Journal of
Essential Oil & Plant Composition

JEOPC, 1(3), 192-197, 2023
https://doi.org/10.58985/jeopc.2023.v01i03.24
ISSN (Print): 2958-9630

Research Article

α-Pinene- and *trans*-caryophyllene-rich volatile female cones oil of *Taxodium distichum* L. from Northern Iran

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Article Information
Received: 25 January, 2023
Revised: 29 March, 2023
Accepted: 20 April, 2023

Academic Editors
Arigò Adriana and Radosław Kowalski

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Keywords
Taxodium distichum, female cones, essential oil, chemical composition

Abstract

The present study aimed to identify the chemical composition of essential oil from fresh female cones of *Taxodium distichum* collected in the winter season. The essential oil was obtained by hydro-distillation method in a Clevenger-type apparatus and then analyzed by gas chromatography-mass spectrometry (GC/MS). Fifteen components were identified, accounting for 99.1% of the total oil volume. Monoterpenoids (75.3%) dominated the identified components in the essential oil, followed by a considerable portion of sesquiterpenoids (23.6%). Monoterpene hydrocarbons (MH) (73.3%) were the principal subclasses of components with *α*-pinene (63.6%), β-myrcene (3.67%), β-pinene (2.8%), and limonene (2.1%) as the main constituents. *Trans*-caryophyllene (13.3%) and caryophyllene oxide (8%) were the representatives of the sesquiterpene hydrocarbon and oxygenated sesquiterpene, respectively.

1. Introduction

Taxodium distichum is known as bald cypress and is distributed in the eastern United States from Maryland and Illinois to South Florida and Central Texas [1]. The plant species are spread as plantations in different regions such as wet or poor drainage soil lands of Guilan and Mazandaran, north of Iran, which have been studied for different purposes [2-7]. The leaves and cones of the species are particularly rich in essential oils and are used as folk medicine to treat skin, gastrointestinal, respiratory, inflammation, and infections [8, 9].

The important uses of essential oils include food, perfumery, cosmetics, cleaning, sanitary, and pharmaceutical industries [10-12]. Furthermore, the essential oils of many plant species have been reported to possess useful biological, pharmacological, and therapeutic activities, including anti-Candida, anti-inflammatory, analgesic, antidiabetic, antidiarrheal, antimutagenic, antioxidant, antipyretic, anti-inflammatory, cytotoxic, apoptotic, by inhalation [13-15], antimicrobial [16], and insecticidal activities [17-19].

An investigation carried out by Jaimand's group [2] considered time lag collection of *T. distichum* fruit essential oil harvested in mid of summer (August 2000) and collected three fractions at three different times by hydrodistillation method. The oils were analysed by GC and GC/MS apparatus and the constituents were identified. The results revealed that the major constituent in the first fraction (after ten min) was α-pinene (61.8%), which decrease in the second fraction (after 60 min) (57.1%) and in the third fraction (after 120 min) (14.8%). The other component terpine-1-ol, was present in the first fraction in 31.5%, and in the second fraction it accounted 32.5%, but in the third fraction its content decreased to 24%. Finally, β-caryophyllene was obtained in the first fraction in small quantities (1.2%), but its content increased in the second and third fractions to 7.2% and 24.6%, respectively.

The essential oil composition of feminine cones, leaves, and branches of bald cypress analyzed was rich in α-pinene (53.7-79.7%) and limonene (3.7-18.7%) [20], while the samples collected from China were analyzed and revealed to contain caryophyllene oxide (41.67%) as the singly abundant constituent with sizeable proportion of bornyl acetate (6.24%), perilla ketone (5.45%), and α-asarone (5.39%) [21].

The chromatogram of cones extract (absolute ethanol) of coppery-red bald cypress that was collected in January 2015 in the Futoški Park (Novi Sad, Serbia) revealed the presence of 53 compounds, of which 33 were identified. So that, the extract contained oxygenated monoterpenes (12.42%), sesquiterpenes (5.18%), oxygenated sesquiterpenes (17.41%), diterpenes (1.15%), and oxygenated diterpenes (30.87%), while the amount of retinoic acid was 0.32%. Monoacylglycerols were detected in 4.32%. The most abundant compounds were: caryophyllene oxide (14.27%), 6,7-dehydro-ferruginol (12.49%), bornyl acetate (10.96%), 6-deoxy-taxodione (9.50%), and *trans*-caryophyllene (4.20%) [22].

