

## First Report on the Volatile Composition of *Tricholoma anatolicum* in Comparison with *Tricholoma caligatum*

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**Abstract:** *Tricholoma anatolicum* collected from Turkey is consumed by public and exported to Japan every year. It was previously identified as *Tricholoma caligatum* until it was recognized as a new species. In the existing literature, there is no information on the aromatic composition of *T. anatolicum*. Therefore, in this study it was aimed to identify the volatile composition of *T. anatolicum* together with *T. caligatum*. Species identification was confirmed using molecular analyses based on ITS rDNA sequencing. Volatile compounds of both mushroom species were extracted using liquid-liquid extraction method and determined by Gas Chromatography-Mass Spectrometry-Flame Ionization Detector (GC-MS-FID). In the two *Tricholoma* species, 31 volatiles were obtained and grouped in seven chemical classes. The amounts of alcohols, volatile acids and esters were found to be higher in *T. anatolicum*, whereas the amounts of terpenes were detected as higher in *T. caligatum*. 1-Octen-3-ol responsible for the mushroom-like odour was only found in *T. anatolicum*.

**Keywords:** *Tricholoma anatolicum*; *Tricholoma caligatum*; Tricholomataceae; volatile; aroma composition; Turkey. © 2019 ACG Publications. All rights reserved.

### 1. Introduction

The genus *Tricholoma* encompasses large number of mycorrhizal species and some of them are prized commercially, particularly *Tricholoma matsutake* and *Tricholoma anatolicum*. The latter species is known as “Cedar Mushroom” in Turkey due to its ectomycorrhizal relationship with *Cedrus libani*. This peculiar species was discovered and described in details by Turkish researchers [1, 2]. The main differences of this Anatolian species from other members of *Tricholoma* are its host preference and its odour and aroma reminding the one of its host tree. This mushroom species is also colloquially

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known in Turkey as “Katran Mantarı” (i. e. tar mushroom) due to its odour comes from Cedar tree [2]. Most of the other *Tricholoma* species form ectomycorrhizal relationship with *Pinus* spp. and are therefore known as “Pine Mushroom”. For example, *T. matsutake*, claimed as the most valuable *Tricholoma* species, has ectomycorrhizal relationship with *Pinus densiflora*. *Cedrus libani* named as “Taurus Cedar” in Turkey has a plump trunk, thick branches, splendid structure and may reach 40 m in height. The pyramidal top in young trees spreads with ages and is shaped like an umbrella. It has used for different purposes from the ancient Egyptian, Phoenicians and Assyrians to day due to its highly valuable essential oil and its fragrant wood. Distribution of Taurus Cedar in Turkey has begins from Köyceğiz and Fethiye province of West Mediterranean and reaches in the Kahramanmaraş province of the East Mediterranean. This broad distribution area is situated between the 28°–37° east longitudes and the 36°20’–38°40’ northern latitudes. The altitude is 1000–2200 m in the Mediterranean Region, 670–920 m in Niksar location of North and 800–1250 m in Erbaa location [3–9]. In accordance with the distribution of cedar forests in Turkey, *T. anatolicum* occurs in cedar forests about 30 years old on well-drained, sandy and non-fertile soil in areas with Mediterranean climate at 1400–1700 m elevation between October and November [2,10]. The commercial importance and value of this species for the Turkish public was discussed in details in a book chapter written by Turkish scientists [11].

Mushrooms have been consumed as food or used as food-flavouring material since ancient times because of their unique flavour. The typical flavour substances of mushrooms can be classified into non-volatile components and volatile compounds. Among these compounds, the aliphatic alcohols and ketones with eight carbon atoms (1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 1-octen-3-one, 3-octanone) are in charge-for the mushroom-like flavour. The volatile composition of mushrooms can be affected by several factors, such as species, the part of mushroom used, maturity of mushroom and the substrate composition for mushroom species cultured or habitat for uncultured mushroom species [12]. Although mushrooms are consumed extensively, the studies on their aroma compositions have been limited.

