Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Chemical Composition, Antimicrobial and Antitumor Potentiality of Essential Oil of *Ferula tingitana* L. Apiaceae Grow in Libya

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Submitted: 20-07-2015 Revised: 03-09-2015 Published: 11-10-2017

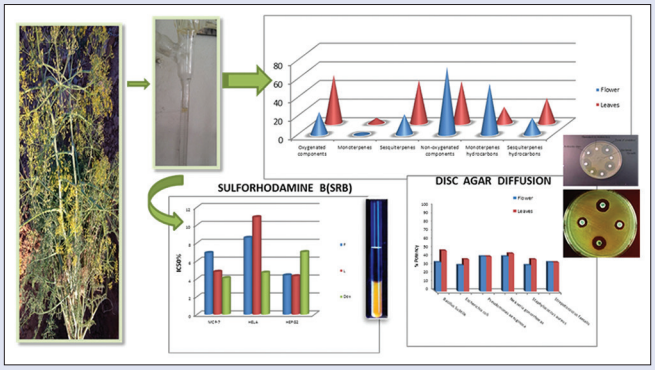
ABSTRACT

Background: *Ferula tingitana* L. (Apiaceae) has been considered to have abortive and menstruation-inducing properties. It used to treat sore throat, fever, indigestion, and pains. **Objectives:** The objective of this study is to establish the chemical composition of the essential oil of flower, leaves of *F. tingitana,* and to throw light on antimicrobial, cytotoxic activities of Libyan plant. **Materials and Methods:** The chemical composition of the essential oil of flower (0.06% w/v) and leaves (0.1% w/v) of *F. tingitana* was comparatively analyzed by gas chromatography/mass spectrometry using nonpolar column DB-5. **Results:** A total of 28–32 components were identified, 15 being common in both samples. The main constituents of both flower- and leave-derived oil samples were α-thujene (13.5%–2.3%), elemol (8.9%–8.3%), eudesmol (0.6%–9.7%) and cadinol (2.2%–13.8%), respectively. The principle difference was a considerably more pronounced sesquiterpenes presence in the leaves-oil, amounting to 74.0%, than in the flower counterpart (39.9%). Caryophyllene (5.6%) and elemol (8.9%) were the major sesquiterpenes detected in flower-oil while leaves-oil showed less amounts of sesquiterpenoid hydrocarbons (27.7%) and represented by eudesmadiene (9.0%). On the contrary, while remaining the dominant group in both oil samples, monoterpenoids are relatively more abundant in flower-derived oil constituting 57.7% versus 24.5% detected in leaves. **Conclusion:** Leaves-oil sample being mostly efficient as antibacterial against *Bacillus subtilis* and *Neisseria gonorrhoeae* with potency 48.3, 41.9% compared to tetracycline standard antibacterial drug. The essential oil samples revealed marked *in vitro* cytotoxicity against breast (MCF7), cervical (HELA) and liver(HEPG2) carcinoma cell lines with IC50% (6.9, 4.8), (8.6, 10.9), and (4.4, 4.2) for the flower-, leaves-derived oil sample, respectively.

Key words: Antimicrobial, apiaceae, cadinol, cytotoxicity, elemol, *Ferula tingitana* L

SUMMARY

- Comparative analysis of the investigated oil samples indicates more pronounced monoterpene contents in *Ferula tingitana* flower. The principle monoterpenoid constituents of both oils are α-thujene. Sesquiterpenes presence is considerably more pronounced in the leaves-derived oil sample
- Elemol, cadinol, α-thujene and α-terpinoline, the major components of the flower- and leaves-derived oil in the present work and were not detected in the Turkish sample. The differences may be due to climatic and other extrinsic conditions such as where and when the samples were collected
- When screened for antimicrobial activity, the flower- and leaves-derived oil of *F. tingitana* exhibited a mild effect against all tested Gram-negative and Gram-positive microorganisms. However, not displayed growth inhibitory effect against the fungus *Aspergillus flavus* and *Candida albicans*
- On assessing the cytotoxic activity, the flower- and leaves-derived oil exhibited specific and significant effects on the viability of the selected human cell lines, viz., hormone-responsive breast carcinoma cell line (MCF7), cervical carcinoma cell line (HELA), and liver carcinoma cell line (HEPG2).



