

Investigations on the chemical composition of volatile oils extracted from the leaves of spontaneous and cultivated *Taxus baccata* L. trees

Mădălina-Elena FRUNZETE¹, Tatiana RODIDEAL¹,
Marius-Nicușor GRIGORE^{2*}, Violeta A. ION³, Liliana BĂDULESCU^{3,4},
Ramona M. CIOCAN⁵, Maria-Magdalena ZAMFIRACHE¹

¹“Alexandru Ioan Cuza” University, Faculty of Biology, Bd. Carol I, Nr. 20 A, 700505, Iasi, Romania; f.madalinaelena@yahoo.com; tatiana.rodideal@yahoo.com; magda@uaic.ro

²Stefan cel Mare University of Suceava, Faculty of Medicine and Biological Sciences, 720229 Suceava, Romania; marius.grigore@usm.ro (*corresponding author)

³University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Studies of Food Quality and Agricultural Products, 59 Mărăști Blvd, District 1, Bucharest, Romania; violeta.ion@qlab.usamv.ro;

liliana.badulescu@qlab.usamv.ro

⁴University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, 0111464, Bucharest, Romania

⁵“Alexandru Vlahuță” Technological High School, 44 Main Street, 717380, Șendriceni, Romania; ramonabiologie54@gmail.com

Abstract

Taxus L. is accepted in the literature as natural resources of biologically active compounds and volatile oils, with applications in medicine, pharmaceuticals, food, cosmetics, and with ecological impact on the natural living environment. In this context, the present work aims to analyze by GS/MS techniques the chemical composition of volatile oil obtained by hydrodistillation of leaves harvested from spontaneous and cultivated female individuals of *Taxus* (dried and fresh plant material) and to spectrophotometrically evaluate the hydrosols resulting from their hydrodistillation. The compounds with the highest concentrations in the volatile oil obtained from the spontaneous taxon *Taxus baccata* L. were hexahydrofarnesyl acetone (33.03% fresh leaves; 20.09% dried leaves); ar-abietatriene (14.98% dried leaves; 3.03% fresh leaves); phthalic acid, hex-3-yl isobutyl ester (10.51% dried leaves); salicylic acid, benzyl ester (8.11% dried leaves). In the cultivated taxon *Taxus baccata* the compounds identified with the highest concentrations were 1-octen-3-ol (25.61% fresh leaves); phytol (12.50% dry leaves); geranyl acetone (11.90% dry leaves); manoyl oxide (11.85% dry leaves; 10.86% fresh leaves); 1,9-decadiene (7.92% fresh leaves). The compounds with the highest concentrations in the oil extracted from the leaves of *Taxus baccata* ‘Robusta’ were hexahydrofarnesyl acetone (17.81% fresh leaves); pentacosane (11.28% dry leaves); heptacosane (11.27% fresh leaves); tetracosane (11.13% dry leaves); tricosane (8.45% fresh leaves). The chemical composition of volatile oils from yew is influenced by many exogenous factors such as soil, light, and endogenous factors such as age, DNA.

Keywords: GC/MS; hexahydrofarnesyl acetone; hydrosol; *Taxus*; volatile oils

Received: 05 Sep 2023. Received in revised form: 02 Dec 2023. Accepted: 14 Dec 2023. Published online: 18 Dec 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Taxus baccata L., a dioecious gymnosperm of the Taxaceae family, can grow as a tree or shrub, reaching a height of 15 m. The flowers of female individuals have a single seed surrounded by a fleshy part called the aril, which is non-toxic compared to the other organs of the plant (Jovanović, 1970; Milutinović *et al.*, 2015). The toxicity of the species is due to the presence of the alkaloids taxine A and taxine B, which can block cardiac sodium and calcium channels, causing vomiting, cardiac arrhythmia, abdominal pain, coma, convulsions, and death (Panzeri *et al.*, 2010). According to Reijnen *et al.* (2017), ingesting approximately 42-91 g of yew leaves can be lethal to a 70 kg adult. Despite its toxicity, the species is intensively exploited for taxol, a compound used in cancer therapy (Wangkheirakpam and Laitonjam, 2016); at the same time, species of the *Taxus* genus are also ecologically and economically significant (Wang *et al.*, 2023).

Identifying biologically active compounds present in different organs of the yew may help understand its toxicity, medicinal potential (Shirmohammadli *et al.*, 2020), and possible uses in the food and cosmetic industry (Turek and Stintzing, 2013). Volatile oils, by-products of plant metabolism, can be synthesized by all plant organs (flowers, buds, leaves, stems, bark, shoots, seeds, fruits, roots, and wood) and are stored in glandular trichomes, secretory ducts, secretory cells, cavities and epidermal cells (Bakkali *et al.*, 2008). Their important roles are to protect plants against attacks by herbivores, bacteria, viruses, and fungi and to attract pollinators (Bakkali *et al.*, 2008).

In general, volatile oil is a complex mixture whose composition can be influenced by many endogenous or exogenous factors. In particular, endogenous factors are represented by their age, anatomical features, physiological characteristics, and the specificity of the biosynthesis pathways, which may change depending on the season, the plant organ, and the degree of adaptation of the plant DNA. Exogenous factors (soil, light, precipitation, season, geographical region) can affect, over some time, some genes responsible for the synthesis of the compounds present in volatile oil (Barra, 2009). Other factors that can influence the chemical composition of volatile oils include pollution, plant diseases and pests, processing and storage conditions of plant material (Figueiredo *et al.*, 2008), extraction method, and solvent used (Lefebvre *et al.*, 2020).

One of the methods by which volatile oil can be obtained is hydrodistillation, which involves three physicochemical processes: hydrodiffusion, hydrolysis, and heat decomposition. A disadvantage of the method, which limits its application to the extraction of thermolabile compounds, is that some volatile components may be lost during the processing of plant material (Azmir *et al.*, 2013). Also, limitations of hydrodistillation compared to other methods, such as microwave accelerated distillation (MAD), include the longer time required for extraction, higher costs to perform, the smaller quantities of oxygenated compounds obtained, and the fact that hydrodistillation causes slower breakdown of oil-presenting cells and glands compared to MAD (Ferhat *et al.*, 2006).

Biologically active compounds present in volatile oil can be converted into each other by chemically or enzymatically triggered cyclization, dehydrogenation, and oxidation reactions, this conversion phenomenon being due to their structural relationship within the same chemical group (Turek and Stintzing, 2013). In some cases, however, these chemical compounds, such as acids with molecules of tiny sizes, are not sufficiently volatile and can be dissolved during hydrodistillation in the hydrosol (Sadgrove *et al.*, 2021).

The aim of the present work is the analysis of the composition of volatile oils by GS/MS determination techniques, the spectrophotometric evaluation of hydrosols resulting from the hydrodistillation of dry and fresh plant material (leaves) belonging to cultivated and spontaneous female individuals of *Taxus baccata* L., as well as the identification, based on information presented in the literature, of their possible applications, according to the main biologically active compounds present in the highest concentrations in their composition.

Materials and Methods

Study area

The taxa investigated are represented by female yew individuals belonging to the spontaneous species *Taxus baccata* L. (T1) from the Yew Tree Reserve - Tudora Forest, Botoșani County, Romania (lat. 47°52'49" N, long. 26°69'19" E) (access to the Reserve was possible based on ANANP agreement no. 8147/26.07.2019 issued by the National Agency for Protected Natural Areas) and cultivated taxa: *Taxus baccata* (T2) and *Taxus baccata* 'Robusta' (T3), purchased from the nursery S.C. Doropad S.R.L. Suceava based in Dorohoi and cultivated in a private space in Vorniceni commune, Botoșani county (lat. 47°98'63" N, long. 26°66'33" E) (Figure 1). The environmental conditions of the areas from which the plant material was collected can be seen in Table 1.

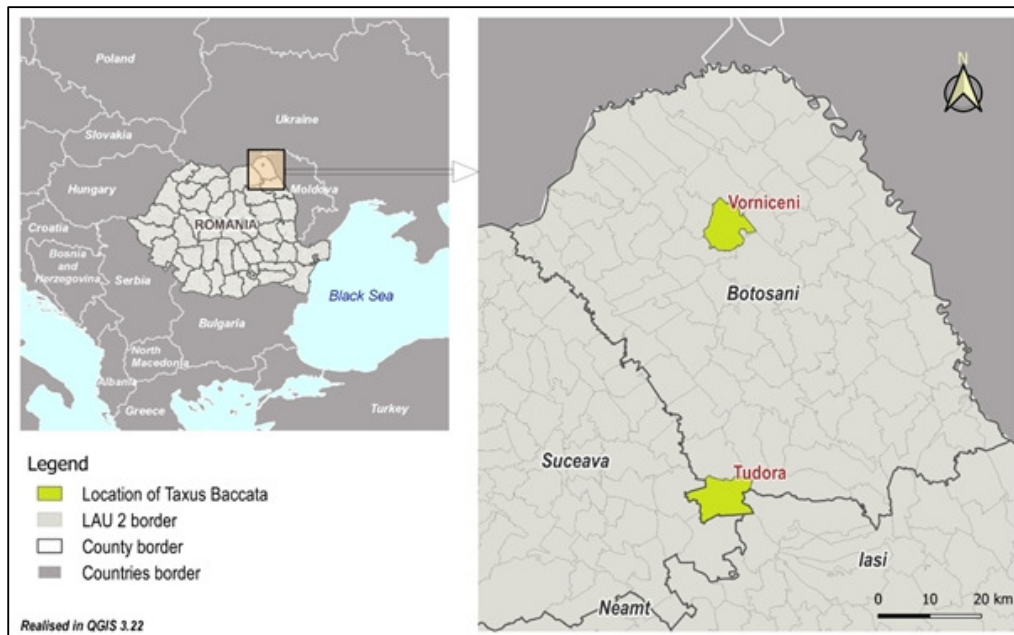


Figure 1. Locations of analyzed spontaneous and cultivated taxa of the genus *Taxus*