Moreover, α-pinene (87.3%), thujopsene (3.7%), myrcene (2.0%), β-pinene (1.7%), and limonene (1.3%), with effective antimicrobial, antispasmodic, and anti-inflammatory properties have been characterized in the fruit essential oil of *T. distichum* growing in Egypt [23]. Cytotoxic effects of the leaves and fruits of *T. distichum* oils collected from Nigeria were also analyzed and assessed [23]. It was shown that the main compounds were α-pinene (60.5%) and thujopsene (17.6%) from the fruits and thujopsene (27.7%), pimara-8(14),15-diene (13.1%), widdrol (12.8%), and β-caryophyllene (11.4%) from the leaves. The oils exhibited pronounced cytotoxic activities against PC-3, Hep G2, and Hs 578T human tumor cell lines at tested concentrations. Only the fruit oil displayed a promising antifungal effect (MIC 19.5 mg mL^{-1}) against *Aspergillus niger* [24].

Despite the previously reported work on *T. distinchum*, there is still little data about the chemical composition of the essential oil of this species specially in Iran. Also, we found no previous reports on the chemical composition within *Taxodium distichum* female cone essential oil harvested in the winter season. So, aiming to contribute to further advance the knowledge in this area, the current study was performed to detect the compounds present in the oils from cones of *T. distichum* collected in the winter season

2. Materials and methods

2.1. Plant material

Female cones of one individual plant representing the local population of cultivated *T. distichum* from Babol were harvested in January 2020. The wetlands of Babol are located in Mazandaran province, northern Iran, between 52° 35'- 52° 45' E and 36° 31' - 36° 37' N. The total surface and the mean altitude of the Babol wetlands are 1470 ha and 14.7 m, respectively. The rainiest month is October. The mean annual precipitation is 738.7 mm and the mean annual temperature is 16.3 °C. The maximum and minimum mean temperatures are 29.3 °C, and 4.5 °C, respectively [25].

The collected materials (female cones) were kept under shade and were air-dried at an ambient temperature about 23 ± 2 °C. The plant material was identified by Sayed Khosrow Hosseinashrafi, Assistant Professor, Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran, and a voucher specimen was deposited in the Herbarium of College of Agriculture and Natural Resources of Islamic Azad University, Karaj Branch, Karaj, Iran, under the code 5238.

2.2. Isolation of essential oils

Shade and air-dried female cones of *T. distichum* (120 g harvested in January 2020) were chopped, then poured into a 2000 mL round bottomed-flask charged with 1500 mL distilled water. Finally, test materials were hydrodistilled for 3 h using a Clevenger-type apparatus [26]. The collected oil sample was dried over anhydrous sodium sulphate (Na_2SO_4), stored in sealed 2 mL glass vials, and kept in a refrigerator at 4 °C until GC/MS analysis. The essential oil content was expressed as % v/w (mL per 100 g based on the dry weight of the plant material).

2.3. Analysis of essential oils

The oils were analysed by gas chromatography-mass spectrometry (GC/MS). Accordingly, 100 µL of dry oil was dissolved with 900 µL of hexane and run on a GC Agilent 7890A coupled to a MS Agilent 5975C mass spectrometer detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS cross-linked capillary column (30 m long and 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. The GC/MS operation conditions were as follows: injector temperature of 260 °C; transfer line of 270 °C; oven temperature program of 60 °C for 4 min, 3 °C/min to 100 °C for 2 min, then 4 °C/min to 250 °C for 5 min; carrier gas was He at 1 mL/min; ionisation energy, 70 eV; scan range, 40–800 u; scan time, 1 s. The split ratio of the sample was 50:1 with a split flow (column flow) of 1 mL/min. The total chromatographic run time was about 52 min [26]. Individual components were identified by using mass spectra with data from literature based on two mass spectrometric libraries (Wiley 275 L, 1998 and NIST-05), mass database matching, and by comparing the retention times and mass spectra of constituents with published data [27, 28]. Retention indices (R_I) were determined with reference to a homologous series of normal alkanes (C_9 to C_{23}) by using the following equation (1) [29]:

$$R_I = 100\,[(n + (N-n) \times \log t_{1R}(x) - \log t_{1R}(C_n)/\log t_{1R}(C_N) - \log t_{1R}(C_n)] \quad (1)$$

where R_I is the retention index of the compound, t_{1R} (min) is the net retention time (t_R-t_0), t_0 (min) is the retention time of the solvent (dead time), t_R (min) is the retention time of the compound, C_n and C_N are the number of carbons in the n-alkanes eluting immediately before and after the compound, respectively.