Abbreviations used: F: Flower-derived oil of *F. tingitana*; L: Leaves-derived oil of *F. tingitana* ; IPP: Isopentenyl pyrophosphate or also isopentenyl diphosphate; DMAPP: Dimethylally pyrophosphate or also dimethylallyl diphosphate; GPP: Geranyl pyrophosphate; GGPP: Geranylgeranyl pyrophosphate; MEP: Methylerythritol phosphate pathway; FPP: Farnesyl pyrophosphate; GC/MS: Analysis gas chromatography/mass spectroscopy; SRB: Sulforhodamine B.

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DOI: 10.4103/pm.pm_323_15

Access this article online
Website: www.phcog.com
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INTRODUCTION

Since the middle ages, essential oils have widely been used for bactericidal, virucidal, fungicidal, medicinal, and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, and agricultural and food industries. Because of the mode of extraction, mostly by hydrodistillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids phenol-derived aromatic components, and aliphatic components.[1] Apiaceae is one of the important flowering plant families that are scattered around the world. This family includes 420 genera, 3100 species. *Ferula* is one the important genera in this family which consists 133 species distributed throughout Mediterranean area and central Asia.[2-4] More than 70 species of *Ferula* have already been investigated chemically leading to the fact

Cite this article as: Elghwaji W, El-Sayed AM, El-Deeb KS, ElSayed AM. Chemical composition, antimicrobial and antitumor potentiality of essential oil of *Ferula tingitana* L. apiaceae grow in Libya. Phcog Mag 2017;13:S446-51.

that germacranes, humulanes, carotanes, and guaianes represent the main sesquiterpene constituents of the genus.[5-13] Several species of this genus have been used in folk medicine.[6] α-pinene and *β*-pinene are reported as a major constituent of most studied *Ferula* taxa and considered as chemotype of *Ferula*.[14] Giant fennel (*Ferula communis* L. characterized by two chemotypes with different biological activities. One chemotype is poisonous, due to prenyl coumarins, and responsible for ferulosis, which mainly affects sheep and goats, cattle, and horses; the other chemotype is nonpoisonous and contains daucane esters. The two chemotypes cannot be distinguished botanically. Major volatiles in the poisonous chemotype the sesquiterpenes aristolene and farnesol while in the nonpoisonous chemotype, the main component was the sesquiterpene allohedycaryol.[15] For rapid and unequivocal discrimination of the two chemotypes, aristolene and allohedycaryol had been used as markers of the poisonous and nonpoisonous *Ferula* chemotypes, respectively.[16] *Ferula tingitana* L. (giant Tangier fennel) is a tall perennial herb. It has alternate leaf arrangement and yellow, unisexual flowers which, like other Apiaceae, grow in umbels. It grows in scrubland and rocky areas. Its range is the Mediterranean coast[17] in Spain, Morocco, Lebanon, Isral, Cyprus, and Turkey.[18-20] Sesquiterpene ester, tingitanol and sesquiterpene coumarin ethers coladonin, feselol isosamarcandin angelate, and daucane esters were isolated from the root.[21-23] *F. tingitana* has been considered to have abortive and menstruation-inducing properties.[24] The species has been suggested as a possible identity for the controversial silphium a plant used as a spice and for various medical purposes in the Mediterranean region.[25] It was said that it could be used to treat cough, sore throat, fever, indigestion, aches and pains, warts, and all kinds of maladies.[26] However, reports neither on composition nor on the bioactivity of the essential oil of Libyan plant could not be traced in the available literature. The aim of the study is to establish the chemical composition of the essential oil of flower, leaves of *F. tingitana* and to throw light on antimicrobial, cytotoxic activities of Libyan plant. Furthermore, compare between Libyan ferula and other varieties (Turkish and Iranian).

MATERIALS AND METHODS

Plant material

Samples of the flower and leaves of *F. tingitana* L., collected in April 2013 from Mislata city which far away from Tripoli by 100 km. Libya, and identified by Dr. Reem Samir Hamdy, Lecturer of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Giza, Egypt. A specimen of the plant was deposited at the Faculty of Pharmacy, Cairo University as a reference material (2142013).