Table 1. Environmental conditions in the study area (according to Mohan, 2006; Gherman, 2007; Birsan *et al.*, 2017)

Environmental conditions	Study area	
	Yew Tree Reserve - Tudora Forest Botoșani County	Vorniceni commune Botoșani county
Altitude	350-530 m	175-200 m
Soil type	Luvisols	Black and cambic chernozems
Mean annual temperature	8-9 °C	8.4-8.7 °C
Mean annual precipitation	600-700 mm	526-609 mm

Plant material

Acquisition of biological material (leaves) was carried out during the period of intense vegetative growth (years 2021 and 2023), according to the phenophase of the proper ontogenetic cycle of the yew described by Robakowski *et al.* (2018). The taxa were identified and authenticated by biologist Dr. Irina Irimia, and vouchers were registered at the herbarium of the Faculty of Biology of “Alexandru Ioan Cuza” University of Iași with identification numbers 186539 (T1), 186537 (T2), and 186538 (T3).

The plant material prepared for analysis was divided into two types of samples: dried leaves initially subjected to heat treatment in an oven (60 °C for 60 minutes) for enzymatic inactivation and subsequently dried at room temperature in areas with adequate ventilation, protected from direct sunlight; fresh leaves, introduced into the work as soon as possible after harvesting.

The processing of the plant material was carried out in the research facilities of the Faculty of Biology of “Alexandru Ioan Cuza” University of Iasi, using equipment provided by the Plant Biology Laboratory and the Integrated Centre for Environmental Science Studies for the North-East Development Region (CERNESIM), organized with funds obtained through grant No. 257/28.09.2010, SMIS/CNR 13984/901.

Extraction of the volatile oil and hydrosols

The dried leaves (150 g/sample) and the freshly harvested leaves (300 g/sample) were distilled in water for 3 hours and 30 minutes after the start of boiling in a NeoClevenger extractor according to the method described by N. Radulović *et al.* (2010); Burzo and Toma (2012); Stefanović *et al.* (2016). After the distillation times were finished the hydrosol (hydrodistillate) was collected in Falcon tubes, and the volatile oil uptake was performed with hexane in specially designed chromatography flasks. The hydrosol was stored in the refrigerator at a temperature of 4 °C until its spectrophotometric evaluation and the volatile oil was frozen at -18 °C until GS/MS analysis.

Volatile oils analysis

The obtained oil samples were diluted in hexane at a dilution of 1000 and analyzed using the gas chromatograph coupled with mass spectrometer/Agilent Series GC/MS system consisting of GC 7890B Gas Chromatograph, MS 5977A System, GS Sampler 80 Injector. The analytical method uses the ultra-inert HP-5MS Capillary Column (30 m × 0.25 mm × 0.25 µm film thickness). Temperature program on the column oven: stationary at 50 °C for 8 min, heated from 50 °C to 280 °C with a speed of 4 °C/min. Helium carrier gas at a flow rate of 1 ml/min. Injection volume 3 µl, using a 50:1 spiking ratio. Ionization energy 70eV, over the mass range 50-550 m/z. The chemical compounds identification of the volatile oils was performed using the spectra library NIST 2015. Percent concentrations for each identified compound were calculated based on GC peak area without correction factors. Analyses were carried out in the laboratories of the Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Qualitative assessment of hydrosol absorption spectra

For the qualitative assessment of the chemical composition of hydrosols, their UV-VIS absorption spectra were determined on the 190-700 nm interval (Baciu *et al.*, 2013) using a Beckman DU - 730 spectrophotometer. The determinations were performed in the Laboratory of Biochemistry and Molecular Biology of the Faculty of Biology of “Alexandru Ioan Cuza” University of Iasi.

Results

Chemical composition of volatile oil

The volatile oils are natural volatile compounds with a complex structure, clean, rarely colored, soluble in organic solvents such as hexane, with a density less than the density of water, and liposoluble. They help to protect plants against attacks by weeds, bacteria, viruses, and fungi and attract pollinators (Bakkali *et al.*, 2008).

Analyzing the composition obtained by GC/MS analysis of the volatile oils extracted by hydrodistillation from the plant material at our disposal, we can deduce that they may be significant in different economic branches, such as pharmaceuticals, cosmetics, medicine, or agriculture.

In the carried-out investigation, a total of 83 compounds were detected for the analyzed leaf samples (dry and fresh) from wild and cultivated *Taxus* individuals (Table 2), of which 73 compounds were identified with ten compounds remaining unidentified: one compound for **T1** in fresh material and nine compounds at **T3** in dry materials (the ordering of the compounds in the table was done, for each taxon, according to the retention time RT).

Table 2. The percentage chemical composition of the volatile oils obtained from the leaves of the studied spontaneous and cultivated yew taxa, harvested during the phenophase of intense vegetative growth

No.	Compound	Chemical class	RI lit	RT	Dry leaves (%)			Fresh leaves (%)		
					T1	T2	T3	T1	T2	T3
1.	2-Butene-1,4-diol	Ac	960	11.882	-	-	-	-	1.7	-
2.	1-Octen-3-ol	Ac	956	12.186	-	0.47	-	1.03	25.61	-
3.	3-Octanol	Ac	976	13.048	-	-	-	-	0.21	-
4.	Pentadecane	A	1121	16.054	0.58	0.28	0.90	-	-	-
5.	Octanol	Ac	1059	16.660	0.27	0.26	-	1.8	2.73	0.92
6.	Linalool	M	1082	17.912	-	-	-	-	0.19	1.38
7.	Decane	A	1200	18.050	-	-	0.70	-	-	-
8.	Nonanal	Al	1081	18.123	-	-	-	-	0.42	-
9.	α -Terpineol	M	1189	21.593	-	-	-	-	-	0.59
10.	Methyl salicylate	E	1181	21.724	-	-	-	-	0.68	-
11.	Myrtenol	M (b)	1191	21.816	-	0.71	-	-	1.83	0.31
12.	Nonane	A	1199	22.038	-	-	-	-	-	0.21
13.	p-Menth-2-en-7-ol. cis	M (c)	1201	22.170	-	-	-	-	0.45	-
14.	Nerol	M (a)	1228	23.084	-	-	-	-	0.91	0.24
15.	p-Mentha-1(7).8(10)-dien-9-ol	M (c)	-	23.810	-	-	-	-	4.05	-
16.	β -cis-Ocimene	H	1024	23.833	-	0.78	-	-	-	-
17.	Geraniol	M (a)	1234	24.094	-	-	-	-	1.16	0.6
18.	trans-2-Decenal	A	1212	24.325	-	-	-	0.64	1.27	0.65
19.	1-Decanol	Ac	1258	24.784	0.83	1.00	-	2.68	0.76	0.53
20.	Unidentified component	-	-	25.037	-	-	1.82	-	-	-
21.	Perilla alcohol	M	1287	25.645	-	-	-	-	0.5	-
22.	Tridecane	A	1300	25.713	-	-	-	2.02	-	2
23.	Unidentified component	-	-	26.654	-	-	1.87	-	-	-
24.	Eugenol	F	1337	27.708	-	0.55	-	-	1.1	-
25.	Farnesane	S (a)	1381	28.331	-	-	-	1.25	-	1.46
26.	β -Damascenone	M (c)	1440	28.654	0.51	-	-	-	-	-
27.	β -Elemene	S	1388	28.862	-	-	-	-	-	2.91
28.	Tetradecane	A	1400	29.108	-	0.71	-	3.7	-	4.16
29.	Benzene. 1,2,4-trimethyl-	H	-	29.240	-	-	-	-	0.71	-
30.	α -Ionone	K	1429	30.058	1.70	-	-	-	-	-
31.	Dehydro- β -ionone	K	1440	30.196	1.05	-	-	-	-	-
32.	10-Undecyn-1-ol	Ac	-	30.646	-	-	-	-	1.4	-
33.	1,9-Decadiyne	H	1011	30.793	-	-	-	-	7.92	-
34.	Geranyl acetone	M (a)	1420	30.862	3.20	11.90	-	-	-	-