3. Results and discussion

In the present study, the amount of essential oil extracted from the *Taxodium distinchum* female cones was 0.8 % (v/w), and the oil was yellowish-pink. Fig. 1 shows the chromatogram of the essential oil of *T. distichum* female cones obtained in winter season and analyzed in the HP-5MS column.

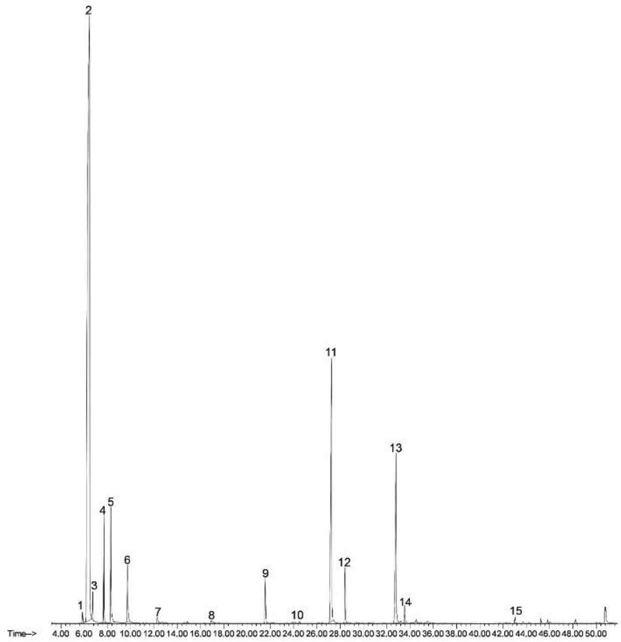

Figure 1. The GC/MS chromatogram of the essential oil of *T. distichum* winter cones (January 2020): **1-** Tricyclene, **2-** α-Pinene, **3-** Camphene, **4-** β-Pinene, **5-** β-Myrcene, **6-** Limonene, **7-** α-Terpinolene, **8-** α-Terpineol, **9-** Bornyl acetate, **10-** Terpinyl acetate, **11-** *trans*-Caryophyllene, **12-** α-Humulene, **13-** Caryophyllene oxide, **14-** Humulene epoxide II, **15-** Pimara-8(14),15-diene.

After the GC/MS analysis, fifteen compounds were identified (Table 1), representing 99.1% of the oil volume from a sample collected in January 2020 (winter). The GC-MS analysis of the essential oil of *T. distichum* winter cones revealed the presence of approximately 21 compounds, of which 15 compounds were identified. This analysis revealed the presence of α-pinene, *trans*-caryophyllene, and caryophyllene oxide in high amounts. The overall constituents of the oil (Table 1) were as follows: seven monoterpene hydrocarbons (73.31%), three oxygenated monoterpenes (2.02%), three sesquiterpene hydrocarbons (15.60%), one oxygenated sesquiterpenes (7.96%), and one diterpene (0.24%). Monoterpene hydrocarbons were the most abundant class of compounds, then sesquiterpene hydrocarbons, followed by oxygenated sesquiterpenes.

The predominant compounds in the essential oil were: α-pinene (63.55%), *trans*-caryophyllene (13.30%), caryophyllene oxide (7.96%), β-Myrcene (3.67%), β-pinene (2.75%), limonene (2.06%), α-humulene

Table 1. The essential oil composition of *T. distichum* female cones that were harvested in January 2020.