Preparation, characterization, and analysis of the essential oil

The flower and leaves of *F. tingitana* L. (500 g) of each were subjected to hydrodistillation. The percentage yield was calculated on fresh weight basis according to the Egyptian Pharmacopœia (2005).[27] The essential oil was dried over anhydrous sodium sulfate and kept refrigerated until analysis.

Sample preparation

Aliquots (5 µl, each) of the dehydrated essential oils were, separately, mixed with approximately 1 ml of CH_2Cl_2 in autosampler vials.

Gas chromatography/mass spectrometry analysis

Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oil was performed using a Thermo Trace GC 2000 (Thermo Quest, TX, USA)/MS Finnigan mat SSQ7000 system. The instrument was equipped with a DB-5 column (30m × 0.25 mm i.d., 0.25 µm film thickness); J&W Scientific, USA.

Operating conditions

Injection volume, 1 µl of CH_2Cl_2 solution of tested samples; oven temperature programming: initial temperature, 40°C (isothermal for 3 min), then increased (4°C/min) to 160°C, followed by further increased to final temperature 280°C (10°C/min); injection temperature: 220°C carrier gas: helium at a flow rate of 1 ml/min; mass spectrometer, electronic ionization mode; ion source, 70 eV; mass range: 40–500 amu.

Identification of the essential oil constituents

This was achieved by library search on a Wiley 275 L GC/MS database, observed Kovats index and by comparing the retention indices and mass fragmentation patterns to those of the available references as well as of published data.[28] The quantitative estimation was carried out by peak area measurement. A series of authentic *n*-alkanes was subjected to GC under the same experimental conditions, and the retention indices of the oil constituents were calculated. The individual components were determined by computerized peak area measurement. Compounds of the essential oil, their retention indices, and relative percentage composition are compiled [Tables 1 and 2].

Evaluation of antimicrobial activity

The disc agar diffusion method[29] was adopted, and zone of inhibitions measured in mm. Results are recorded in Table 3. Potencies relative to the appropriate antibiotic tetracycline and amphotericin B are represented in Table 3.

Microorganisms, culture media, and standard antimicrobial agents

The antimicrobial activity was performed against a set of 8 representative Gram-positive and negative bacterial and two fungal strains of standard properties [Table 3]. These were maintained in the Micro Analytical Center, Faculty of Science, Cairo University. The tested Gram-positive bacteria were (*Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 12600 and *Streptococcus faecalis* ATCC 19433). The Gram-negative bacteria included (*Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 11775, and *Neisseria gonorrhea* ATCC 19424), and fungi (*Aspergillus flavus* and *Candida albicans* ATCC 26555). The tested bacteria were grown on nutrient agar (Oxoid, England) and fungi on Sabouraud's glucose agar (Oxoid, England). Tetracycline (Oxoid, UK) and Amphotericin B (Sigma Chemical Co., St. Louis, MO, USA) were used as standard antibacterial and antifungal agents.

Antimicrobial assay

The prepared essential oil of the flower and leaves were separately tested against the selected strains at concentration of 20 mg/ml adopting the disc agar diffusion method.[29] Discs were impregnated with tetracycline and amphotericin B as antibacterial and antifungal standards, respectively. Test solutions were prepared by dissolving in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/ml; aliquots, 10

Table 1: Yield and physical characters of volatile oil of *Ferula tingitana* L

Characters	Oil samples	
	F	L
Color	Yellow	
Odor	Aromatic	
Optical rotation	+4.7	+4.5
Specific gravity	0.9	0.9
Percentage of yield based on fresh weight v/w	0.06	0.1

F: Flower-derived oil of *Ferula tingitana*; L: Leaves-derived oil of *Ferula tingitana*

Table 2: Identified components in the hydrodistilled essential oil of flower and leaves of *Ferula tingitana* L

