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CHEMICAL COMPOSITIONS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL OF *TRACHYSPERMUM AMMI* L. GROWING IN TUNISIA

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In order to increase our knowledge about *Trachyspermum ammi* L., this work assesses the chemical composition as well as the *in vitro* biological activities of its essential oil. Thus, the essential oil obtained by hydrodistillation of the aerial parts of this species was analyzed by GC-FID and GC-MS. The main components of *T. ammi* L. oil were Thymol (60.90%), carvacrol methyl ether (18.93%), γ-terpinene (7.50 %), *p*-cymene (4.59 %), carvacrol (1.99 %) and α-pipene (1.31 %). The essential oil of *T. ammi* stood out for its antioxidant activity expressed on µg. mL^{-1}, 6.53 and 5.24, for DPPH and ABTS assays, respectively. Antimicrobial activity against several micro-organisms, including some ones infesting historical art craft, was also determined. These results suggested that essential oil of *T. ammi* processes antimicrobial and antioxidant properties, and are therefore a potential source of active ingredients for food and pharmaceutical industry.

INTRODUCTION

In this study, the attention was focused on *Trachyspermum ammi* (named ajwain caraway and also *Carum copticum* L.),[1] which is one of the most important genera within the *apiaceae* family. It is a highly reputable plant as a source of constituents with promising bioactivity to be exploited at pharmaceutical level. It is an annual herb up to 90 cm tall, native to arid and semiarid regions of the Mediterranean zone, notably Egypt.[2] It is also widely distributed and cultivated in Iraq, Iran, Afghanistan, Pakistan and India. The plant is a valuable herbal that has been used by humans in a variety of ways. *T. ammi* is known for its antiviral, anti-inflammatory, antifungal, molluscicidal, antihelminthic, plant nematicidal,

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antipyretic, antiaggregatory and antimicrobial activity.[3]

To the best of our knowledge, there are no published reports on phytochemical constituents of Tunisian *T. ammi* described here. This paper represents the first report of Tunisian *T. ammi* essential oil using gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC-FID). The evaluation of antioxidant and antimicrobial activities are also investigated. These data will offer a strong frame work for new discoveries, particularly the pharmaceutical, cosmetic and agri-food processing industries.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. ammi* were collected from the Institut National De La Recherche En Génie Rural Eaux et Forêts (INGREF) arboretum (Tunisia) in March 2015. Identification was performed in the Laboratory of Forest Ecology of INGREF by Pr. Youssef Ammari. A voucher specimen MO2015 is deposited in the Herbarium of this Laboratory. Leaves, branches and female cones were separated, dried at room temperature for 7 days, and used for analyses.

Essential oil isolation and analysis

The aerial part of *Trachyspermum ammi* was subjected to hydro-distillation in a Clevenger's type apparatus for 3 h for isolation of essential oils. The essential oils were measured directly in the extraction burette and content (%) was calculated as volume (mL) of essential oil per 100 g of dry plant material.[4] The oils were dehydrated over anhydrous $MgSO_4$ and kept in a cool and dark place prior to analysis. The essential oil was analyzed as previously described.[5] Identification of constituents was made as elsewhere reported.[6] Component relative concentrations were calculated based on GC peak areas without using correction factors.

Antioxidant activities

DPPH assay. The DPPH radical scavenging capacity was measured according to Boukhris *et al.*,[7] with some modification.[8,9] Each experiment was analyzed in triplicate.

ABTS assay. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM $(NH_4)_2S_2O_8$ and allowing the mixture to stand in the dark at room temperature for 16 h before use.[8] The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in $\mu g.mL^{-1}$) was calculated from the graph plotted with inhibition percentage against the extract concentration.

Antimicrobial activity

Test Microorganisms. The essential oil of *T. ammi* was tested against a panel of pathogenic microorganisms including *Staphylococcus aureus* ATCC 2319 (American Type Culture Collection, Rockville, MD, USA), *Escherichia coli* (ATCC8739), *Salmonella thyphimurium* (NCTC 6059) and *Pseudomonas aeruginosa* (ATCC 27850) as a reference strain and two clinical strains *Enterobacter* and *Klebsiella pneumonia* supplied by the microbiology laboratory of the regional hospital of Béja (Tunisia). The former strains were isolated, identified and characterized by conventional biochemical methods.[10] A fungi *Candida albicans* were also included in the study. Bacterial strains were cultured overnight at 37°C in blood agar plates (Oxoid, Basingstoke, UK). *C. albicans* was grown in Sabouraud Dextrose Agar (Oxoid). Tests were performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Tetracycline (10µg disc) was used as a positive control and discs with DMSO (5%, v/v) as negative control. Each experiment was performed in quadruplicate.