No.	Compound	Chemical class	RI lit	RT	Dry leaves (%)			Fresh leaves (%)		
					T1	T2	T3	T1	T2	T3
35.	Unidentified component	-	-	31.022	-	-	-	4.02	-	-
36.	Germacrene D	S (a)	1480	31.726	-	-	-	-	5.13	-
37.	β -Ionone	K	1457	31.913	5.24	-	-	-	-	-
38.	α -Bergamotene	S (a)	1430	32.179	-	-	2.87	1.42	3.36	3.19
39.	Humulene	S (a)	1447	32.474	-	-	-	-	-	5.87
40.	α -Farnesene	S (a)	1458	32.566	-	-	-	0.9	5.4	6.47
41.	Unidentified component	-	-	33.546	-	-	4.13	-	-	-
42.	cis- α -Bisabolene	S (m)	1521	33.606	-	-	-	0.59	1.8	-
43.	Nerolidol	S (a)	1522	34.218	-	-	-	3.02	0.39	0.55
44.	cis-3-Hexenyl benzoate	E	1544	34.429	-	-	-	-	0.46	-
45.	Hexadecane	A	1600	35.252	-	-	-	0.5	-	-
46.	Pentadecanal	Al	1693	35.664	1.55	-	-	0.79	-	-
47.	α -Bisabolol	S (m)	1683	37.732	-	-	-	-	0.33	-
48.	Heptadecane	A	1700	38.068	-	-	-	0.57	-	-
49.	Unidentified component	-	-	38.380	-	-	3.59	-	-	-
50.	Hexadecanal	Al	1795	38.473	2.41	-	-	1.16	-	-
51.	Hexa-hydro-farnesol	S (a)	1563	38.927	-	-	-	2.26	0.4	1.1
52.	α -Cedrene epoxide	S (b)	1569	39.337	4.29	-	-	-	-	-
53.	Hexylcinnamic aldehyde	Al	1728	39.432	4.41	-	-	-	-	-
54.	Unidentified component	-	-	39.551	-	-	4.73	-	-	-
55.	Benzyl Benzoate	E	1725	39.880	-	2.54	-	-	0.84	-
56.	Octyl caprylate	E	-	40.196	-	-	-	-	-	0.35
57.	Octadecane	A	-	40.790	1.47	-	-	-	-	-
58.	1.14-Tetradecanediol	Ac	1924	41.173	-	-	-	0.9	-	-
59.	Hexahydrofarnesyl acetone	S	1754	41.966	20.09	3.37	-	33.03	5.15	17.81
60.	Salicylic acid, benzyl ester	E	1860	42.568	8.11	-	-	0.87	0.93	-
61.	trans-2,3-Dimethoxycinnamic acid	CA	-	42.997	4.31	-	-	-	-	-
62.	Nonadecane	A	1900	43.297	2.26	-	-	0.96	-	-
63.	Farnesyl acetone	S (a)	1902	43.827	4.63	-	-	-	-	-
64.	Isophytol	D	1938	44.484	-	-	-	0.72	-	-

No.	Compound	Chemical class	RI lit	RT	Dry leaves (%)			Fresh leaves (%)		
					T1	T2	T3	T1	T2	T3
65.	Phthalic acid, hex-3-yl isobutyl ester	E	1908	44.930	10.51	-	-	1.4	-	-
66.	Unidentified component	-		44.948	-	-	4.07	-	-	-
67.	geranyl- α -terpinene	D	1962	45.025	-	-	-	-	1.71	-
68.	Manoyl oxide	D (t)	1992	46.123	-	11.85	2.53	-	10.86	1.46
69.	Hexadecanoic acid	FA	1968	46.382	1.51	-	-	-	-	-
70.	ar-abietatriene	D	2004	47.152	14.98	-	-	3.03	0.53	1.16
71.	Unidentified component	-		48.068	-	-	1.57	-	-	-
72.	Heneicosane	A	2100	48.095	1.89	-	-	2.52	0.55	2.96
73.	Phytol	D	2045	48.397	0.74	12.50	-	0.97	0.87	0.49
74.	Unidentified component	-		48.927	-	-	2.41	-	-	-
75.	Unidentified component	-		49.854	-	-	5.86	-	-	-
76.	Docosane	A	2200	50.281	-	-	-	1.92	0.69	3.63
77.	Phytol, acetate	D	2168	50.758	-	4.27	-	-	-	-
78.	Tricosane	A	2300	52.414	2.96	4.89	6.98	6.36	1.72	8.45
79.	Tetracosane	A	2400	54.469	-	8.11	11.13	3.1	0.93	5.34
80.	Pentacosane	A	2500	56.443	-	9.22	11.28	5.05	1.31	7.37
81.	Glycerol 2-palmitate	FA	-	56.625	-	9.06	17.89	-	-	-
82.	Hexacosane	A	2600	58.342	-	9.36	8.68	3.45	0.93	6.57
83.	Heptacosane	A	2700	60.180	-	8.17	6.99	7.37	2.11	11.27
Total:					100	100	100	100	100	100

Abbreviations: RI lit - Retention index from literature data; RT - Retention time; T1-*Taxus baccata* L. (spontaneous); T2-*Taxus baccata* (cultivated); T3 - *Taxus baccata* 'Robusta' (cultivated); Ac- Alcohol; A-Alkanes; Al-Aldehydes; CA- Carboxylic acid; D-Diterpenes; E-Esters; F-Phenols; FA- Fatty acid; H-Hydrocarbons; K-Ketones; M-Monoterpenes; S-Sesquiterpenes; (a)-acyclic; (b)-bicyclic; (m)-monocyclic; (t)-tricyclic.

Twenty-four compounds were detected in the volatile oil by spontaneous taxon T1 on dry material, 20 in the oil of taxon T2, and 19 in the oil of taxon T3 on cultivated taxa, whereas 32 compounds were detected in the oil of spontaneous taxon T1 on fresh material, and 42 in the oil of taxon T2 and 36 in the oil of taxon T3 on cultivated taxa.

In volatile oil obtained from dried leaves collected from spontaneous yew species, T1, the compounds identified in the highest concentrations were: hexahydrofarnesyl acetone (20.09%); ar-abietatriene (14.98%); phthalic acid, hex-3-yl isobutyl ester (10.51%); salicylic acid, benzyl ester (8.11%); β -ionone (5.24%); farnesyl acetone (4.63%); hexyl cinnamic aldehyde (4.41%). Following hydrodistillation of the fresh material, the most quantitatively well-represented compounds were: hexahydrofarnesyl acetone (33.03%); heptacosane (7.37%); tricosane (6.36%); pentacosane (5.04%); hexacosane (3.45%); tetracosane (3.10%); ar-abietatriene (3.03%); nerolidol (3.02%).

Common compounds identified in the volatile oils obtained from dried leaves and fresh leaves collected from spontaneous yew species (T1) are present in higher concentrations in oil obtained from dried material compared to oil obtained fresh material, such as pentadecanal (1.55% - dry leaves, 0.79% - fresh leaves); hexadecanal (2.41% - dry leaves, 1.16% - fresh leaves); nonadecane (2.26% - dry leaves, 0.96% - fresh leaves); phthalic acid, hex-3-yl isobutyl ester (10.51% - dry leaves, 1.4% - fresh leaves). At the same time, some chemical compounds such as β -damascenone (0.51%), α -ionone (1.70); dehydro- β -ionone (1.05%); β -ionone (5.24%); α -cedrene epoxide (4.29%); octadecane (1.47%); trans-2,3-dimethoxycinnamic acid (4.31%); hexadecanoic acid (1.51%); phyto acetate (4.27%) were identified only in the volatile oil obtained by hydrodistillation of the dried leaves of this taxon, while other compounds, such as hexadecane (0.5%); heptadecane (0.57%); 1,14-tetradecanediol (0.9%); isophytol (0.72%) were present only in the volatile oil obtained by hydrodistillation of its fresh material.

In the case of the oil obtained from the dried leaves of the cultivated taxon *Taxus baccata* L. (T2) highest concentrations were observed for the following compounds: phytol (12.50%); geranyl acetone (11.90%); manoyl oxide (11.85%); hexacosane (9.36%); pentacosane (9.22%); glycerol 2-palmitate (9.06%); heptacosane (8.17%); tetracosane (8.11%); tricosane (4.88%). Oil obtained from fresh leaves of the same taxon recorded the highest concentrations for 1-octen-3-ol (25.61%); manoyl oxide (10.86%); 1,9-decadiyne (7.92%); α -farnesene (5.40%); hexahydrofarnesyl acetone (5.15%); germacrene D (5.13%); p-mentha-1(7),8(10)-dien-9-ol (4.05%); α -bergamotene (3.36%).

Benzyl benzoate is present in higher concentrations in the volatile oil extracted from dried leaves, 2.54%, and eugenol is found in higher concentrations in the oil obtained from hydrodistillation of fresh leaves - 1.1%. β -cis-ocimene (0.78%) was identified only in the volatile oil obtained from dried leaves, in contrast, 2-butene-1,4-diol (1.70%); 3-octanol (0.21%); nonanal (0.42%); methyl salicylate (0.68%); p-menth-2-en-7-ol. cis (0.45%); perilla alcohol (0.50%); benzene, 1,2,4-trimethyl- (0.71%); 10-undecyn-1-ol (1.40%); cis-3-hexenyl benzoate (0.46%); α -bisabolol (0.33%); geranyl- α -terpinene (0.71%) were identified only in oil obtained by hydrodistillation of fresh leaves harvested from T2.

