Organic and Medicinal Chemistry
International Journal
ISSN 2474-7610

Research Article
Volume 12 Issue 3 - July 2023
DOI: 10.19080/OMCIJ.2023.12.555838

Organic & Medicinal Chem IJ

Phytochemical Analysis of *Tsuga Dumosa*

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Submission: July 13, 2023; **Published:** July 24, 2023

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Abstract

There is increasing evidence showing the potential of plant constituents as antioxidant agents. Plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Essential oils (EO) from Tsuga dumosa, have various medicinal properties, including antibacterial and antioxidant activity. The plant Tsuga dumosa leaves extracted by hydro distillation method for 6 hours using Clevenger apparatus. The oil was analyzed by Gas Chromatography-Mass spectrophotometry (GC-MS). A total of 35 compounds were identified constituting 98.93% of the total oil. The main compounds were Bornyl acetate (18.87%), α-pinene (17.67%), Limonene (17.05%), Intermedeol (14.13%) and minor compounds was trans-Piperitol acetate (0.09 %), Thunbergol (0.13%), Cryptone (0.14%).

Keywords: Tsuga Dumosa; Phytochemical Analysis; Essential Oil; GC-MS

Abbreviations: E0: Essential Oils; GC-MS: Gas Chromatography-Mass Spectrophotometry; RI: Retention Indices

Introduction

Medicinal plants have the ability to inhibit the growth of a wide range of pathogenic microorganisms due to the presence of essential oils. The antimicrobial impact of essential oils and its various components extracted from medicinal plants has been well documented. Many plant species and herbs exert antioxidant activity due to their essential oil fractions. Some scientists reported the antioxidant activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, and onion against both bacteria and molds. The composition, structure, as well as functional groups of the oils play an important role in determining their antioxidant activity. The aromatic oils from plant leaves are used as pharmaceutical raw material in the formulation of many drugs. [1]

The genus Tsuga (Pinaceae) is comprised of nine species. *T. dumosa D. Don* is an economically as well as medicinally important conifer. It is commonly known as "*Hemlock Spruce*" and locally called as "*Dhupi*" or "*Thingre Salla*". The plant has been extensively used for timbering and lumber products because of its resistance to decay. The bark of this plant is a rich source of tannin, hence can be used for dying. Himalaya, the youngest mountain system of the world, constitutes an important link between the vegetation of the southern peninsular India on the one hand, the eastern Malaysian, the north-eastern Sino-Japanese and the northern Tibetan areas on the other. Biodiversity is essential for human survival and economic well-being and for the ecosystem function and stability [2-6]. The present paper deals with the estimation of essential oil present in the leaves of plants.

Materials and Methods

Plant Material

The leaves of *T. dumosa* was collected in the month of January 2020 from Thalkedar near Pithoragarh, Uttarakhand, India in the Kumaon Himalayas. The plant was first identified in the Department of Botany, Kumaun University, Nainital. The collected plant material was first washed with cold water to remove the soil particles and then shade dried.

Chemicals

Isolation of Essential Oil

The leaves of *T. dumosa* were extracted by hydro-distillation method for 6 hours using Clevenger apparatus. The oil was dried over anhydrous sodium sulphate and stored at room temperature in a sealed vial until analysis was performed. The percentage oil yield was calculated based on the dry weight of the plant. The oil yield was (0.09%).

GC and GC/MS Analyses and Identification

Essential oil analyses were performed by GC-MS and GC-FID on a Shimadzu QP-2010 instrument, equipped with FID, in the same conditions. The percentage composition of the oil sample was computed from the GC peak areas without using correction for response factors. The oil was analyzed using a Shimadzu GC/MS Model QP 2010 Plus, equipped with Rtx-5MS (30 m × 0.25 mm; 0.25 mm film thickness) fused silica capillary column. Helium (99.99%) was used as a carrier gas adjusted to 1.21 ml/min at 69.0 K Pa, spitless injection of 1 mL, of a hexane solution injector and interface temperature was 270^{0}C, oven temperature programmed was 50-280^{0}C at 3 0C/min. Mass spectra was recorded at 70 eV. Ion source temperature was 230^{0}C.

The identification of the chemical constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature.[7] Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following Kovats formula. [8]

$$KI = 100[n + (N - n)X \quad \frac{\log t_R^1(unknown) - \log t_R^1(C_n)}{\log t_R^1(C_N) - \log t_R^1(C_n)}$$

t_R^1 - the net retention time (t_R - t_0)

t_0 - the retention time of solvent (dead time) t_R - the retention time of the compound.

C_N - number of carbons in longer chain of alkane C_n - number of carbons in shorter chain of alkane

n - is the number of carbon atoms in the smaller alkane N - is the number of carbon atoms in the larger alkane

Results and Discussion

The GC and GC-MS analyses of essential oil of T. dumosa resulted in the identification of 35 compounds (Table 1). The oil yield was (0.09%) by raw material weight. Both, the major as well as minor constituents were identified by their retention indices and comparison of their mass spectra. A total of 35 compounds were identified constituting 98.93 % of the total oil. The main compounds were Bornyl acetate (18.87%), α-pinene (17.67%), Limonene (17.05%), Intermedeol (14.13%), Camphene (9.85%), β-Courbonne (3.22%), β-Pinene (2.41%) and 1- Borneol (2.13%). The main minor compounds were trans-Piperitol acetate (0.09 %), Thunbergol (0.13%), Cryptone (0.14%), Germacrene-D (0.14%), 3Z-Cembrene A (0.16%), α- Pinene oxide (0.17%), Cis-Piperitol (0.17%), alpha-Terpinyl acetate (0.17%) and α-Pinenoxid (0.18%). The presence of 18.87% Bornyl acetate, 17.67% α-pinene and 17.05% Limonene show good source of these natural compound.