Compound[a]	RI[b]	F	L
Cyclofenchene	896	1.2	1.3
Thujene <alpha>	930	13.5	2.3
Pinene <alpha>	935	-	4.9
Sabinene	975	7.5	-
Myrcene <alpha>	990	8.1	-
Phellandene <alpha>	1002	-	0.7
3-carene	1008	13.9	-
Limonene	1029	-	2.1
n-pentylcyclopentane	1033	0.7	2.3
Penta methylcyclopentadiene	1044	4.2	5.2
3-nonenol	1152	-	0.3
Ocimene <beta>	1056	4.6	0.7
Terpinolene <alpha>	1086	1.1	5.2
Terpinene-4-ol	1177	1.0	0.4
Decanal	1201	0.3	1.8
Carveol <trans>	1215	0.5	-
Fenchyl acetate	1218	0.5	0.6
Perillyl alcohol	1294	0.3	-
Copaene <alpha>	1374	0.6	-
α-isocomene (berkheyaradulene)	1387	3.2	-
Cubebene	1389	2.2	0.8
β-bourbonene	1387	-	0.8
Dodecanal	1408	0.3	0.5
β-ylangene	1410	-	4.2
Gurjunene <alpha>	1412	0.5	-
Caryophyllene	1417	5.6	-
Junipene	1427	3.1	-
Aromadendrene	1439	-	2.8
α-Humulene <alpha>	1452	-	1.4
Isoaromadendrene	1460	0.5	-
Clovene	1465	-	4.8
Elemene <alpha>	1477	-	4.9
Bicyclogermagene	1485	-	2.6
7-α-Eudesma-3,5-diene	1489	-	9.0
Muurolene <alpha>	1500	0.5	-
Cuparene	1504	1.4	-
Bisabolene <beta>	1505	0.4	1.8
Elemol	1548	8.9	8.3
Germacrene D-4-ol	1574	1.1	7.7
Caryophyllene oxide	1582	0.7	-
Epi-globulol	1590	-	2.2
Guaiol	1600	5.6	-
γ-eudesmol	1630	0.6	9.7
δ-cadinol	1638	2.3	13.8
Bulnesol	1670	2.2	-
Identified components		97.2	97.9
Oxygenated components		24.4	52.4
Monoterpenes		2.5	5.9
Sesquiterpenes		21.9	46.5
Nonoxygenated components		72.8	45.5
Monoterpenes hydrocarbons		54.8	18.0
Sesquiterpenes hydrocarbons		18.0	27.5

[a]Compound listed in order of their reported RI; [b]RI measured relative to *n*-alkanes on DB-5 column. F: Flower-derived oil of *Ferula tingitana*; L: Leaves derived oil of *Ferula tingitana*; RI: Retention indices

µl each, were aseptically transferred into sterile discs of Whatman filter paper 8 mm diameter. Results are recorded [Table 3] as a Mean zone of inhibition in mm.

Evaluation of cytotoxic activity

Potential cytotoxicity of the essential oils of *F. tingitana* was tested using sulforhodamine B (SRB) method of Skehan *et al.*[30-32] Human tumor cell lines: hormone-responsive breast (MCF7), cervical (HeLa), and liver carcinoma (HePG2) were used and maintained in the laboratory of the Cancer Biology Department of National Cancer Institute, Cairo, Egypt.

In vitro cytotoxicity screening

Samples of flower-, leaves-derived oil at different concentrations (0.0–50.0 µg/ml) in DMSO were tested for cytotoxicity, against the aforementioned human tumor cell lines adopting SRB stain assay. The relation between surviving fractions and oil concentration was plotted to get the survival curve of each tumor cell line after the application of the specific concentration. The results were compared to those of the standard cytotoxic drug, doxorubicin (10 mg Adriamycin hydrochloride, in 5 ml intravenous injection, Pharmacia, Italy) at the same concentrations. The dose of the test solutions which reduces survival to 50% (IC_{50}) was calculated [Table 4].

RESULTS

Hydrodistillation of the flower and leaves of *F. tingitana* were yielded 0.06% w/v and 0.1% w/v, respectively of clear yellow-colored oil exhibiting a characteristic agreeable odor. The yield of leaves derived oil exceed that obtained from flower about two times as flower-oil sample as depicted in Table 1.