*T. anatolicum* was known as *T. caligatum* in Turkey until being proposed as a new species by Turkish and Italian mycologists [1]. The differences between those two species were outlined in details in a study carried out by Turkish scientists [2]. While *T. caligatum* has strong and similar to *Inocybe corydalina* odour, sweetish-bitter to bitter taste and tends to form mycorrhiza with hardwoods or pine trees, *T. anatolicum* has fragrant and similar to *C. libani* odour, very mild, pleasant taste and is in mycorrhizal relationship with *C. libani*. Additionally, there are many morphological differences between those species concerning details as colour, pileus, stipe, spore and hyphae. Aroma composition of some *Tricholoma* species has been assessed until today by different researchers, who tackled *T. matsutake* [13, 14], *T. equestre* [15], *T. ustaloides*, *T. sulphureum* [16], and *T. terreum* [17]. The chemical composition of *T. anatolicum* was also determined by two different research groups from Turkey [18, 19]. However, no study has been conducted so far to investigate the aromatic composition of *T. anatolicum*. Therefore, the focus of this study was to determine aroma compounds of *T. anatolicum* in comparison with *T. caligatum*.

## 2. Materials and Methods

### 2.1. Fungi Materials

Fresh *Tricholoma* species were used as material for aromatic studies in this study. While *T. anatolicum* was collected from Feke-Adana (37°47’20” N, 35°44’33” E, 1397 m) province of Turkey under *C. libani*, *T. caligatum* was obtained from Pozantı-Adana (37°25’24” N, 34°52’45” E, 850 m) province of Turkey under *Pinus brutia* (Figure S1). Dried samples were used for molecular analyses including DNA isolation and sequencing. Voucher specimens have been deposited in the Herbarium of Osmaniye Korkut Ata University, Osmaniye, Turkey (voucher numbers: *T. anatolicum* - FBozok 00204, *T. caligatum* - FBozok 00205).

## 2.2 Chemicals

Dichloromethane ( $\geq 99.9\%$  purity), sodium sulphate anhydrous (99%) and internal standard (4-nonanol) were obtained from Merck (Darmstadt, Germany). A mixture of n-alkane standards ranging from C<sub>8</sub>-C<sub>40</sub> were used for the determination of retention indices and they were provided from Sigma Aldrich (St Louis, MO, USA) (E)-3-penten-ol (96% purity), limonene (97% purity), 3-octanone ( $\geq 98\%$  purity), 2-hexanol ( $\geq 98\%$  purity), 3-methyl-2-buten-1-ol ( $\geq 98\%$  purity), nonanal ( $\geq 99.5\%$  purity), 3-octanol ( $\geq 98\%$  purity), (E)-2-octenal (97% purity), (Z)-furan linalool oxide ( $\geq 97\%$  purity), (E)-limonene oxide (97% purity), 1-octen-3-ol ( $\geq 98\%$  purity), linalool ( $\geq 98.5\%$  purity),  $\gamma$ -butyrolactone ( $\geq 99\%$  purity), 2(5H) furanone ( $\geq 98\%$  purity), (Z)-pyran linalool oxide ( $\geq 97\%$  purity), 1-phenylethanol ( $\geq 99\%$  purity), hexanoic acid ( $\geq 99\%$  purity), (Z)-nerolidol ( $\geq 96\%$  purity), (E)-methyl cinnamate ( $\geq 98\%$  purity), decanoic acid ( $\geq 99\%$  purity), dodecanoic acid ( $\geq 99\%$  purity), linoleic acid ( $\geq 98.5\%$  purity), pentadecanoic acid ( $\sim 99\%$  purity), hexadecanoic acid ( $\geq 99\%$  purity) were obtained from Sigma Aldrich (St Louis, MO, USA). (Z)-2-octen-1-ol (97% purity) was obtained from Alfa Aesar (Haverhill, USA).