Antimicrobial screening. The minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using a broth micro-dilution method. Each experiment was performed in triplicate.

Data analysis

All determinations were conducted in triplicates and results for each measured parameter were expressed as mean ± SD. Quantitative differences was assessed by Tukey's test (at $P < 0.05$) followed by Dunn's post–hoc multiple comparison test (SPSS. v15).

RESULTS AND DISCUSSION

Essential oil yields and chemical polymorphism

The yield of the essential oil extracted by hydrodistillation from the dried aerial part of *Trachyspermum ammi* (*T. ammi*) growing in Tunisia was 2.4 g which represents 2.4 ± 0.21%. The value was, significantly, higher than those reported in literature data for the same species of *T. ammi*.

The chemical composition of *T. ammi* essential oil was investigated by means of GC-FID and GC-MS in order to determine its qualitative and quantitative profile (Fig. 1 a and b). Table 1 shows the composition of *T. ammi* essential oil sample by single compounds and then class of substances. The compounds are listed in order of elution. A total of twenty five compounds were identified, which constitute approximately 98.52% of the entire fraction. Data in Table 1 are expressed as relative percentage of the peak areas. Among identified components of ajwain oil (*T. ammi)*, thymol (60.90%) was the most abundant component, followed by carvacrol methyl ether (19%), γ-terpinene (7.50%), *p*-cymene (4.59%), carvacrol (1.99%) and α-pipene (1.31%). Phenols were the components present in the highest percentage: tymol with 60.90% while carvacrol methyl ether represented 18.93%, confirming that *Trachyspermum ammi* is a tymol chemiotype, according to literature data for this species in the world.[11,12]