The volatile oil extracted from the dried leaves of the cultivated taxon T3 (*Taxus baccata* 'Robusta') showed the following compounds in the highest concentrations: glycerol 2-palmitate (17.89%); pentacosane (11.28%); tetracosane (11.13%); hexacosane (8.68%); heptacosane (6.99%); tricosane (6.98%). Volatile oil obtained from the fresh leaves of the same taxon recorded the highest concentrations for hexahydrofarnesyl acetone (17.81%); heptacosane (11.27%); tricosane (8.45%); pentacosane (7.37%); hexacosane (6.57%); α -farnesene (6.47%); humulene (5.87%); tetracosane (5.34%); tetradecane (4.16%); docosane (3.63%). Only one compound was identified in the volatile oil of dried leaves harvested from T3, namely decane (0.70%), while α -terpineol (0.59%); nonane (0.21%); β -elemene (2.91%); octyl caprylate (0.35%) were identified only in the volatile oil obtained from fresh leaves of this taxon.

The compounds identified in the volatile oils obtained by hydrodistillation of dry and freshly sampled material from the wild and cultivated *Taxus* taxa analyzed were classified into ten main classes, shown in Figure 2. The classes of compounds identified with the highest concentrations in the volatile oil obtained by hydrodistillation of dried leaves are represented by diterpenes (T2 - 28.62%); fatty acids and esters (T1 - 20.13%); monoterpenes (T2 - 12.61%); aldehydes (T1 - 8.37%); ketones (T1 - 7.99%) and carboxylic acids and phenols (T1 - 4.31%). In the volatile oil obtained by hydrodistillation of fresh leaves, the highest concentrations were recorded by sesquiterpenes (T1 - 42.47%), aldehydes (T3 - 51.96%), alcohols (T2 - 32.41%), and hydrocarbons (T2 - 8.63%).

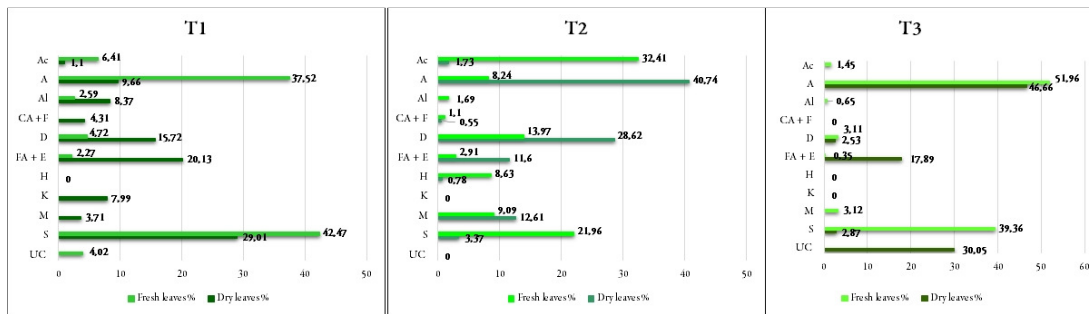


Figure 2. The main classes of compounds identified in the volatile oil obtained from the leaves of the spontaneous and cultivated yew taxa collected in the intense vegetative growth phenophase. **T1** -*Taxus baccata* L. (spontaneous); **T2** -*Taxus baccata* (cultivated); **T3** - *Taxus baccata* 'Robusta' (cultivated); Ac-Alcohol; A-Alkanes; Al-Aldehydes; CA-Carboxylic acid; D-Diterpenes; E-Esters; F-Phenols; FA- Fatty acid; H-Hydrocarbons; K-Ketones; M-Monoterpenes; S-Sesquiterpenes.

The practical results obtained in this respect can be correlated with those presented in the literature (Erdemoglu *et al.*, 2003; Radulović *et al.*, 2010) that classify the compounds present in the volatile oils extracted from yew into 8 or 10 classes of chemical compounds. Also, our results confirm the data presented in the literature on the variation in the chemical composition of volatile oils produced by plants as a function of environmental and internal factors.

Qualitative assessment of hydrosol absorption spectra

To qualitatively estimate the composition of hydrosols resulting from hydrodistillation of dried and fresh leaves collected from the three yew taxa, which were in the period of intense vegetative growth, a spectrophotometric analysis of their hydrosols was performed (Figure 3). This revealed higher values of the concentration of constituent compounds corresponding to the 220 - 240 nm spectral region, an area corresponding to aromatic compounds, as well as in the 260 - 280 nm region, an area corresponding to the presence of phenolic compounds (Kumar, 2006; Pretsh *et al.*, 2009; Butnariu, 2014). Of the analyzed three yew taxa, the cultivated species T2 shows the highest absorbance, especially in the 220 - 240 nm region, so it can be considered that its hydrosol is the most concentrated in aromatic compounds.

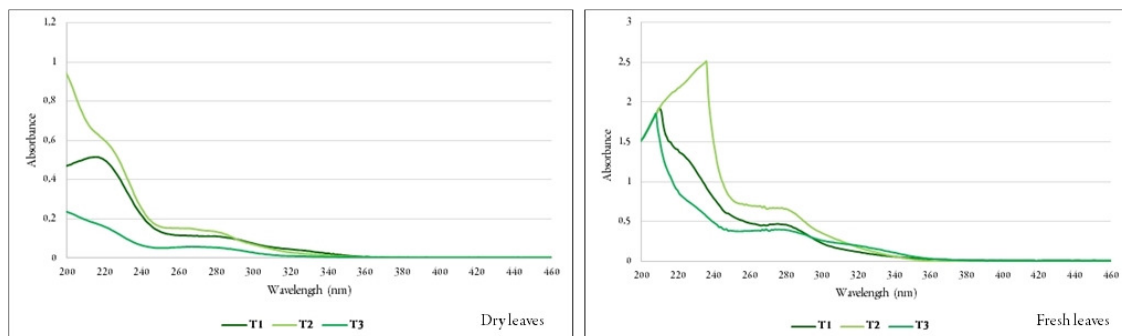


Figure 3. Absorption spectra of hydrosols obtained by hydrodistillation of leaves of the three spontaneous and cultivated yew taxa analyzed, harvested in the intense vegetative growth phenophase. **T1** -*Taxus baccata* L. (spontaneous); **T2** -*Taxus baccata* (cultivated); **T3** - *Taxus baccata* 'Robusta' (cultivated).

Discussion

The consulted literature provides little data on the composition of the volatile oils produced by the vegetative and reproductive organs of the species *Taxus baccata*, which is why our discussions also refer to other related species of the genus *Taxus*. Huong *et al.* (2020) consider that oils obtained from species of the genus *Taxus* can be classified, in terms of chemical composition, into five categories: oils containing large amounts of terpene hydrocarbons, oils whose main compounds are aliphatic alcohols, oils containing large amounts of fatty acids, oils in which oxygenated monoterpenes and sesquiterpenes predominate, and oils dominated by non-terpene compounds.

The chemical composition of the volatile oil obtained from *T. baccata* was also investigated by Erdemoglu *et al.* (2003) by analyzing the chemical composition of oils obtained from dried leaves and fresh leaves of specimens growing in Rize, Turkey. Before extraction in the hydrodistillation apparatus, they added emulsion for enzymatic hydrolysis of the samples. Using GS/MS analysis they found 63 chemical compounds representing 88.6% of the oil obtained from fresh plant material and 65 chemical compounds representing 86.6% of the oil obtained from dried leaves. The main groups of compounds identified in the two oils were fatty acids and esters - 61.0% (dry leaf oil) and 62.5% (fresh leaf oil); aliphatic compounds - 6.1% (dry leaf oil) and 13.6% (fresh leaf oil); monoterpenes were identified in concentrations of 2.6% (dry leaf) and 2.9% (fresh leaves), diterpenes in concentrations of 3.3% (dry leaves) and 2.4% (fresh leaves), alkanes and alkenes in concentrations of 5.0% (dry leaves) and 2.7% (fresh leaves), while terpenes were identified in concentrations of 1.8% (dry leaves) and 0.3% (fresh leaves), and sesquiterpenes in concentrations of 1.6% (dry leaves) and 0.8% (fresh leaves). In our case, the main groups of compounds identified in the six oils were alkanes in the dry leaves of T2 (40.74%), T3 (46.66%), and in the fresh leaves of T1 (37.52%), T3 (51.96%). We identified diterpenes in the dry leaves of T2 (28.62%), T1 (15.72%), and fresh leaves of T2 (13.97%). Fatty acids and esters were found in high concentrations in the case of volatile oils obtained from dry leaves of T1 (20.13%) and T3 (17.89%). We observed high concentrations of sesquiterpenes in one oil obtained from dry leaves of T1 (29.01%) and in all oils obtained from fresh leaves with the following concentrations 42.47% (T1), 39.36% (T3) and 21.96% (T2). Alcohol in the concentration of 32.41% was found only in the oil obtained from fresh leaves of T2, whereas in the oil obtained from dry leaves of T3, we found one unidentified compound in high concentration (30.05%). The most abundant chemical compounds identified by Erdemoglu *et al.* (2003) in the volatile oil obtained from dried leaves were hexadecanoic acid (22.5%); decanoic acid (12.6%); tetradecanoic acid (8%); hexahydrofarnesyl acetone (4.7%); ethyl hexadecanate (3.7%); decanol (3.6%); ethyl linolenate (3.2%); phytol (1.8%); (E)-geranyl acetone (1.6%); β -ionone (1.2%). In the volatile oil obtained from fresh leaves, the researchers identified the following compounds in higher concentrations: hexadecanoic acid (19.6%); decanoic acid (19.5%); decanol (5.4%); ethyl linolenate (4.2%); ethyl palmitate (3.1%); tetradecanoic acid (2.1%); octanol (2.0%); phytol (1.9%); hexahydrofarnesyl acetone (1.6%); ethyl (E)-cinnamate; 1-octen-3-ol (1.3%). We identified hexadecanoic acid in the low concentration (1.51%) only in the oil obtained from dry leaves of T1. We also identified high concentrations of hexahydrofarnesyl acetone in the case of oils obtained from fresh leaves of T1 (33.03%), T3 (17.81%), and T2 (5.15%). We did not find in any of our oils some compounds identified in high concentration by Erdemoglu *et al.* (2003), such as decanoic acid, tetradecanoic acid, ethyl hexadecanate.