## 2.3. Aroma Compounds Analysis

### 2.3.1. Extraction

Fresh mushroom sample (25 g) was homogenized with a mechanic blender and then transferred into a 250 mL erlenmeyer flask. As internal standard, dichloromethane (50 mL) and 40  $\mu$ g of 4-nonanol were added into flask and the mixture was stirred at 4°C for 30 minutes under nitrogen gas [14, 20], then the mixture was centrifuged at 4°C (9000 g, 15 min). After all, the organic phase was recovered. Before gas chromatography/mass spectrometry (GC/MS) analysis, the organic extract was dried over sodium sulphate and concentrated to a volume of 0.5 mL with a Vigreux distillation column [14, 20]. Each sample was extracted in triplicate. The concentration of volatile compounds were quantified from the flame ionization detection (FID) peaks areas and the internal standard, 4-nonanol. The response factor was set to 1 for all compounds [21].

### 2.3.2. GC/MS-FID Analysis

The volatile compounds were analyzed by using GC/MS-FID. The gas chromatography (GC) system consisted an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (Wilmington, DE, USA) and an Agilent 5973-Network mass selective detector (MSD). Volatile compounds were separated on DB-Wax (30 m length  $\times$  0.25 mm i.d.  $\times$  0.5  $\mu$ m thickness; J&W Scientific Folsom, CA, USA) column and a total of 3  $\mu$ L sample of extract was injected. Injector and FID detectors were set at 250°C and flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperature of the DB-Wax column was increased from 40°C (after 3 min holding) to 90°C at a rate of 2°C/min, then at a rate of 3°C/min to 130°C, and at a rate of 4°C/min to 240°C with a final hold at 240°C for 12 min. For the mass selective detector, the same oven temperature programs were used. MS scan conditions as follows: source temperature 120°C, interface temperature 250°C, EI energy 70 eV, mass scan range 29-350 a.m.u and a scan rate 1.0 scan/s. Volatile compounds were identified by retention indices (RI), mass spectral database (Wiley 6, NIST 98). Identification of some volatile compounds was confirmed by the injection of their chemical standard into GC-MS system. Retention indices of the volatile compounds were calculated by using an n-alkane series (C<sub>8</sub>-C<sub>22</sub>) [14, 20].

## 2.3. Statistical Analysis

The independent-samples analysis of variance tests (*t-test*) were carried out to compare the significant differences of the mean values of the volatile compounds with  $p < 0.05$ . SPSS Statistics software version 20.0 (Chicago, IL, USA) was used for statistical analysis.

## 2.4. Molecular Analyses

Species names of the *Tricholoma* samples collected were confirmed by obtaining DNA sequences of ITS rDNA gene region amplified using ITS1F and ITS 4 primers [22] and then by comparing with sequences in Genbank database. Eurx genematrix universal DNA purification kit was used to isolate DNA from dried samples. PCR conditions were set up as follows: 95°C for 5 min and 30s at 9°C, 60s at 54°C, 90 s at 72°C by 30 cycles and final extension 72°C for 5 min. PCR amplifications were verified by electrophoresis on 1.5% agarose gel and then DNA sequence analyses of successful amplifications were performed by BigDye Terminator v3.1 Sequencing Kit using ITS1F-ITS4 primers. Sequencing reactions were run on ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA, US) and then Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA) was used for editing and aligning of raw sequence chromatograms. The sequences obtained from this study were deposited in GenBank with MH170219 and MH173092 accession numbers.

