

Terpenoid composition and antimicrobial activity of essential oil from *Torilis japonica* (Houtt.) DC.

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Abstract : Chemical composition of *Torilis japonica* (Houtt.) DC. growing wild in the central Himalayan region, was analyzed by capillary GC and GC-MS. The oil was found to be rich in sesquiterpine hydrocarbons (59.47%). A total of 44 compounds were identified representing 94.28% of the oil. Germacrene D (25.86%), α -humulene (14.64%), β -caryophyllene (11.33%), nootkatone (10.80%) and caryophyllene oxide (5.07%) were found to be the principal constituents. *T. japonica* essential oil demonstrated bactericidal and fungicidal activity against six bacterial strains and two fungal strains respectively by agar well diffusion method.

Keywords : *Torilis japonica*, Apiaceae, terpenoid composition, α -humulene, germacrene D, antimicrobial activity.

Introduction

Torilis japonica (Houtt.) DC. belonging to the Apiaceae family, is an annual herbaceous plant, with short hair, 30–70 cm tall. It is a very common and widespread plant, which grows in waste places, near roads, in hedges and grassy areas^{1,2}. The fruits of *T. japonica* have been used as traditional medicine for the treatment of skin disease, testitis, arthroneuralgia, impotence and renal disorder in Japan³. Fresh or dried herb or fruit of *T. anthriscus* is used as a medicinal agent, especially in Korea and China, to treat amnesia, pruritis, acidosis, women's diseases and scabies^{4,5}. The literature search did not reveal any report on the composition of the essential oil as well as on the antimicrobial screening of *T. japonica* from India. Therefore, the aim of the present investigation is to carry out a detailed analysis of the oil and also to assess the antibacterial activity of the oil against six bacterial and two fungal strains.

Results and discussion

The yield of the essential oil obtained from aerial part of the *T. japonica* (Houtt.) DC. was 0.02% (v/w). The constituents of the essential oil were identified in the

order of their elution in Rtx-5 column in GC and GC-MS. Forty four compounds were characterised in the oil, accounting for 94.28% of the total oil (Table 1). The oil of *T. japonica* is characterized by a high content of germacrene D (25.86%), α -humulene (14.64%) and β -caryophyllene (11.33%), followed by nootkatone (10.80%), caryophyllene oxide (5.07%), (*E,E*)- α -farnesene (4.09%), humulene epoxide II (2.61%) and α -vetivone (2.39%). The oil of *T. japonica* aerial parts contained major percentage of sesquiterpene hydrocarbons. Fujita⁶ reported the presence of fifty three compounds, the main components being germacrene D (57.9–71.8%), α -humulene (2.4–13.2%), bicyclogermacrene (1.9–5.4%), β -caryophyllene (1.5–4.6%) and δ -cadinene (1.0–1.9%)⁶. The major constituents in *T. japonica* from another report were β -eudesmene (22.9%), α -selinene (12.9%), eudesm-7(11)-en-4-ol (10.4%) and β -elemene (8.2%). Bicyclogermacrene, β -eudesmene and α -selinene were totally absent in our reported plant.

The oil of *Torilis japonica* was tested for antimicrobial activity against six standard strains of bacteria and two standard fungal strains. The results of *in vitro* examination (Table 2) showed that the oil had moderate acti-

Table-1 (contd.)