In another paper, Radulović *et al.* (2010) described the chemical composition of volatile oil obtained by hydrodistillation from fresh leaves and apical parts of branches with cortex of *T. baccata* L. species from Serbia. By the GS/MS analysis, they identified 62 chemical compounds, of which the highest concentrations were recorded for hexahydrofarnesyl acetone (18.3%); myrtenol (18.3%); (Z)-3-hexenol (6.0%); senecioic acid (5.9%) and trichozan (5.5%). The groups of compounds identified by Radulović *et al.* (2010) showed the following concentrations: 28.2% terpenes, 19.1% monoterpenes (myrtenol 18.3%; geraniol 0.7%; 4-vinylphenol 0.3%), 18.4% carotenoid-derived compounds (hexahydrofarnesyl acetone 18.3%; isophorone 0.

1%) and n-alkanes (tricosan 5.5%; heneicosan 2.7%; docosan 2.6%; tetracosan 2.1%; eicosan 1.6%; nonadecan 0.3%), 12% green leaf volatile compounds, 3.2% aldehydes, 7.2% fatty acids and fatty acid esters (hexadecanoic acid 2.2%; oleic acid 1.4%; stearic acid 1.3%; δ -octadecalactone), 5.9% hemiterpenoids, 3.2% n-aldehydes (pentadecanal 0.9%; docosanal 0.5%; tetradecanal 0.4%) and diterpenoids ((E)-phytol 1.3%; ar-abietatrien 0.9%), 1.8% 1-alkenes (1-docosene 0.5%; 1-eicosene 0.3%) and other compounds 3. According to the authors, the absence of sesquiterpenes in the oil obtained by hydrodistillation is because these compounds were not volatile under hydrodistillation conditions or because the biosynthesis pathways of sesquiterpenoids were not operational in the *Taxus* species from Serbia. In comparison with Radulović *et al.* (2010), as can be seen in Figure 2, we found sesquiterpenes in all oils obtained by hydrodistillation of fresh and dry leaves collected from the cultivated and spontaneous species of *Taxus* from Romania.

Another study on the chemical composition and variability of volatile oils of *Taxus baccata* L. from Serbia was conducted by Stefanović *et al.* (2016); the mentioned researchers identified 91 chemical compounds in the volatile oil of shoots and leaves taken from 3 populations of *Taxus baccata* L., of which 87 were identified. The composition of the oil obtained by the cited authors was 86.92% aliphatic alcohols, terpenes, aliphatic hydrocarbons, and aliphatic aldehydes. Monoterpenes constituted 14.41% of the oil composition, while sesquiterpenes constituted 2.31%. To determine variations in the chemical composition of the oil produced by the three populations studied, the researchers selected 22 compounds, of which 9 showed statistical differences, concluding that, in terms of chemical composition, population I (Starovlaško-Raška) and II (Kopaonik) were similar, and population III (Carpathian-Balkan Mountains) distinct. In our case, pentadecane and tricosane were identified in all oils obtained from dry leaves of yew. Apart from these compounds, we did not find common compounds in the volatile oils obtained from the dry leaves of T1 and T3. In the dry leaves of T1 and T2, we found 5 common compounds (octanol, 1-Decanol, geranyl acetone, hexahydrofarnesyl acetone, phytol) and in the oil obtained from dry leaves of T2 and T3 we found 6 common compounds (manoyl oxide, tetracosane, pentacosane, glycerol 2-palmitate, hexacosane and heptacosane). We found 16 common compounds in the volatile oils obtained from fresh leaves of yew. We did not find pentadecane in oils obtained from fresh leaves. Also, we identified 3 common compounds (cis- α -Bisabolene, 1-Octen-3-ol, salicylic acid, benzyl ester) in the oils from T1 and T2, 5 common compounds (linalool, myrtenol, nerol, geraniol, α -Farnesene and Manoyl oxide) in the oils from T2 and T3. In the volatile oils of T1 and T3, the common compounds were tridecane, farnesane, and tetradecane. We can conclude that in the case of volatile oils obtained from dry leaves, the chemical composition of T1 and T3 differ, whereas the chemical composition of T2 and T3 are more similar. Whereas in the case of volatile oils obtained from fresh leaves, the chemical composition of all yew taxons is more similar. The chemical composition of the volatile oil obtained from the leaves of *Taxus baccata* species from Algeria was analyzed by Benlembarek *et al.* (2021). The researchers identified by GC/MS analysis the following compounds in the highest concentrations: undecanon-2 (76.96%); nonanon-2 (5.57%); tridecanon-2 (4.43%); decanon-2 (3.92%); methyl dehydro-abietate (1.9%); α -pinene (1.13%). The compounds thus identified were grouped into the following classes: ketones (9.97%), hydrocarbons and monoterpenes (2.66%), esters (1.94%), alcohols (1.18%), hydrocarbons and sesquiterpenes (0.75%), aldehydes (0.18%).

After the analysis of volatile oil obtained from plant material (fresh leaves and shoots) taken from *Taxus canadensis* Marsh by GS/MS, Jean *et al.* (1993) identified the following compounds in the highest concentrations: (E)Q-hexenal (24.13%); 1-octen-3-ol (44.64%); cyclooctanone (4.65%); citral (3.00%); hexene (2.14%); (Z)-3-hexenol (1.97%); occidentalol (1.41%); thujatric acid (1.18%); hexanol (1.02%).

The chemical composition of the volatile oil obtained from mature leaves and wood belonging to the species *Taxus chinensis* (Rehder & E.H. Wilson) Rehder from Vietnam, analyzed GC/MS by Huong *et al.* (2020) revealed that the most abundant groups of compounds present in the leaf volatile oil were: monoterpene hydrocarbons (54.2%), oxygenated monoterpenes (26.1%), diterpenes (5.1%), sesquiterpene hydrocarbons (4.1%) and oxygenated sesquiterpenes (0.7%), while in the oil obtained from wood, the most abundant groups

of compounds were: oxygenated sesquiterpenes (39.1%), monoterpene hydrocarbons (31%), oxygenated monoterpenes (16%), sesquiterpene hydrocarbons (4.4%), diterpenes being absent.

Similarly, Zhang *et al.* (2012), analyzing the chemical composition of volatile oil extracted from the leaves of *Taxus media* and *Taxus chinensis* var. *mairiei* species harvested from Changshan, China, found that their chemical composition varies, authors considered that these variations may be due to the different genotypes of the species, age, and geographical regions where the species grow. In another paper, Wei and Yin (2019) analyzed the chemical composition of oil obtained from 50-year-old aerial stems of *Taxus chinensis* var. *mairiei* species harvested from four geographical regions of China, with GS/MS analysis allowing the identification of 62 chemical compounds. According to the researchers, most of the compounds identified were benzenes, acids, esters, ketones, and alkanes, and variations in chemical composition could be attributed to environmental factors.

The chemical composition of volatile oil extracted from leaves of *Taxus cuspidata* species collected from Yeungnam University campus analyzed by Bajpai *et al.* (2013) allowed the identification by GC/MS analysis of 34 compounds, which belonged to diterpenes, aliphatic and aromatic hydrocarbons, mono- and sesquiterpenes, aliphatic and aromatic acids, phenolic acids, alkaloids and esters, as well as imidazole, quinoline and isoquinoline derivatives.

Dhakal *et al.* (2022) systematized scientific papers published between 1975 and 2021 on the chemical composition and biological properties of volatile oils belonging to *Taxus* species, concluding that although this taxon is known worldwide for the presence of taxol with anticarcinogenic activity, it can also be used in the therapy of lung diseases, epilepsy, malaria, and hysteria. According to the researchers, the chemical composition of volatile oils is generally dominated by alcoholic compounds, myrtenol, caryophyllene, elemicin, trans-2-hexenal, α -pinene, laminitol, palmitic, linoleic, oleic, taxoleic and α -linolenic acids.