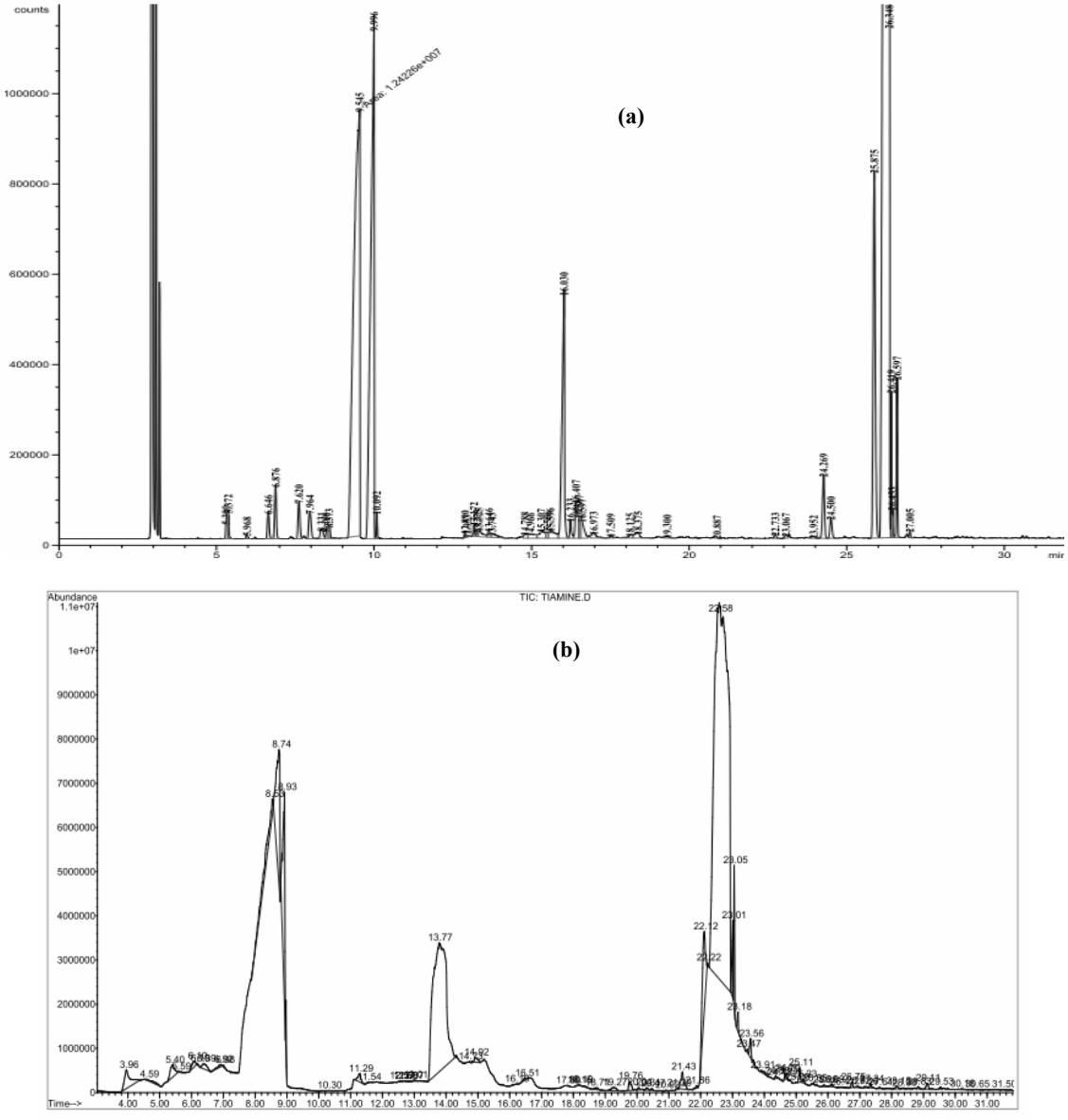

Fig. 1 – GC-FID and TIC (b) chromatograms of essential oil of *T.ammi*.

Table 1

Constituents identified from the essential oil of *T.ammi*

No.	RT(min)	Constituents	KI	Ps%
1	3.93	α-pipene	939	1.31
2	5.40	sabinene	980	0.96
3	6.39	β-terpinene	998	0.28
4	8.74	γ-terpinene	1042	7.50
5	8.93	*p*-cymene	1045	4.59
6	10.30	alloocimene	1070	0.02
7	11.29	Dehydro-*p*-cymene	1088	0.26
8	11.54	β-thujone	1093	0.04
9	12.87	α-thujone	1117	0.05
10	13.77	carvacrol methyl ether	1134	19
11	14.92	terpinene-4-ol	1155	0.20
12	16.19	camphene	1178	0.02
13	17.80	*cis*-piperitol	1207	0.02
14	18.19	Cuminal	1215	0.08
15	18.71	*cis*-dihydrocarvone	1224	0.07
16	19.76	*m*-ethylcumene	1243	0.25
17	20.04	Seudenone	1249	0.04
18	20.47	Safranal	1256	0.03
19	21.43	Piperitenone	1274	0.58
20	22.58	Thymol	1295	60.90
21	23.05	Carvacrol	1304	1.99
22	23.18	Methoxymesithylene	1306	0.20
23	25.23	Durophenol	1344	0.07
24	30.18	Isodurenol	1434	0.06
		Total		**98.52**
		Aromatic compounds		**87.41**
		Monoterpene hydrocarbons		**10.09**
		Monoterpene oxygenated		**0.53**
		Other compounds		**0.49**

KI: Kovalts index, Ps: mean percentage of each compound, RT: retention time.

Regarding the constituents of oils, these results showed some differences in composition with respect to data in literature, such as those reported by Park *et al.*,[11] These authors found that the *T. ammi* essential oil was characterized by the dominance of thymol (41.77 %) followed by γ-Terpinene (27.77 %) and *p*-Cymene (24.40 %). Our results were in accordance with those found by Vitali *et al.*, [13] who mentioned that the essential oil of *T. ammi* growing in Iran, analyzed by GC–FID and GC–MS, showing that thymol (67.4%), *p*-cymene (17.9%) and γ-terpinene (11.3%) as the major constituents. However, different chemical profiles for ajwain oil were recently reported from Iran[14] and India.[15] In these works, the authors found γ-terpinene (48%) and *p*-cymene (76.3%) more abundant than thymol (17.4% and 13.3%, respectively). We assume that these quantitative differences may be related to the geographic origin, genetic variability and harvesting time of the samples.[11,12]

