SHORT COMMUNICATION

Evaluation of the chemical composition of essential oil of *Thuja occidentalis* leaves grown in Peshawar, Pakistan by gas chromatography mass spectrometry

Muhammad Nasimullah Qureshi[1]*, Muhammad Siddique[2], Inayat-ur-Rahman[2] and Farina Kanwal[2]

[1]Department of Chemistry, Abdul Wali Khan University Mardan, Pakistan
[2]Medicinal Botanic Centre, PCSIR Laboratories Complex Peshawar, Pakistan

Abstract: Essential oil extracted from the fresh leaves of *Thuja occidentalis* were evaluated for its chemical composition employing GC-MS. Total of twenty nine components were identified and determined quantitatively using the area normalization procedure. Alpha-pinene and (+)-4-carene were found in high amount with a percentage concentration of 54.78 and 11.28 respectively. Other compounds which yielded appreciable amounts are: alpha-cedrol (6.87%), terpinolene (5.88%), p-menth-1-en-8-ol acetate (5.21%), beta-myrcene (4.04%), beta-pinene (2.26%), germacrene D (1.72%), sabinene (1.65%) and D-Limonene (1.62%).

Keywords: *Thuja occidentalis*, essential oil, volatile compounds, GC-MS.

INTRODUCTION

Medicinal plants have been in use for their therapeutically active compounds against different diseases (Qureshi *et al.*, 2012a; Qureshi *et al.*, 2011a; Qureshi *et al.*, 2013; Qureshi *et al.*, 2012b; Qureshi *et al.*, 2011b; Sultana *et al.*, 2008; ur Rahman *et al.*, 2012). Essential oils are natural water immiscible liquids found in various parts of plants. Plants use them for their defense against different diseases, predators and also in pollination (Knobloch *et al.*, 1989; Lang and Buchbauer, 2011; Miguel, 2010). Chemically these oils composed of various organic compounds volatile in nature and have some fragrance (Patel *et al.*, 2011). These have been used as raw materials in food, cosmetics, perfumes, aromatherapy, phototherapy, spices etc (Wang *et al.*, 2012).

Thuja trees have evergreen scale like leaves mostly grown for ornamental purposes. Thuja leaves have shown various pharmacological activities such as anti-infammatory, anti-cancer, anti-hypertension and for the treatment of flu, cough, bleeding arthralgia, insecticidal etc (Comerford, 1996; Hashemi and Safavi, 2012; Kéïta *et al.*, 2001; Naser *et al.*, 2005; Pavela, 2008). It has been employed for curing gout, rheumatism, diarrhea and chronic tracheitis (Biswas *et al.*, 2011; Naser *et al.*, 2005; Tsiri *et al.*, 2009). The leaves oil has been used as a constituent in the production of perfumes, insecticides, soaps and deodorants.

**Corresponding author:* e-mail: mnasimuq@yahoo.com

MATERIALS AND METHODS

Chemicals and reagents

Sodium sulphate anhydrous (analytical grade) and n-hexane (analytical grade) was procured from Merck (Darmstadt, Germany). Helium gas (99.9999%) was obtained from Pak gas (United Arab Emirates). Deionized water was used through out the experimental work. Fresh leaves of *Thuja occidentalis* were collected form local area of Peshawar, Pakistan and were identified by a taxonomist of MBC, PCSIR Laboratories Complex Peshawar.

Extraction of oil

Essential oil was extracted from the fresh leaves by hydro distillation in Clevenger apparatus with sample to water ratio of 1 to 10. The oil obtained was dried over anhydrous sodium sulphate and preserved at 4°C until GC-MS analysis.

Gas Chromatography Mass Spectrometry

A GC-MS QP 2010 plus (Shimadzu, Tokyo, Japan) with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. The carrier gas used was Helium and all the analytes were separated on a chromatographic capillary column (DB 5ms; Technokroma) of specifications: length; 30m, i.d.; 0.25mm, thickness; 0.25µm. Temperature of the ion source (EI) and interface was 240°C. Solvent cut time was 2.5min and 1µL of sample was used for analysis. Mode of operation of the injector was split with a 1:50 split ratio. Temperature at the injection point was 240°C. The temperature gradient initiated at 40°C and rose to

Table 1: Quantification of components of essential oil extracted from *Thuja occidentalis*