Table 1. Chemical composition of aerial part of <i>Torilis japonica</i>			
Sl. No.	Compounds ^a	RI _{Exp} ^b	RI ^c Percentage (%)
1.	α -Pinene	937	932 0.10
2.	Sabinene	979	969 0.12
3.	6-Methyl hept-5-en-2-one	985	981 0.10
4.	<i>p</i> -Cymene	1027	1020 0.11
5.	(<i>Z</i>)- β -Ocimene	1036	1032 t
6.	(<i>E</i>)- β -Ocimene	1048	1044 0.11
7.	γ -Terpinene	1059	1054 t
8.	(2 <i>E</i>)-Nonene-1-al	1065	1157 0.30
9.	δ -Elemene	1338	1335 0.11
10.	α -Copaene	1376	1374 t
11.	Daucene	1379	1380 0.46
12.	β -Bourbonene	1382	1387 0.19
13.	β -Elemene	1393	1389 1.01
14.	β-Caryophyllene	1422	1417 11.33
15.	β -Copaene	1429	1430 0.29
16.	(<i>E</i>)- α -Bergamotene	1434	1432 0.34
17.	(<i>Z</i>)-Muurolo-3,5-diene	1448	1448 0.10
18.	α-Humulene	1453	1452 14.64
19.	<i>allo</i> -Aromadendrene	1460	1458 0.10
20.	Germacrene D	1483	1484 25.86
21.	α -Zingiberene	1498	1493 0.82
22.	(<i>E,E</i>)- α -Farnesene	1509	1505 4.09
23.	Cubebol	1516	1514 t
24.	δ -Cadinene	1524	1522 0.13
25.	Unknown sesquiterpene alcohol ^d	1558	– 3.52
26.	Isoelemicin ^e	1563	– 0.51
27.	<i>trans</i> -Nerolidol	1565	1561 0.86
28.	Germacrene D-4-ol	1570	1574 t
29.	Spathulenol	1579	1577 t
30.	Caryophyllene oxide	1584	1590 5.07
31.	Globulol	1596	1590 0.86
32.	Humulene epoxide II	1610	1608 2.61
33.	10- <i>epi</i> - γ -Eudesmol	1625	1622 0.64
34.	<i>allo</i> -Aromadendrene epoxide	1642	1639 1.75
35.	β -Eudesmol	1650	1649 0.10
36.	α -Cadinol	1652	1652 0.62
37.	Selin-11-el-4 α -ol	1665	1658 0.10
38.	Bulnesol	1675	1670 1.60
39.	α -Bisabolol	1688	1685 0.51
40.	Curcumenol	1734	1733 0.16
41.	(2 <i>E</i> ,6 <i>E</i>)-Farnesol	1740	1742 0.19
42.	Nootkatone	1798	1806 10.80
43.	α -Vetivone	1845	1842 2.39
44.	Phytol isomer	2118	– 1.68

Total	94.28%
Grouped components	
Monoterpene hydrocarbons	0.44%
Oxygenated monoterpenes	0.40%
Sesquiterpene hydrocarbons	59.47%
Oxygenated sesquiterpenes	32.29%
Diterpenoid	1.68%
Total identified	94.28%

^aMode of identification : Retention Index, on Equity-5 column, MS (GC-MS). ^bRetention indices determined on the Equity-5 column using an *n*-alkane homologous series (C₉–C₃₃). ^cRetention indices from the literature⁸. ^dMS (70 eV) : 222 (C₁₅H₂₆O; M⁺), 207 (100%), 189, 161, 149, 137, 109, 91, 81, 55, 41. ^eCorrect isomer (*Z*- or *E*-) not identified with almost same retention index in operating conditions, t = trace (<0.10%).

vity against the tested pathogens on the basis of zone of inhibition and MIC values. The essential oil of the plant showed mean zone of inhibition for the bacterial strains ranging from 11.67±0.15 mm (*Escherichia coli*) to 8.33±0.30 mm (*Klebsiella pneumoniae*) with the MIC values 150 µL/mL and 175 µL/mL respectively. The oil exhibited maximum activity against *Escherichia coli* with inhibition zone of 11.67±0.15 mm diameter and MIC 150 µL/mL followed by *Pseudomonas aeruginosa* (ZI; 10.33±0.14 mm, MIC; 150 µL/mL), *Staphylococcus aureus* (ZI; 9.67±0.12 mm, MIC; 175 µL/mL), *Proteus vulgaris* (ZI; 9.67±0.04 mm, MIC; 150 µL/mL), *Salmonella enteric* (ZI; 8.67±0.06 mm, MIC; 175 µL/mL) and *Klebsiella pneumonia* (ZI; 8.33±0.30 mm, MIC; 150 µL/mL).

The oil of the plant exhibited comparable activity against *Candida albicans* (ZI; 9.67±0.29 mm, MIC; 175 µL/mL) and *Pichia guilliermondii* (ZI; 7.67±0.05 mm, MIC; 175 µL/mL). Earlier report on the antibacterial activity of *T. japonica* showed that the oil was effective against *S. typhimurium*, *B. subtilis* and *L. monocytogenes*⁷.

Experimental

Plant collection and identification :

Torilis japonica was collected in July 2011 from Champawat (Uttarakhand, India). The plant was identified by Professor Y. P. S. Pangtey, Botany Department,

Table 2. Antimicrobial activity of essential oil of *Torilis japonica*

Sl. No.	Microorganism	MTCC	<i>Torilis japonica</i> (TLM)		Standard antibiotics			
			ZI ^a (mm)	MIC ^b (μL/mL)	Gentamycin		Kanamycin	
	Bacteria				ZI ^a (mm)	MIC ^b (μg/mL)	ZI ^a (mm)	MIC (μg/mL)
1.	<i>Klebsiella pneumoniae</i>	3384	8.33±0.30	150	10.67±0.02	50	10.67±0.17	50
2.	<i>Staphylococcus aureus</i>	3103	9.67±0.12	175	17.33±0.10	50	16.33±0.35	50
3.	<i>Salmonella enteric</i>	3224	8.67±0.06	175	14.67±0.03	50	13.33±0.41	75
4.	<i>Escherichia coli</i>	443	11.67±0.15	150	10.67±0.03	50	9.67±0.24	100
5.	<i>Pseudomonas aeruginosa</i>	424	10.33±0.14	150	13.33±0.07	50	12.67±0.71	50
6.	<i>Proteus vulgaris</i>	1771	9.67±0.04	150	15.67±0.03	50	13.33±0.59	75
	Fungus				ZI ^a (mm)Standard Nystatin MIC ^b (μg/mL)			
7.	<i>Pichia guilliermondii</i>	4052	7.67±0.05	175	15.00±0.09	250		
8.	<i>Candida albicans</i>	227	9.67±0.29	175	13.00±0.01	250		