As it was reported by Russo *et al.*,[16] who studied the *T. capitatus* essential oil and its antifungal activity, the biogenetic precursor of the phenols were present in a 3,52-6,08% range *p*-cimene and 2,69-3,71% γ-terpinene. The variations between the main compounds of thyme essential oil can be explained by the biosynthetic relationship between the two phenols. The metabolic pathways for the carvacrol and thymol formation begin with the autoxidation of γ-terpinene to *p*-cymene and the subsequent hydroxylation to thymol. Instead the carvacrol originates from unsaturation of γ-terpinene to *p*-cymene followed by the hydroxylation to C-2 aromatic ring. So is evident the key role played by γ-terpinene in the process of flavoring and by *p*-cymene as precursor of oxygenates compounds. On the other hand, and in a last research published in 1998, Russo and his co-workers[17] mentioned that γ-terpinene originates in the biosynthetic chain which, from acetil-Co A, leads to the synthesis of terpenoids through the formation of geranyl-pyrophosphate, just by cyclization of the latter.

Table 2

Antioxidant activities of Trachyspermum ammi aerial parts essential oil

	DPPH Assay (IC_{50} µg.mL^{-1})	ABTS Assay (IC_{50} µg.mL^{-1})
essential oil	**6.53 ±0.01**	**5.24±0.00**

Results are expressed as mean± standard deviation of 3 determinations.

Table 3

Antimicrobial activity of essential oil of T.ammi

		Essential oil			Tétracyclin[d] (10µg / disc)
	Strains	**[a]ZI (mm)**	**MIC[b] (%)**	**MBC/MFC[c] (%)**	**D (mm)**
Gram negative bacilli	***Escherichia coli***	45±2.52[e]	12.5	25	30
	Entérobacter	31.25±4.78[f]	25	50	28
	Klebsiella pneumoniae	16.25±3.20[g]	12.5	25	11
	Salmonella typhimurium	30±4.14[hf]	12.5	50	25
	Pseudomonas aeruginosa	27±1.04[i]	6.25	12.5	18
Gram positive cocci	***Staphylococcus aureus***	26.25±2.50[i]	25	50	15
Fungi	***Candida albicans***	*50 ±1.2*	25	50	17

[a] ZI, disc diffusion method as recommended by NCCLS. Diameter of zone of inhibition (mm) including disk diameter of 6 m [b]MIC was considered as the lowest concentration of each extract showing a clear zone of inhibition. [c] No bacterial/fungal growth on solid medium was taken as MBC/MFC. [d]Tetracyclin was used as a positive control. Means (±SD) within the same row are significantly different at *CI=95%.*

Antioxidant activities

Antioxidant activities of the essential oils of *T. ammi* oil were tested by the DPPH radical scavenging and ABTS radical cation. As reported in Table 2, the *T. ammi* essential oil had a good antioxidant activity, 6.53 and 5.24 µg. mL^{-1} for DPPH and ABTS assays, respectively. These minor differences in the scavenging activities may be attributed to the intrinsic mechanisms of the radical antioxidant reactions in the different assays, or to factors such as stereo-selectivity of the radicals and the solubility of antioxidant components. Our data are in accordance with those previously reported for the *T. ammi* oil,[18,19] where a noticeable free radical scavenging and antioxidant activity is shown, and confirm that *T. ammi* is a natural source of important antioxidant substances to be used as efficient antioxidant agents comparable to commercially used antioxidants.

It is worth noting that the antioxidant activity of essential oil could be attributed to their chemical composition which is determined by the genotype and influenced by environmental and agronomic conditions,[20,21] The relationship between the antioxidant activity and their chemical profiles was previously reported by Barra *et al.*[22] Generally the antioxidant activities of plant essential oils were attributed to their major compounds.[23] Thus, the level of the antioxidant activity for the studied species may be attributed primarily to the concentration of phenolic compounds, *i.e*, thymol and carvacrol with its derivatives. These results were in accordance with Bounatirou *et al.*[24] and Sokmen *et al.*,[25] who mentioned that essential oils rich in phenolic compounds such as carvacrol and thymol had strong antioxidant potentials in the case of *Thymus capitatus* Hoff. and Link,[24] and *Origanum acutidens* Hand.-Mazz.[25]