In total, 32 components could be identified in flower and 28 in leaves, corresponding to 97.2% and 97.9% of the oil derived from the respective organs. To the best of our knowledge, this is the first report dealing with the essential oil composition of Libyan *F. tingitana* flower as opposed to the leaf-derived volatiles.

Comparative analysis of the investigated oil samples, as presented in Table 2, indicates more pronounced monoterpene contents in *F. tingitana* flower reaching 57.3% while the leaf-derived oil contains 23.9%. The principle monoterpenoid constituents of both oils are α-thujene (13.5% and 2.3%, for flowers and leaves, respectively) and α-terpinolene (1.1%, 5.2%). Sesquiterpenes presence is considerably more pronounced in the leaves-derived oil sample, amounting 74.0% than in its flower counterpart containing 39.9% of C_{15} terpenes. The presence of a higher concentration of sesquiterpenes in both oils can induce their usage as fixative essential oils for the formulation of fragrances. Cadinol (13.8%), elemol (8.3%) and germacreneD-4-ol (7.7%) were the major identified oxygenated sesquiterpenes in leaves-derived oil. Eudesma-3,5diene (9.0%) and Eudesmol (9.7%) prevailed in leaves-derived oil while the latter is minor quentities in flower-derived oil (0.6%).

In the flower-derived oil, ten sesquiterpene hydrocarbons, amounting to 18.0%, were identified. *β*-caryophyllene, showing the highest percentage (5.6%), is accompanied by minor quantities of functionalized sesquiterpenes with the same basic structure, namely, *β*-caryophyllene oxide (0.7%), which are detected only in the flower-derived oil. Flowers have a high risk of pathogen attack because of their rich nutrient, moisture content, and high frequency of insect visitors. (E)-*β*-caryophyllene, thus, appears to serve as a defense against pathogens that invade flower tissues.[33] Caryophyllene and its oxide report properties of pharmacological interest as anti-inflammatory, antitumor, antibacterial, and antiseptic.[34,35] On the contrary, while remaining the dominant group in both oil samples under investigation, monoterpenes are relatively more abundant in flower-derived oil constituting 57.3% as opposed to 23.9% detected in leaves.

This observation could be accounted for the formation of active isoprene units-basic C_5 terpenes building block. Isoprene itself does not undergo the building process, but rather activated forms, isopentenyl pyrophosphate (IPP or also isopentenyl diphosphate), and dimethylallyl pyrophosphate (DMAPP or also dimethylallyl diphosphate), are the components proceeding through two alternative pathways.

Table 3: Results of the antimicrobial testing of the essential oil of *Ferula tingitana* L

Microorganisms	Diameter of inhibition zone in mm/(% potency)			
	F	L	Tetracycline	Amphotericin B
Bacillus subtilis ATCC 6051	10 (34.5)	14 (48.3)	29 (100)	-
Staphylococcus aureus ATCC 12600	9 (32.1)	11 (39.3)	28 (100)	-
Streptococcus faecalis ATCC 19433	10 (32.3)	10 (32.3)	31 (100)	-
Pseudomonas aeruginosa ATCC 10145	12 (40)	12 (40)	30 (100)	-
Escherichia coli ATCC 11775	9 (30)	11 (36.7)	30 (100)	-
Neisseria gonorrhoeae ATCC 19424	12 (38.7)	13 (41.9)	31 (100)	-
Aspergillus flavus	0.0	0.0	-	17 (100)
Candida albicans ATCC 26555	0.0	0.0	-	20 (100)

F: Flower-derived oil of *Ferula tingitana*; L: leaves-derived oil of *Ferula tingitana*

Table 4: IC50% of the essential oil of flower and leaves of *Ferula tingitana* L. compared to standard doxorubicin

Tested human cell line	IC_{50} µg/ml		
	Doxorubicin	F	L
Breast carcinoma MCF-7	4.1	6.9	4.8
Cervical carcinoma HeLa	4.7	8.6	10.9
Liver carcinoma HePG2	7.0	4.4	4.3