S. No.	Name of Pesticide	Retention Time (min.)	Area	Concentration (%)
1	Alpha-Phellandrene	8.625	34389	0.26
2	Alpha-Pinene	8.936	7111689	54.78
3	Sabinene	10.595	213746	1.65
4	Beta-Pinene	10.783	293955	2.26
5	Beta-Myrcene	11.399	524389	4.04
6	(+)-4-Carene	12.250	1463840	11.28
7	O-Cymene	12.741	18388	0.14
8	D-Limonene	13.191	210649	1.62
9	Cis-beta-Ocimene	14.077	7116	0.05
10	Gamma-Terpinene	14.594	49995	0.39
11	Terpinolene	15.910	763033	5.88
12	Beta-Linalool	16.692	128331	0.99
13	1-Terpinene-4-ol	19.553	76027	0.59
14	p-Menth-1-en-8-ol	19.968	16815	0.13
15	n-Octyl acetate	20.375	33658	0.26
16	Bergamot mint oil	21.295	58084	0.45
17	Bornyl acetate	22.060	58257	0.45
18	p-Menth-1-en-8-ol acetate	23.239	676660	5.21
19	Nerol acetate	23.404	6178	0.05
20	Geraniol acetate	23.740	25617	0.20
21	Caryophyllene	24.550	49119	0.38
22	Beta-Farnesene	24.646	10432	0.08
23	Germacrene D	25.494	223708	1.72
24	Farnesene	25.707	8933	0.07
25	Beta-Sesquiphellandrene	26.044	3442	0.03
26	O-Methoxycinnamic aldehyde	26.534	1190	0.01
27	Caryophyllene oxide	26.962	15807	0.12
28	Alpha-Cedrol	27.362	892136	6.87
29	Cembrene	32.924	6263	0.05

90°C at the rate of 3°C/min. The temperature was increased to 240°C at the rate of 10°C/min and was kept at this temperature for 20 minutes. Total time for analysis was 51.67 minutes. Mass spectrometric scanning was performed in the range of *m/z* 40 to *m/z* 500. The system was controlled and the data was acquired using the GC-MS solutions software provided by the supplier. Eluted compounds were identified by comparing the mass spectra obtained with those of standard mass spectra from the NIST library (NIST 05).

RESULTS

Twenty nine compounds were identified and quantitatively determined in the under studied essential oil with major components as Alpha-pinene (54.78%) and (+)-4-carene (11.28). As shown in table 1, other compounds which yielded appreciable amount are: alpha-cedrol (6.87%), terpinolene (5.88%), p-menth-1-en-8-ol acetate (5.21%), beta-myrcene (4.04%), beta-pinene (2.26%), germacrene D (1.72%), sabinene (1.65%) and D-limonene (1.62%). Concentrations of the rest of the constituents of the oil were found below 1%.

DISCUSSION

Essential oil from the leaves of *T. occidentalis* was extracted using the most often applied method i.e. the hydro distillation in Clevenger apparatus. Chemical composition of the essential oil extracted from the leaves of Thuja has been evaluated employing gas chromatography hyphenated to mass spectrometry. Percentage compositions of each component are shown in table 1 and the gas chromatograph obtained is shown in fig. 1. Total of twenty-nine components were identified and determined quantitatively using the area normalization procedure. Alpha-pinene and (+)-4-carene were found in high amount with other compounds which yielded appreciable amount are: Alpha-cedrol,

terpinolene, p-menth-1-en-8-ol acetate, beta-myrcene, beta-pinene, germacrene D, sabinene and D-limonene. As shown in the published literature that these compounds are naturally occurring antimicrobial agents and antioxidants (Wang *et al.*, 2012).

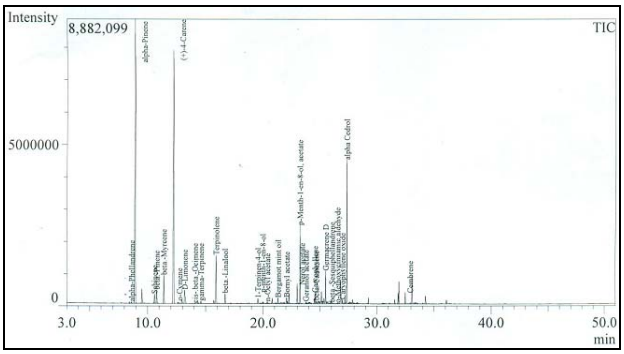

Fig. 1: GC-MS chromatogram of essential oil from leaves of *Thuja occidentalis*. Chromatographic conditions: inj. vol.: 1µL, carrier gas: Helium, column: DB 5ms capillary column (length; 30m, i.d.; 0.25mm, thickness; 0.25µm, MS scanning: *m/z* 40-500.

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