Antimicrobial activities

T.ammi EO exhibited a great antimicrobial potential against whole Gram negative bacilli and Gram positive cocci bacteria and yeast, *C.albican* (Table 3). The maximum growth inhibitions of tested microorganisms were in the range of 16.25 to 50 mm. *C.albicans* and *E.coli* were the most sensitive strains with an inhibition zones of 50±1.2 mm and 45±2.52 mm respectively, against only 30 mm and 17 mm for tetracycline. The minimal inhibitory activity was recorded with *K.pneumonia* at 6.25%, for which the inhibition zone did not exceed 17 mm but still significantly higher (p<0.05) than that of the positive control that exhibited a ZI of 11 mm. It is worth mentioning that for all tested microorganisms the ZI of *T.ammi* essential oil highly exceed that of tetracycline used since the ancient times as a strong antibiotic against different kind of infections,[26] which is promising. Nonetheless, diverse publications have shown that the clinical outcome of treatments with this agent is limited due to the development of drug resistance and the high side effects of the treatment.[27] Moreover, we noticed a positive inhibitory effect of the essential oil extract on the highly multidrug resistant bacteria: *P. aeroginosa* for which the minimal bactericidal concentration was only 12.5% and a ZI of 27±1.04 mm. Previous works,[11] has demonstrate that various compounds, including alcohols, aldehydes, fatty acid derivatives, terpenoids and phenolics that exist in plant Jointly or independently, contribute to antibacterial or antifungal activity of their by-products. In the present study and based on the GC result, the strong antimicrobial activity could be attributed essentially to its dominant component: Thymol which is well-known chemical with a pronounced antimicrobial potential that could probably act on the membrane integrity either for bacteria or for fungi. Antimicrobial activities of γ-terpinene and *p*-cymene have also been previously reported by other investigator.[28] Nevertheless this cannot neglect the contribution of phenolic molecules present in *T.ammi* EO. Previous works,[29] has shown that the site and the number of hydroxyl groups on the phenol components increases the toxicity against the microorganisms. Our result is in accordance with that of Hassanshahian *et al.*,[30] who studied the antimicrobial activity of *T.ammi* essential oil from seeds . However it disagreed with the result of Senator *et al.*,[31] who investigated the essential oil from aerial parts of *Crithmum maritimum* L. (Apiaceae) growing in Turkey. The former authors had demonstrated that the oil have a appreciable activity, mostly against Gram positive bacteria. This could be due to the difference in yield and composition of secondary metabolites that highly depend on physiological variations, environmental conditions, geographic variations and genetic factors. Our results are promising since the essential oil is able to inhibit the growth of both Gram positive and Gram negative bacteria and fungi. Indeed Gram positive and Gram negative bacteria are both affected by the emergence and rise of antimicrobial resistance. As this problem continues to grow, the updating of knowledge concerning treatment of disease caused by such pathogens is required. Further studies will be necessary to understand the mechanisms of action underlying the effects of the extract and its active compounds.

CONCLUSIONS

To the best of our knowledge, the essential of *Trachyspermum ammi* L. aerial part growing in the North of Tunisia (Mateur), was not the subject of previous studies. Thus this is the first report on the volatile composition of this genus. The essential oil showed a very high antioxidant activity when measured by DPPH and ABTS radicals. In addition, this essential oil reveals a very important *in vitro* antimicrobial activity on the studied bacterial, confirmed by a low minimum inhibitory concentration (MIC). Our results, therefore, suggest that the *T ammi* could be used as a natural antimicrobial agent for the treatment of several infection diseases.

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REFERENCES

1. R. Bairwa, R. S. Sodha and B. S.Rajawat, *Pharmacogn. Rev.,* **2012**, *6*, 56.
2. M. Ashraf, *Crit. Rev. Plant. Sci.,* **2002**, *2*, 1.