No.	Compound	(Group)	RT (min)	Area (%)	KI* exp	KI lit
1	Tricyclene	(MH)	5.852	0.37	922	926
2	α-Pinene	(MH)	6.454	63.55	942	939
3	Camphene	(MH)	6.740	0.61	951	954
4	β-Pinene	(MH)	7.694	2.75	978	979
5	β-Myrcene	(MH)	8.286	3.67	993	991
6	Limonene	(MH)	9.723	2.06	1031	1024
7	α-Terpinolene	(MH)	12.276	0.30	1089	1088
8	α-Terpineol	(OM)	16.935	0.24	1192	1188
9	Bornyl acetate	(OM)	21.563	1.68	1285	1288
10	Terpinyl acetate	(OM)	24.490	0.10	1352	1349
11	*trans*-Caryophyllene	(SH)	27.255	13.30	1414	1419
12	α-Humulene	(SH)	28.412	1.77	1453	1454
13	Caryophyllene oxide	(OS)	32.797	7.96	1584	1583
14	Humulene epoxide II	(SH)	33.544	0.53	1608	1608
15	Pimara-8(14),15-diene	(DH)	43.018	0.24	1964	1969
(MT)	Monoterpenes			75.33		
(MH)	Monoterpene hydrocarbons			73.31		
(OM)	Oxygenated monoterpenes			2.02		
(ST)	Sesquiterpenes			23.56		
(SH)	Sesquiterpene hydrocarbons			15.60		
(OS)	Oxygenated sesquiterpenes			7.96		
(DH)	Diterpenes hydrocarbons			0.24		
(TI)	**Total identified**			**99.13**		

*KI exp and KI lit: experimental and literature Kovats indices, respectively on HP-5MS column in reference to *n*-alkanes; RT: retention time.

(1.77%), and bornyl acetate (1.68%), while the other components were present in less than 1%. The cones essential oil of *T. distichum* was mainly composed of monoterpene hydrocarbons (73.3%), represented mainly by α-pinene (63.55%). The moderate and minor monoterpenoid hydrocarbons detected were β-myrcene (3.67%), β-pinene (2.75%), limonene (2.06%), camphene (0.61%), tricyclene (0.37%), and α-terpinolene (0.30%).

Sesquiterpenes were also abundant constituents (23.56%) with *trans*-caryophyllene (13.3%) being the main representative. The high content of α-pinene among the monoterpenoids, in the female cones and leaves oils, is in agreement with the earlier reports of samples analyzed in Egypt, Iran, Nigeria, and India [2, 20, 23, 24, 30, 31], and caryophyllene oxide among the sesquiterpenoids is in agreement with the earlier report of sample analyzed in Iran [2].

In the previously reported composition of examined *T. distichum* essential oil aims to compare the contents of seven major components, e.g. tricyclene, α-pinene, β-pinene, myrcene, limonene, α-terpineol, and caryophyllene oxide, from plants collected from different geographic regions, hierarchical cluster analysis was carried out [31]. The major oxygenated monoterpenes were bornyl acetate (1.68%) and α-terpineol (0.24%); also the major sesquiterpene hydrocarbons were humulene epoxide II (0.53%) similar to the results found in a study [24] on *T. distichum* fruit oil. Pimara-8(14),15-diene as the diterpenes were the other constituents present in the cones oil that were observed in the leaves, fruits, and branches oil of *T. distichum* [20, 24].

4. Conclusions

The identification of the chemical composition of *T. distichum* female cones essential oil harvested in the winter season from northern Iran showed the presence of α-pinene, *trans*-caryophyllene, and caryophyllene oxide as the major constituents. It can be suggested that the essential oil of *T. distichum* from Iran could be utilized as a potential source of α-pinene and *trans*-caryophyllene, which possesses antimalarial activity, antimicrobial, and anti-inflammatory activities and is used as an important substance in the synthesis of a variety of synthetic aroma chemicals for applications in the perfume, cosmetics, and pharmaceutical industries. It can be also recommended that different province, times, and seasons of harvesting of the plant (*T. distichum*) can be analyzed and compared.

Authors' contributions

Seyyed Khalil Hosseinihashemi designed the experiment, collected female cones, analyzed essential oil, and prepared MS, Luiz Cláudio Almeida Barbosa aided in interpretation of the results and consulted in technical details and the paper preparation, and Reihaneh Kermani assisted with laboratory experiments.

Acknowledgements

The authors are grateful for the support of the Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran. LCAB thanks the Brazilian Research Council (CNPq) and Federal University of Minas Gerais for support.

Funding

The authors declare that there is no specific funding associated with the work featured in this article.

Conflicts of interest

The authors have declared that no conflict of interest exists.

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