F: Flower-derived oil of *Ferula tingitana*; L: Leaves-derived oil of *Ferula tingitana*

In plants, both the cytosolic mevalonate (MVA) and the plastidic methylerythritol phosphate (MEP) pathways generate the five-carbon compound IPP and its isomer DMAPP. Plants have enzymes called terpene synthases, which catalyze the formation of diverse hemi-, mono-, sesqui-, and diterpene, plant volatiles from DMAPP, geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP), respectively.[36]

In cytosol, the condensation of one DMAPP molecule with two IPP molecules results in FPP, the MVA pathway[37] localized in the cytosol, provides the isoprene units for sesquiterpenes biosynthesis.

A plastidic prenyltransferase synthesizes GPP from the condensation of one IPP molecule and one DMAPP molecule. A second type of plastidic prenyltransferase condenses DMAPP with three IPP molecules to produce GGPP, the plastid-localized MEP pathway, is though to feed the biosynthesis of monoterpenoids and diterpenoids, which is initiated from C5-sugars. In different plant tissues, monoterpenes are biosynthesized exclusively through (MEP) pathway whereas sesquiterpenes are generated by the classical mevalonic acid pathway as well as by the MEP route.[38]

The aforementioned cell compartmentation[39] seems to be in accord with the postulated direct light interference with the non-MVA pathway[40] and consequently with the obviously prevalent cross-talk between the cytoplasmic and plastidial biosynthetic routes.[41] Hence, the significantly higher amount of C_{15} terpenoids observed previously in the plant organs less photosynthetically active and less exposed to the light.

Light may directly interfere with the regulation of the MEP pathway and with the cross-talk between the cytoplasmic MVA and the plastidial MEP pathways.[42] Activation of the MEP pathway by light is documented by rapid emission of isoprenoids known to be derived from MEP pathway on irradiation of green tissue, isoprene, and monoterpenes.[43] Rates of biosynthesis of plant volatiles in leaf were highest when leaves are young, not fully expanded and need the most protection, which plant volatiles provide directly because of their toxicity or indirectly through the summoning of herbivores' predators.[44-46] High rates were also observed when flowers are ready for pollination, and they decrease drastically after fertilization.[45,47] Biosynthetic rates are correlated with levels of transcripts of genes encoding the final biosynthetic enzymes, or the concentration of the substrates of these enzymes, or both.[45,48] In *F. tingitana*, leaves–derived oil contain C_{15} terpenes oxygenated in higher amount than in the counterpart its flower which may be explained by the distribution of the foliage.

DISCUSSION

Comparing the chemical composition of hydrodistilled of flower- and leaves-derived oil of *F. tingitana,* α-thujene, α-terpinoline, decanal, fenchyl acetate, elemol, cadinol, and eudesmol were present in both samples in comparable amounts. Carene, ocimene, myecene, and caryophyllene were detected in flower-derived oil not detected in leaves-oil.

An alternative goal of the hereby presented study was to draw conclusion as to the presumed disparities between our observation and previously published data concerning the chemistry of *F. tingitana.* To the best of our knowledge, this is the first report on the chemical composition of the essential oil of *F. tingitana* flower and leaves growing in Libya. Nevertheless, the composition of the essential oil from *F. tingitana*, cultivated in different regions of Turkey, was previously studied.[49] It was rich in monoterpenes represented by Naphthalene (15%), α-Pinene (11%). Meanwhile, sesquiterpenes were predominated by Daucene (6%) (Z)-*β*-Farnesene (5%) Germacrene D (5%).[49,50] On the contrary, naphthalene (absent in the Libyan sample). Moreover, the hydrocarbons, *β*-caryophyllene, elemene, α-humulene, and eudesma – 3, 5 diene were present Libyan samples. Moreover, the hydrocarbon, α-pinene were present in both Turkish[49] and Libyan *Ferula* in comparable amounts. α-pinene and *β*-pinene are reported as a major constituent of most studied *Ferula* taxa, and considered as chemotype of *Ferula*.[14]

Elemol, cadinol, α-thujene, and α-terpinoline, the major components of the flower- and leaves-derived oil in the present work and, respectively, were not detected in the Turkish sample.[49] The differences may be due to climatic and other extrinsic conditions such as where and when the samples were collected.