According to Sharma *et al.* (2022), the pharmacological value and medicinal properties of the volatile oil obtained from the plant material represented by the organs of the species *Taxus baccata* L., are due to the presence in its composition of numerous groups of compounds such as terpenes, aldehydes, alcohols, hydrocarbons, flavonoids (quercetin 3-O-glucosyl-rutinoside, myricetin-3-rutinoside, kaempferol 3-O-rutinoside, kaempferol, quercetin 7-O-glucoside, kaempferol 7-O-glucoside, myricetin, quercetin, betulozide, sciadopitysin, bilobetin) and alkaloids (taxine A, B, and M, 10-deacetylbaaccatine II, cephalomannine, baaccatine II).

Khan *et al.* (2006) after analyzing the chemical composition of the volatile oil extracted from the leaves of *Taxus wallichiana* Zucc. species harvested from the Northern Himalayan region of India found that due to its specific chemical composition, the volatile oil of *Taxus wallichiana* has potential for use in the perfume industry.

The volatile oil obtained from *Taxus media* and *Taxus chinensis* var. *mairiei* were found to exhibit antibacterial activity against bacterial strains of *Escherichia coli* and *Staphylococcus aureus* by Zhang *et al.* (2012). Bajpai *et al.* (2013) have tested the antibacterial activity of the volatile oil obtained from *Taxus cuspidata*, recording the highest inhibitory effects of it applied in concentrations of 1.000 $\mu\text{g}/\text{disc}$ on bacterial strains *Bacillus cereus* ATCC 13061 (diameter of the inhibition zone 34.0 ± 1.2 mm), *Listeria monocytogenes* ATCC 7644 (diameter of the inhibition zone 27.0 ± 0.3 mm) and, *Staphylococcus aureus* ATCC 12600 (diameter of the inhibition zone 34.0 ± 0.8 mm), moderate inhibitory effects were also recorded for the bacterial strains *Staphylococcus typhimurium* ATCC 43174 (inhibition zone diameter 22.0 ± 0.4 mm) and, *Escherichia coli* ATCC 43889 (inhibition zone diameter 24.0 ± 0.6 mm), the minimum inhibitory concentration was 250 $\mu\text{g}/\text{mL}$ and was recorded for *Bacillus cereus* and *Staphylococcus aureus* strains. According to Bajpai *et al.* (2013), *Taxus cuspidata* volatile oil at concentrations of 250 and 500 $\mu\text{g}/\text{mL}$ in *Bacillus cereus* and *Escherichia coli* strains caused cell membrane damage, cell swelling, abnormal rupture, and complete lysis or death, and the volatile oil caused pore formation that allowed ATP to be released into the extracellular medium. Analyzing

potassium ion efflux, the cited authors found that the K⁺ ion efflux of Gram-positive bacteria was 1.2 times higher than that of Gram-negative bacteria, stating that in bacterial cells treated with volatile oil, the loss of cytoplasmic constituents indicated significant and irreversible damage to the plasma membrane as a result of the action of volatile oil. In another of these studies Benlembarek *et al.* (2021) tested the antibacterial and antifungal activity of *T. baccata* volatile oil; the pure extracted oil showed inhibitory action on the bacterial strain *Pseudomonas aeruginosa* (inhibition zone diameter of 15.3 mm) and the fungal strains *Puccinia sp.* (inhibition zone diameter 15.07 mm), *Fusarium oxysporum* (inhibition zone diameter 14.05 mm), *Alternaria alternata* (inhibition zone diameter 13.14 mm), *Fusarium gramineum* (inhibition zone diameter 12.14 mm). Regarding antioxidant activity (DPPH), Benlembarek *et al.* (2021) found that volatile oil exerted low antioxidant activity.

As the literature indicates, the compounds identified in the highest concentrations in the oils of the three *Taxus* taxa studied, listed in Table 2, have numerous biological properties and possible practical applications. Thus, according to Paparella *et al.* (2021), the compound β -ionone has a repellent or attractant effect on insects, antibacterial and antifungal activity, and potential activity in cancer therapy. We found these biologically active compound in a concentration of 5.24% in the volatile oil obtained from dry leaves of T1.

Guan *et al.* (2014) also reports that the compound β -elemene has been approved for use in China for the treatment of various cancers, particularly brain tumors, and the chemical also has potential in breast cancer therapy as it can induce cytoprotective autophagy and apoptosis. In our case this compound in a concentration of 2.91% was identified only in fresh leaves of T3. Labbozzetta *et al.* (2022) investigated the mechanism by which phytol and heptacosan enhance P-glycoprotein-mediated drug transport in the context of the need to identify new therapies that can be applied in drug-resistant acute myeloid leukaemia; the authors thus suggest that two compounds, phytol and heptacosan act as non-toxic modulators of P-glycoprotein by different mechanisms and can reverse P-glycoprotein-mediated drug resistance in tumour cells. Compound α -humulene shows, according to Chen *et al.* (2019), an effect against hepatocellular carcinoma through its property to inhibit protein kinase B (Akt), thereby reducing carcinoma cell proliferation and inducing apoptosis, and Legault *et al.* (2003) suggest that the cytotoxic activity of compound α -humulene may involve glutathione and reactive oxygen production. According to Ansari and Emami (2016), the compound β -ionone exhibits antiproliferative, antimetastatic activity and the property of inducing apoptosis *in vivo* and *in vitro*, and Pereira *et al.* (2018) stated that linalool did not show toxicity *in vivo* and *in vitro* studies, suggesting that this compound could be used, due to its properties (anticancer, anti-inflammatory, analgesic, anti-anxiolytic, antimicrobial and antidepressant activity) in obtaining efficient preparations in the cosmetic and pharmaceutical industry. The research by Cordeiro *et al.* (2020) demonstrates the antibiofilm activity of the myrtenol compound, making it a good candidate for creating a drug for the antibiotic treatment of *Streptococcus aureus* infections.

According to Stevanović *et al.* (2018), the biological activity of volatile oils is influenced by the interaction of phytochemicals and their bioavailability in the gastrointestinal tract of animals. Following the analysis of literature data, ElGawad *et al.* (2021) concluded that the phytotoxicity of volatile oils is due to oxygenated terpenes, mono- and sesquiterpenes. Among these, pinene, 1,8-cineole, linalool, and carvacrol are the most effective monoterpenes exerting phytotoxicity, while the most effective sesquiterpenes are represented by caryophyllene and its derivatives, germacrene, spathulenol, and hexahydrofarnesyl acetone. According to this information, we can conclude that the volatile oils obtained from the dry and fresh leaves of spontaneous and cultivated species of yew from Romania can exercise phytotoxicity, due to the high concentration of hexahydrofarnesyl acetone (Table 2).

In the review paper by Bhardwaj *et al.* (2020), it is stated that the phytotoxicity of conifer oils is due to the presence of α -limonene, β et α -pinene, linalool compounds, which exert herbicidal activity and inhibit seed germination. The authors also state that the oils of these species are rich in monoterpenes, sesquiterpenes, diterpenes, ketones, alcohols, and esters, biologically active compounds used in the cosmetic, food, and

pharmaceutical industries. Due to their antiseptic, anti-cancer, and anti-inflammatory properties, these oils are used in traditional medicine for skin, digestive, bronchitis, fever, and asthma.

According to Dhifi *et al.* (2016), the qualitative and quantitative chemical composition of volatile oils shows an increased variability, which is influenced by intrinsic factors, which are related to the producing plant and its interaction with environmental factors (climate, soil), the age of the plant, the time of day when the plant material was harvested and extrinsic factors, which are related to the environment and the way the volatile oil was extracted. The cited authors consider these factors hard to isolate, as they are often interlinked and influence each other. These observations align with our findings and are substantiated by the empirical data we have gathered. The environmental parameters, namely altitude, soil type, mean annual precipitation, and temperature, varied across the locations from which we obtained plant samples (Table 1). Although we employed a uniform extraction method, utilizing both dried and fresh plant materials, as other scientists, we can conclude that the variability in chemical compositions may be attributable to numerous factors (internal and environmental). The complexity of these factors makes it challenging to isolate their influences.

After consulting the information presented by the specialized literature regarding the composition of the volatile oils extracted from different organs of the yew trees, we observed the variety of applied working methods that refer to the investigated taxa, the location where they grow, the type of material analyzed (leaves, shoots or rhytidoma); the state of the material to be extracted (fresh or dry), type of extraction solvents, and the methods used in the investigation of the biological activity (antibacterial, antifungal, antiproliferative) of volatile oils. Due to these aspects, the comparison of the results thus obtained and communicated becomes difficult.

Hydrosols/hydrosols are the secondary compounds of the hydrodistillation of different parts of aromatic plants, which separate from the volatile oil phase at the end of the distillation process (Politi *et al.*, 2022). In the literature, hydrosols are found under various names, including hydrolate, hydrochlorate, hydroflorate, aromatic water, floral water, and essential aromatic water (Rajeswara Rao, 2013; D'Amato *et al.*, 2018). In the scientific community, hydrosols have been defined for a long time as waste products of steam distillation. Currently, researchers are reconsidering them, analyzing their allelopathic, antimicrobial properties, and antifungal capacity (D'Amato *et al.*, 2018). The aromatic profile of hydrosols extracted by hydrodistillation of plant material can be significantly different from that of the respective volatile oils due to the lack of hydrophobic, water-insoluble isoprenoid (hydrocarbon) compounds (Rajeswara Rao, 2013). The chemical composition of hydrosols and volatile oils can vary; thus, in a comparative study of 44 hydrosols with corresponding volatile oils, it was found that in 42% of cases, the main compounds of volatile oils and hydrosols were different (Inouye *et al.*, 2008; Politi *et al.*, 2022). The evaluation of hydrosols resulting from hydrodistillation of leaves (dry and fresh material) in the yew taxa by different methods such as LC-QTOF-MS/MS (Azhar *et al.*, 2021), GC-MS (Ulusoy *et al.*, 2009), HPLC (Timung *et al.*, 2016), HS-SPME-GC-MS (Politi *et al.*, 2022), can be a future research step, along with testing their possible antimicrobial, antifungal, and allelopathic effects.