^aInhibition zone diameter (mm), size of well 3 mm not included in ZI.^bMinimum inhibitory concentration values in 1000 μL/mL to 5 μL.

Kumaun University, Nainital, which has been deposited in the herbarium repository of Botanical Survey of India, Dehradun for future reference (Acc. No. 113646).

Isolation of essential oil :

The essential oil was extracted from fresh aerial parts of *Torilis japonica* by hydrodistillation, for 3 h using a Clevenger apparatus. The percentage essential oil content was estimated on fresh weight basis. The obtained oil sample was dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analyses.

Essential oil analysis :

The oil was analysed by using a Shimadzu 2010 auto system GC fitted with Rtx-5 column and FID detector. The column temperature was programmed at 80 °C (hold time for 2 min) to 210 °C (hold time 5 min) at 3 °C min⁻¹ and than 210–300 °C at 20 °C min⁻¹ with final hold time of 15 min, using N₂ at 30.0 mL/min column head pressure as carrier gas, the injector temperature was 270 °C and detector temperature 280 °C. The GC-MS used was Autosystem 2010 GC (Rtx-5, 30 m×0.25 mm, i.d. 0.25 μm) coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column (30 m×0.25 mm with film thickness 0.25 μm). The column temperature was 80 °C (hold time of 2 min) to 210 °C (hold time 5 min) at 3 °C min⁻¹ and than

210–300 °C at 20 °C min⁻¹ with final hold time of 21 min, using helium as carrier gas. The injector temperature was 230 °C and 0.2 μL in *n*-hexane, with split ratio of 1 : 30 MS were taken at 70 eV with a mass range of 40–650 amu.

Identification of the components :

Identification of constituents was done by on the basis of Retention Index, MS Library search (NIST and Wiley) and also by comparing with the MS literature data⁸.

Antimicrobial activity :

The essential oil was screened for antiimicrobial activity against bacteria *Klebsiella pneumoniae* (MTCC3384), *Staphylococcus aureus* (MTCC3103), *Salmonella enterica* (MTCC3224), *Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424), *Proteus vulgaris* (MTCC1771), and fungus *Pichia guilliermondii* (MTCC4052) and *Candida albicans* (MTCC227). The bacterial and fungal inoculated culture was prepared by emulsifying single colony in 5 mL of sterilized required nutrient broth. Tubes with required nutrient broth and inoculated bacterial cultures were incubated overnight at 37 °C for 24 h. Turbid cultures were then used as stock inoculums. The bacterial strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, as Microbial Type Culture Collection (MTCC). All

bacterial strains were cultured and maintained in Microbiology Laboratory, Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital by regular sub culturing and preserved as glycerol stock at $-20\text{ }^{\circ}\text{C}$ for further use.

Zone of inhibition (ZI) and minimum inhibitory concentration (MIC) :

Antimicrobial activity of the essential oil against above mentioned microorganism and a few reference materials were determined by Agar Well Diffusion method^{9,10}. The organisms were cultured in nutrient broth (bacterial strains) and malt yeast broth (fungal strains) and the tests were carried out on Mueller Hinton agar and Potato Dextrose agar plates respectively. The inoculums of the microbial strains were prepared from 24 h broth cultures, the cultures were adjusted to 10^6 CFU/mL with sterile water. The different concentration range from 5 to 1000 $\mu\text{L/mL}$ of essential oils was prepared by dissolving them in hexane. Muller Hinton agar and Potato Dextrose agar were poured into petridishes. After solidification, 100 μL of test strains were spread on the media plates separately. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the inoculation, a well was made in the plates with sterile borer (3 mm). The oil samples (30 μL /well) of different concentrations (5 μL to 1000 μL) were introduced into the well and plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. All samples were tested in triplicate. Microbial growth was determined by measuring the zone of inhibition (ZI). Gentamicin and Kanamycin for bacte-

rial strains and Nystatin for fungal strains were used as positive control. Hexane was used as negative control. The MIC value was defined as the lowest concentration of the volatile oil required for inhibiting the growth of each microorganism.

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