When screened for antimicrobial activity, the flower- and leaves-derived oil of *F. tingitana* exhibited a mild effect against all tested Gram-negative and Gram-positive microorganisms as compared to the standard antibacterial drug, tetracycline [Table 3]. However, tested essential oils not displayed growth inhibitory effect against the fungus *A. flavus* and *C. albicans* as compared to the standard antifungal drug, amphotericin B. The leaves-derived oil is slightly more effective against Gram-negative and Gram-positive bacteria than oil of flower. Antimicrobial activity of essential oil is difficult to correlate to a specific compound due to their complexity and variability and in general, is attributed to phenolic hydroxyl groups able to form hydrogen bonds with active site of target enzymes, although other active terpenes, alcohols, aldehydes, and esters can contribute to the overall antimicrobial effect of essential oil.[51]

The oil samples showed moderate antibacterial activity on *B. subtilis* and *N. gonorrhoeae*. *B. subtilis*, which are multiple antibiotic bacteria, because it is a biogenic amine procedure in food.[52] Furthermore, *B. subtilis* has become an important agent of nosocomial infection.[53] The essential oil also inhibited the growth of multiple antibiotic resistant Staphylococcus strain, tested. The effect of essential oil on *S. aureus* and *P. aeruginosa* were high. *S. aureus* is a one of the most common causes of both hospital and community-acquired infection worldwide.[54] *S. aureus* is a major cause of cutaneous infections, furunculosis, impetigo and arthritis, and toxinoses, such as food poisoning, septic shock, scalded skin syndrome, and toxic shock syndrome. The presence of antibiotic resistant Staphylococci is of concern due to the possible spread of resistance determinants among the *staphylococcus* species.

On assessing the cytotoxic activity, the flower- and leaves-derived oil exhibited specific and significant effects on the viability of the selected human cell lines, *namely.*, hormone-responsive breast carcinoma cell line (MCF7), cervical carcinoma cell line (HeLa), and liver carcinoma cell line (HePG2). $IC_{50}$was 4.8 μg/ml, 4.2 μg/ml, and 10.9 μg/ml for the leaves-derived oil of *F. tingitana* on breast, liver, and cervical carcinoma cell line, respectively, which is comparable to the standard cytotoxic drug, doxorubicin [Table 4]. Lower effects were obtained on testing the flower-derived oil. The previous studies revealed that many essential oil components exhibited cytotoxic activity. The cytotoxic effect of the leaves-derived oil could be attributed to its relatively high content of oxygenated sesquiterpenes (46.5%) including γ-cadinol (13.8%) which has been reported to exert cytotoxic activity.[55] Alpha-cadinol has been reported to have cytotoxicity against human colon adenocarcinoma cell line HT-29.[56] Compounds which are similar in structure (oxygenated sesquiterpenes) as eudesmol and γ-cadinol may possess the same effect. α-Cadinol was said to act as antifungal agent[57] and as hepatoprotective[58] and was proposed as a possible remedy for drug–resistant–tuberculosis.[59]

CONCLUSION

To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way.[60] The quantitative composition and relative proportions of the oil components are widely influenced by the genotype, ontogenic development, and the environmental and growing conditions or on the plant species, the chemotypes and the climatic condition. In conclusion, the stage of development obviously influenced the yield and composition of the hydrodistilled volatiles of the flower and leaves of *F. tingitana* and consequently affected its antimicrobial and cytotoxic potency. The latter could not be exclusively correlated to the efficiency of a specific constituent but rather to a synergistic effect of all components. The difference in composition observed in comparison with samples obtained from other localities may be referred to a number of extrinsic factors which affect growth conditions and production of secondary metabolites. Finally, the transport, storage, and emission of plant volatiles are neglected areas of study that must be addressed to complete our understanding of how plants use these specialized metabolites in diverse ecosystems. Plant volatiles synthesis or emission may be influenced by environmental factors such as light, temperature, and moisture[46,61] and often follow a rhythmic pattern, which may be regulated by a circadian clock or light.[45,62]

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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