menad, R. Mekkiou, J.-C. Chalchat, S. Benayache, F. Benayache, *Int. J. Med. Arom. Plants* 2012, *2*, 688.
[19] H. H. El-Kamali, M. O. Yousif, O. I. Ahmed, S. S. Sabir, *Ethnobot. Leaflets* 2009, *13*, 467.
[20] B. K. Chhetri, N. A. A. Ali, W. N. Setzer, *Medicines* 2015, *2*, 67.
[21] R. P. Adams, 'Identification of Essential Oils Components by Gas Chromatography/Mass Spectrometry', 4th edn. Allured Publishing Corporation, USA, 2007.
[22] A. P. Wilson, 'Cytotoxicity and viability assays', in 'Animal Cell Culture: A Practical Approach', 3rd edn., Ed. J. R. W Masters, Oxford University Press, 2000.
[23] R. K. Saini, A. S. Choudary, Y. C. Joshi, P. Joshi, *E-J. Chem.* 2005, *2*, 224.

Received May 6, 2016
Accepted January 11, 2017

 © 2017 Wiley-VHCA AG, Zurich, Switzerland

View publication stats