3. N. Mathew, S. Misra-Bhattacharya, V. Perumal and K. Muthuswamy, *Molecules,* **2008,** *13*, 2156.
4. "Council of Europe European Pharmacopoeia", third edition, Council of Europe, Strasbourg, 1997.
5. M. Boukhris, M. Bouaziz, I. Feki, H. Jemai, A. El Feki and S. Sayadi, *Lipids Health Disease,* **2012**, *11*, 81.
6. J. Valente, M. Zuzarte, R. Resende, M. J. Goncalves, C. Cavaleiro, C. F. Pereira, M. T. Cruz and L. Salgueiro, *Ind. Crops Prod.,* **2015**, *65*, 361.
7. M. Boukhris, M. S. J. Simmonds, S. Sayadi and M. Bouaziz, *Phytother. Res.,* **2013**, *27*,1206.
8. G. Rigane, R. Ben Salem, S. Sayadi and M. Bouaziz, *J. Food Sci.,* **2011**, *76*, 965.
9. A. M. Oueslati, G. Rigane, H. Ghazghazi, R. Ben Salem, S. Hamdi, M. Hannachi, H. Jouili and Y. L'ammari, *J. New Sci.*, **2016**, *25*, 1151.
10. M. S. Khan, A. Zaidi, P. A. Wani and M. Oves, *Environ. Chem. Lett.*, **2009**, *7*, 1.
11. I. K. Park, J. Kim, S. G. Lee and S. C. Shin, *J. Nem.,* **2007**, *39*, 275.
12. I. Ashrafi Tamai, T. Zahraei Salehi, A. R. Khosravi, A. Sharifzadeh and A. Balal, *J. Med. Plants Res.,* **2013**, *12*, 137.
13. L. A. Vitalia, D. Beghelli, P. C. Biapa Nya, O. Bistoni, L. Cappellacci, S. Damiano, G. Lupidi, F. Maggi, G. Orsomando, F. Papa, D. Petrelli, R. Petrelli, L. Quassinti, L. Sorci, M. M. Zadeh and M. Bramucci, *Arab. J. Chem.,* **2015**, *in press.*
14. M. R. Moein, K. Zomorodian, K. Pakshir, F. Yavari, M. Motamedi and M. M. Zarshenas, *J. Evid. Based Complementary Altern. Med.,* **2015**, *20*, 50.
15. A. Kedia, B. Prakash, P. K. Mishra, A. K. Dwivedy and N. K. Dubey, *Ind. Crops Prod.,* **2015**, *69*, 104.
16. S. J. Russo and E. J. Nestler, *Nature Rev. Neur.*, **2013**, *14*, 609.
17. M. T. Russo, G. C. Galletti, P. Bocchini and A. Carnacini, *J. Agric. Food Chem.,* **1998**, *46*, 3741.
18. B. Nickavar, A. Abrisham Adeli and A. Nickavar, *Iran. J. Pharm. Res.,* **2014**, *13*, 127.
19. N. Goswami and S. Chatterjee, *Bio. Med. Res. Int.,* **2014**, *582767*, 1.
20. S. Burt, *Int. J. Food Microbio.,* **2004**, *94*, 223.
21. E. Derwich, R. Chabir, R. Taouil and O. Senhaji, *Int. J. Pharm. Sci. Drug Res.,* **2011**, *3*, 130.
22. A. Barra, V. Coroneo, S. Dessi, P. Cabras and A. Angioni, *Nat. Prod. Commun.,* **2010**, *5*, 329.
23. L. Riahi, H. Chograni, M. Elferchichi, Y. Zaouali, N. Zoghlami and A. Mliki, *Ind. Crops Prod.,* **2013**, *46*, 290.
24. S. Bounatirou, S. Smiti, M. G. Miguel, L. Faleiro, M. N. Rejeb, M. Neffati, M. M. Costa, A. C. Figueuredo, J. G. Barroso and L. G. Pedro, *Food Chem.,* **2007**, *5*, 146.
25. M. Sokmen, J. Serkedjleva, D. Dalerera, M. Gulluce, M. Pollsslou, B. Tape, H. A. Akpulat, F. Sahin, and A. Sökmen, *J Agric Food Chem,* **2004**, *52*, 3309.
26. M. D. Vanessa, E. K. Christine, K. Lindsay, M. Mariya, W. L. S. Wilson, S. Carsten, F. Duane, Z. Grant, C. Fabrice, D. Regis, G. Brian, N. P. Hendrik and D. W. Gerard, *Nature*, **2011**, *477*, 457.
27. G. G. Nahum, K. Uhl and D. L. Kennedy, *J. Obs. Gyneco.*, **2006**, *107,*1120.
28. I. H. Choi, J. H. Kim, S. C. Shin and I. K. Park, *Russ. J. Nema.,* **2007**, *15*, 35.
29. M. M. Cowan, *Clin. Microbiol. Rev.,* **1999**, *12*, 564.
30. M. Hassanshahian, Z. Baya, S. Saeidi and Y. Shiri, *Int. J. Adv. Biol. Biom. Res.,* **2014**, *2*, 18.
31. F. Senatore, F. Napolitano and S. Ozcan, *Flav. Frag. J.,* **2000**, *15*, 186.