Table 1: Essential oil composition of *Tsuga dumosa*.

SN	Compound	Area %	Mol. formula	Mol. Wt.	RI	Mode of identify- cation
1	Tricyclene	0.75	$C_{10}H_{16}$	136	920	a, b
2	α-pinene	17.67	$C_{10}H_{16}$	136	935	a, b
3	Camphene	9.85	$C_{10}H_{16}$	136	940	a, b
4	β-Pinene	2.41	$C_{10}H_{16}$	136	976	a, b
5	Myrcene	0.36	$C_{10}H_{16}$	136	990	a, b
6	(+)-3-Caren	1.06	$C_{10}H_{16}$	136	1007	a, b
7	Limonene	17.05	$C_{10}H_{16}$	136	1033	a, b
8	α-Pinenoxid	0.18	$C_{10}H_{16}O$	152	1097	a, b
9	α- Pinene oxide	0.17	$C_{10}H_{16}O$	152	1111	a, b
10	cis-p-menth-2-en-1-ol	0.63	$C_{10}H_{18}O$	154	1124	a, b
11	Camphor	0.36	$C_{10}H_{16}O$	152	1146	a, b
12	1-Borneol	2.13	$C_{10}H_{18}O$	154	1165	a, b
13	4-Terpineol	0.22	$C_{10}H_{18}O$	154	1179	a,b
14	Cryptone	0.14	$C_9H_{14}O$	138	1190	a, b
15	α- Terpineol	0.95	$C_{10}H_{18}O$	154	1196	a, b
16	Cis-Piperitol	0.17	$C_{10}H_{18}O$	154	1198	a, b
17	Bornyl acetate	18.87	$C_{12}H_{20}O_2$	196	1285	a, b
18	Thujyl Acetate	0.52	$C_{12}H_2OO_2$	196	1298	a, b
19	Patchoulane	0.21	$C_{15}H_{26}$	206	1390	a, b
20	trans-Piperitol acetate	0.09	$C_{12}H_{20}O_2$	196	1349	a, b

How to cite this article: Pramod K O, Bipin C J. Phytochemical Analysis of Tsuga Dumosa. Organic & Medicinal Chem IJ. 2023; 12(3): 555838. DOI: 10.19080/OMCIJ.2023.12.555838

21	alpha-Terpinyl acetate	0.17	$C_{12}H_{20}O_2$	196	1350	a, b
22	β-Bourbonene	3.22	$C_{15}H_{24}$	204	1380	a, b
23	(E)-Caryophyllene	0.45	$C_{15}H_{24}$	204	1412	a, b
24	Germacrene-D	0.14	$C_{15}H_{24}$	204	1475	a, b
25	α-Humulene	0.23	$C_{15}H_{24}$	204	1452	a, b
26	Selina-4,11-diene	0.61	$C_{15}H_{24}$	204	1476	a, b
27	Guaia-1(10),11-diene	0.54	$C_{15}H_{24}$	204	1490	a, b
28	β-Bisabolene	0.46	$C_{15}H_{24}$	204	1508	a, b
29	(E)-Nerolidol	0.23	$C_{15}H_{26}O$	222	1560	a, b
30	Caryophyllene oxide	1.29	$C_{15}H_{24}O$	220	1581	a, b
31	Humulene epoxide II	0.42	$C_{15}H_{24}O$	220	1591	a, b
32	Intermedeol	14.13	$C_{15}H_{26}O$	222	1668	a, b
33	α-Bisabolol	1.96	$C_{15}H_{26}O$	222	1688	a, b
34	3Z-Cembrene A	0.16	$C_{20}H_{32}$	272	1967	a, b
35	Thunbergol	0.13	$C_{20}H_{34}O$	290	2211	a, b
		97.93				

Conclusions

Our study concludes that the oil extract has a good number of essential oils. The essential oil from *Tsuga dumosa* showed a qualitative and quantitative make-up of constituents. A total of 35 compounds were identified constituting 98.93% of the total oil. Clinically, these plant leaves can be a good source of herbal medicine for the treatment of diseases indigenously.

Acknowledgement

The authors are thankful to the Head, Department of Botany, Kumaun University, Nainital for the identification of Plant. The authors are grateful to Prof. Veena Pande, Department of Biotechnology, Sir J. C. Bose Technical Campus, Kumaun University, Nainital (Bhimtal) for the evaluation of antioxidant property of EO and AIRF, Jawaharlal Nehru University, New Delhi for GC-MS. The authors are grateful to Departments of Chemistry, of L.S.M. Govt Post Graduate College, Pithoragarh, and L.B.S. Govt Post Graduate College, Halduchaur (Nainital), Uttarakhand for providing the necessary facilities.

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