Conclusions

The quantitative and qualitative analysis of volatile oils and hydrosols obtained by hydrodistillation from leaves collected during the period of intense vegetative growth of the plants revealed a useful potential of the investigated yew taxa. In the volatile oil extracted from the leaves of the spontaneous taxon (T1), 24 chemical compounds (dry leaves) and 31 chemical compounds (fresh leaves) were identified, respectively, of which the highest percentage concentration was found for the compound hexahydrofarnesyl acetone (33.03%); in the oil extracted from the leaves of the cultivated taxon (T2), 20 chemical compounds (dry leaves) and 42 chemical compounds (fresh leaves) were identified, with the compound 1-Octen-3-ol having the highest

percentage concentration (25.61%), and 19 chemical compounds (dry leaves) and 36 chemical compounds (fresh leaves) were identified in the oil extracted from the leaves of the cultivated taxon (T3), of which, as in the case of the spontaneous taxon T1, the highest percentage concentration was recorded by the compound hexahydrofarnesyl acetone (17.81%). Qualitative evaluation of the hydrosols obtained by hydrodistillation, carried out by analysis of their absorption spectra, revealed the presence in their composition of aromatic compounds, which were identified in the 220-240 nm region, and phenolic compounds, detected in the 260-280 nm region of their absorption spectra.

By their chemical composition, the volatile oils extracted from the leaves of the yew taxa analyzed record classes of chemical compounds such as terpenes, aldehydes, alcohols, and hydrocarbons that may constitute resources of biologically active compounds usable in the pharmaceutical and medical industries. From our knowledge, this is the first report on the chemical composition of volatile oils biosynthesized in the leaves of the spontaneous species *Taxus baccata* L., from the Yew Reserve - Tudora Forest, Romania, as well as in the leaves of yew taxa cultivated in the country for ornamental purposes. As a result, we consider it necessary to further investigate the chemical composition of hydrodistillation products (volatile oils and hydrosols) in more samples of plant material (vegetative and reproductive organs) collected from a higher number of spontaneously growing/cultivated yew specimens in Romania and to test them as resources of biologically active compounds of value for pharmacy, cosmetics, medicine, as well as products with possible allelopathic effects of importance in organic agriculture (possible herbicide potential) and in the natural protection of wild +/- endangered species.

Authors' Contributions

Conceptualization: M.E.F., T.R., R.M.C.; Data curation: M.E.F., V.A.I., R.M.C.; Formal analysis: M.E.F., T.R., R.M.C.; Methodology: M.E.F., T.R., M.N.G.; Supervision: M.M.Z., M.N.G., L.B.; Validation: M.M.Z., M.N.G., L.B.; Writing - original draft: M.E.F., T.R., R.M.C.; Writing - review and editing: M.M.Z., M.N.G. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research did not receive a specific grant from any public, commercial, or non-profit funding agency. The authors are grateful to Dr. Cehan Alexandra for producing, in QGIS 3.22 software, Figure 1 - Location map of yew taxa analyzed and thank Prof. Dr. Habil. Marius I. Mihășan from the Laboratory of Biochemistry and Molecular Biology of the Faculty of Biology of the University "Alexandru Ioan Cuza" of Iasi for his help in the spectrophotometric analyses carried out in this study.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Ansari M, Emami S (2016). b-Ionone and its analogs as promising anticancer agents. *European Journal of Medicinal Chemistry* 1231:141-154. <http://dx.doi.org/10.1016/j.ejmech.2016.07.037>
- Azhar MA, Rahaman NWA, Aziz MAA, Isa KM (2021). Identification of chemical compounds from agarwood hydrosol (*Aquilaria malaccensis*) fruits via LC-QTOF-MS/MS analysis, *Earth and Environmental Science* 765(012010):1-16. <https://doi.org/10.1088/1755-1315/765/1/012010>
- Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering* 117:426-436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Baciu A, Ranga F, Fetca F, Zavoi S, Socaciu C (2013). Fingerprinting Food supplements and their botanical ingredients by coupled UV/Vis/FTIR spectrometry. *Bulletin UASVM Food Science and Technology* 70(1):8-15.
- Bajpai VK, Sharma A, Moon B, Baek KH (2013). Chemical composition analysis and antibacterial mode of action of *Taxus cuspidata* leaf essential oil against foodborne pathogens. *Journal of Food Safety* 1745-4565. <https://doi.org/10.1111/jfs.12089>
- Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils-a review. *Food and Chemical Toxicology* 46:446-475. <https://doi.org/10.1016/j.fct.2007.09.106>
- Barra A (2009). Factors affecting chemical variability of essential oils: a review of recent developments. *Natural product communications* 4(8):1147-1154. <https://doi.org/10.1177/1934578X0900400827>
- Benlembarek K, Lograda T, Ramdani M, Figueredo G, Chalard P (2021). Chemical composition, antibacterial, antifungal, and antioxidant activities of *Taxus baccata* essential oil from Algeria. *Biodiversitas* 22(12):5475-5483. <https://doi.org/10.13057/biodiv/d221231>
- Bhardwaj K, Islam MT, Jayasena V, Sharma B, Sharma S, Sharma P, Kuca K, Bhardwaj P (2020). Review on essential oils, chemical composition, extraction, and utilization of some conifers in Northwestern Himalayas. *Phytotherapy Research* 1-22. <https://doi.org/10.1002/ptr.6736>
- Birsan C, Mardari C, Copoț O, Apăștinei L (2017). Development of *Taxus baccata* L. population under tree canopy in the Tudora Reservation. *Acta Oecologica Carpatica* 11:37-50.
- Burzo I, Toma C (2012). Țesuturile secretoare și substanțe volatile din plante [Secretory tissues and volatile substances in plants]. Ed. Universității „Alexandru Ioan Cuza” din Iași, pp 148.
- Butnariu M (2014). Detection of the polyphenolic components in *Ribes nigrum* L. *Annals of Agricultural and Environmental Medicine* 21(1):11-14.
- Chen H, Yuan J, Hao J, Wen Y, Lv Y, Chen L, Yang X (2019). α -Humulene inhibits hepatocellular carcinoma cell proliferation and induces apoptosis through the inhibition of Akt signaling, *Food and Chemical Toxicology* 134:1-11. <https://doi.org/10.1016/j.fct.2019.110830>
- Cordeiro L, Figueiredo P, Souza H, Sousa A, Júnior FA, Filho JB, Lima E (2020). Antibacterial and antibiofilm activity of myrtenol against *Staphylococcus aureus*. *Pharmaceuticals* 13(6):133. <https://doi.org/10.3390/ph13060133>
- D'Amato S, Serio A, López CC, Paparella A (2018). Hydrosols biological activity and potential as antimicrobials for food application. *Food Control* 86:126-137. <https://doi.org/10.1016/j.foodcont.2017.10.030>
- Dhakal S, Khosla PK, Getahun T (2022). Chemical compositions and biological activities of the oils from the genus *Taxus* and factors limiting the regeneration of endangered yews: a review. *Turkish Journal of Chemistry* 46(6):1776-1801. <https://doi.org/10.55730/1300-0527.3480>
- Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016). Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines* 3(4):25. <https://doi.org/10.3390/medicines3040025>

- ElGawad AMA, Gendy AENGE, Assaeed AM, Rowaily SLA, Alharthi AS, Mohamed TA, Nassar MI, Dewir YH, Elshamy AI (2021). Phytotoxic effects of plant essential oils: a systematic review and structure-activity relationship based on chemometric analyses. *Plants* 10(1):36. <https://dx.doi.org/10.3390/plants10010036>
- Erdemoglu N, Sener B, Demirci B, Baser KHC (2003). The glycosidically bound volatile compounds of *Taxus baccata*. *Chemistry of Natural Compounds* 39(2):195-198.
- Ferhat MA, Meklati BY, Smadja J, Chemat F (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *Journal of Chromatography A* 1112:121-126. <https://doi.org/10.1016/j.chroma.2005.12.030>
- Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour and Fragrance Journal* 23:213-226. <https://doi.org/10.1002/ffj.1875>
- Gherman Ghe (2007). Vorniceni însemnată vatră strămoșească [Vorniceni, a significant ancestral home]. Ed. Agata pp 9-44.
- Guan C, Liu W, Yue Y, Jin H, Wang X, Wang XJ (2014). Inhibitory effect of β -elemene on human breast cancer cells. *International Journal of Clinical & Experimental Pathology* 7(7):3948-3956.
- Huong LT, Thuong NTH, Chac LD, Dai DN, Ogunwande IA (2020). Antimicrobial activity and chemical constituents of essential oils from the leaf and wood of *Taxus chinensis* (Rehder & E.H. Wilson) Rehder (Taxaceae) from Vietnam. *Journal of Biologically Active Products from Nature* 10(1):8-17. <https://doi.org/10.1080/22311866.2020.1749128>
- Huong LT, Thuong TH, Chac LD, Dai DN, Ajeniya AOG, Ogunwande IA (2020). The stem essential oil of *Taxus chinensis* (Rehder & E.H. Wilson) Rehder (Taxaceae) from Vietnam. *American Journal of Essential Oils and Natural Products* 8(3):09-12.
- Inouye S, Takahashi M, Abe S (2008). A comparative study on the composition of forty-four hydrosols and their essential oils. *International Journal of Essential Oil Therapeutics* 2:89-104.
- Jean FI, Garneau FX, Collin GJ, Bouhajib M, Zamir LO (1993). The essential oil and glycosidically bound volatile compounds of *Taxus canadensis* Marsh. *Journal of Essential Oil Research* 5(1):7-11. <https://doi.org/10.1080/10412905.1993.9698163>
- Jovanović B (1970). *Taxus* L. In: Josifović M (Ed). *Flora of Serbia* 1, SASA, Belgrade, pp 164-166.
- Khan M, Verma SC, Srivastava SK, Shawl AS, Syamsundar KV, Khanuja SPS, Kumar T (2006). Essential oil composition of *Taxus wallichiana* Zucc. from the northern Himalayan region of India. *Flavour and Fragrance Journal* 21:772-775. <https://doi.org/10.1002/ffj.1682>
- Kumar S, Abedin M, Singh AK, Das S (2020). Role of phenolic compounds in plant-defensive mechanisms. In: Lone R, Shuab R, Kamili AN (Eds). *Plant phenolics in sustainable agriculture*. Springer, Singapore, pp 517-532. <https://doi.org/10.1007/978-981-15-4890-1>.
- Labbozzetta M, Poma P, Tutone M, McCubrey JA, Sajeve M, Notarbartolo M (2022). Phytol and heptacosane are possible tools to overcome multidrug resistance in an *in vitro* model of acute myeloid leukemia. *Pharmaceuticals* 15(36):1-19. <https://doi.org/10.3390/ph15030356>
- Lefebvre T, Destandau E, Lesellier E (2020). Selective extraction of bioactive compounds from plants using recent extraction techniques: a review. *Journal of Chromatography A* 1-50. <https://doi.org/10.1016/j.chroma.2020.461770>
- Legault J, Dahl W, Debiton E, Pichette, A., Madelmont JC (2003). Antitumor activity of balsam fir oil: production of reactive oxygen species induced by α -Humulene as possible mechanism of action. *Planta Medica* 69(5):402-407.
- Milutinović MG, Stanković MS, Cvetković DM, Topuzović MD, Mihailović VB, Marković SD (2015). Antioxidant and anticancer properties of leaves and seed cones from European yew (*Taxus baccata* L.). *Archives of Biological Sciences, Belgrade* 67(2):525-534. [10.2298/ABS161021105E](https://doi.org/10.2298/ABS161021105E)
- Mohan Ghe, Ardelean A (2006). Parcuri și rezervații naturale din România [Natural parks and reserves in Romania]. Ed. Victor B Victor, București, pp 118-119.
- Panzeri C, Bacis G, Ferri F, Rinaldi G, Persico A, Uberti F, Restani P (2010). Extracorporeal life support in a severe *Taxus baccata* poisoning. *Clinical Toxicology* 48:463-465. <https://doi.org/10.3109/15563650.2010.487487>
- Paparella A, Harpaza LS, Ibdah M (2021). β -Ionone: its occurrence and biological function and metabolic engineering. *Plants* 10(4):745. <https://doi.org/10.3390/plants10040754>

- Pereira I, Severino P, Santos AC, Silva AM, Souto EB (2018). Linalool bioactive properties and potential applicability in drug delivery systems. *Colloids and Surfaces B: Biointerfaces* 171:566-578. <https://doi.org/10.1016/j.colsurfb.2018.08.001>
- Politi M, Ferrante C, Menghini L, Angelini P, Flores GA, Muscatello B, Braca A, De Leo M (2022). Hydrosols from *Rosmarinus officinalis*, *Salvia officinalis*, and *Cupressus sempervirens*: Phytochemical analysis and bioactivity evaluation. *Plants* 11(3):349. <https://doi.org/10.3390/plants11030349>
- Pretsch E, Bühlmann P, Badertscher M (2009). Structure determination of organic compounds, Tables of spectral data, Springer -Verlag Berlin Heidelberg.
- Radulović N, Blagojević P, Palić R, Zlatković B (2010). Chemical composition of the essential oil hydrodistilled from Serbian *Taxus baccata* L. *Journal of Essential Oil Research* 22(5):458-461. <https://doi.org/10.1080/10412905.2010.9700371>
- Rajeswara Rao, BR (2013). Hydrosols and water-soluble essential oils: medicinal and biological properties. Recent progress in medicinal plants. *Essential oils I* 36:119-140.
- Reijnen G, Bethlehem C, Remmen JMBL, Smit HJM, Luin M, Reijnders UJL (2017). Post-mortem findings in 22 fatal *Taxus baccata* intoxications and a possible solution to its detection. *Journal of Forensic and Legal Medicine* 52:56-61. <http://dx.doi.org/10.1016/j.jflm.2017.08.016>
- Robakowski P, Pers-Kamczyc E, Ratajczak E, Thomas PA, Ye ZP, Rabska M, Iszkulo G (2018). Photochemistry and antioxidative capacity of female and male *Taxus baccata* L. acclimated to different nutritional Environments. *Frontiers in Plant Science* 9:742. <https://doi.org/10.3389/fpls.2018.00742>.
- Sadgrove NJ, Gonzalez GFP, Leuner O, Melnikovova I, Cusimamani EF (2021). Pharmacology of natural volatiles and essential oils in food, therapy, and disease prophylaxis. *Frontiers in Pharmacology* 1-16. <https://doi.org/10.3389/fphar.2021.740302>
- Sharma A, Sharma A, Thakur S, Mutreja V, Bhardwaj G (2022). A brief review on phytochemistry and pharmacology of *Taxus baccata* L. *Materials Today: Proceedings* 48:1569-1574. <https://doi.org/10.1016/j.matpr.2021.09.468>
- Shirmohammadli Y, Hosseinihashemi SK, Jalaligoldeh A, Efhamisisi D, Mousavinezhad SH, Lashgari A (2020). Chemical composition of *Taxus baccata* L. leaves and male cones water: methanol extracts. *Celal Bayar University Journal of Science* 16(3): 251-255. <https://doi.org/10.18466/cbayarjbs.689482>
- Stefanović M, Ristić M, Popović Z, Matić R, Nikolić B, Vidaković V, Petković DO, Bojović S (2016). Chemical composition and interpopulation variability of essential oils of *Taxus baccata* L. from Serbia. *Chemistry & Biodiversity* 1-36. <https://doi.org/10.1002/cbdv.201500326>
- Stevanović ZD, Neumüller JB, Lijaković IP, Raj J, Vasiljević M (2018). Essential oils as feed additives—Future Perspectives. *Molecules* 23:1717. <https://doi.org/10.3390/molecules23071717>
- Timung R, Purohit S, Barik CR, Goud V (2016). Composition and anti-bacterial activity analysis, *Industrial Crops and Products* 94:178-188. <http://dx.doi.org/10.1016/j.indcrop.2016.08.021>
- Turek C, Stintzing F (2013). Stability of essential oils: a review. *Comprehensive Reviews in Food Science and Food Safety* 12:40-53. <https://doi.org/10.1111/1541-4337.12006>
- Ulusoy S, Tinaz GB, Canbay HS (2009). Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute. *Current Microbiology* 59:554-558. <https://doi.org/10.1007/s00284-009-9475-y>
- Wang T, Lingyu L, Qin Y, Lu B, Xu D, Zhuang W, Shu X, Zhang F, Wang N, Wang Z (2023). Effects of seasonal changes on chlorophyll fluorescence and physiological characteristics in the two *Taxus* species. *Plants* 12(2636):11-17. <https://doi.org/10.3390/plants12142636>
- Wangkheirakpam SD, Laitonjam WS (2016). Studies on the uses of some plants for medicinal and dyeing properties. *International Journal of Chemistry* 5(1):93-102.
- Wei Q, Yin CW (2019). Chemical Composition of Essential Oils from the Stems of *Taxus chinensis* var. *mairei*. *Journal of Essential Oil-Bearing Plants* 22(4):1144-1149. <https://doi.org/10.1080/0972060X.2019.1668864>
- Zhang J, Yuan K, Jin Y (2012). Comparison of chemical composition and antimicrobial activities of the essential oil of *Taxus media* and *Taxus chinensis* var. *mairei* leaves, *Advanced Materials Research* 343-344:1092-1097. <https://doi.org/10.4028/www.scientific.net/AMR.343-344.1092>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.