## 3. Results and Discussion

The volatile compound analysis of two *Tricholoma* species was carried out using GC-MS/FID and compounds identified were shown in Table 1. A total of 31 volatile compounds including 8 alcohols (1, 4, 5, 7, 11,13, 15,18), 4 aldehydes and ketones (3, 6, 8, 16), 7 terpenes (2, 9, 10, 12, 17, 20, 25), 1 ester (21), 9 volatile acids (19, 22, 23, 24, 27, 28, 29, 30, 31) and 1 lactone (14), 1 indole derivative compound (26) were identified (identification numbers correspond to those reported in Table 1 and are presented in supporting information as Figure S2 and Figure S3). Two different *Tricholoma* species showed different volatile profiles. In the *T. anatolicum* (25) was identified the higher number of volatiles than the *T. caligatum* (15). In *T. anatolicum*, 1-octen-3-ol was detected as the most abundant compound (46%), followed by methyl cinnamate (30.2%), linoleic acid (10.6%) and (E)-2-octen-1-ol (5.1%). In contrast, 2,5-dimethyl, 1-H-indole (41.5 %) known as indole derivatives compound was the most dominant component in the *T. caligatum* and it was followed by methyl cinnamate (39%).

As seen in Table 1, among alcohols, 1-octen-3-ol known as eight carbons (C8) derivate was found to be major volatile compound in *T. anatolicum*. This component was also reported as the most abundant aroma compound in other *Tricholoma* species, especially in *T. matsutake* in the previous studies [34, 35]. Aroma threshold value of 1-octen-3-ol is 200 µg/kg [36] and it is an important component due to its low perception threshold value and its specific sensory characteristic. The concentration of 1-octen-3-ol extremely exceeds its threshold value in *T. anatolicum*, whereas it could not be detected in *T. caligatum*. Same result was obtained from a study carried out by French group on *T. caligatum* [37]. In a study performed on *T. terreum* and *T. fractium*, 1-octen-3-ol compound commonly found in *Tricholoma* species could not be detected [12]. The major component 1-octen-3-ol possesses a mushroom-like aroma and is known as ‘mushroom alcohol’. It has been reported that this compound is derived from linoleic acid through oxygenation of the fatty acid and subsequent cleavage of the fatty acid hydroperoxide. However, the precise pathway to 1-octen-3-ol in mushrooms has not been fully elucidated [38, 39]. A research group [38] reported that 1-octen-3-ol formation may be limited by either competitive product inhibition [13-hydroxyperoxydes (13-HPOD)] or low substrate availability due to lipophilic interaction of the fatty acid substrate with the crude mycelium homogenate. In our study, the absence of 1-octen-3-ol in *T. caligatum* may be due to lack of linoleic acid or formation of 13-HPOD. (E)-2 Octen-1-ol was the second most abundant C8 derivatives compound and it was only found in *T. anatolicum*. The other C8 derivatives compounds; 3-octanol and 1-octanol were at lower content. It is generally accepted that C8 compounds are the major volatiles contributing to mushroom, mushroom-like/buttery, mushroom-like/chemical flavour [14, 40, 41]. The presence and concentration of these compounds depend on, genera, species, strains, maturity and geographical origin of the mushroom [34, 42]. Five other alcohols; (E) 3-penten-2-ol, 2-hexenol, 2-methyl-2-buten-1-ol, and 1-phenylethanol were also identified with lower content. In addition, 1-phenylethanol was only detected in *T. anatolicum*.

3-Octanone is one of the important C8 aliphatic compounds and it was the most abundant ketone in *T. anatolicum*, but it was not identified in *T. caligatum*. Aroma threshold value of 3-

octanone is 500 µg/kg [36] and it has mushroom-like-buttery notes [13]. However, it was found under its threshold value in *T. anatolicum*. 3-Octanone was found to be most abundant ketone in six different mushroom species in a previous study conducted by Portuguese scientists [12]. On the other hand, this compound was not detected in a study carried out by another Portuguese group which are worked with eleven different mushroom species [15]. Two aldehydes; nonenal and 2-octenal were only identified in *T. anatolicum* at very low content.

**Table 1.** Volatile composition of *T. anatolicum* and *T. caligatum* (µg/kg)

Peak no	LRI	RI	Volatile Compounds (µg/kg)	<i>T. anatolicum</i>	<i>T. caligatum</i>	Sig.	ID
1	1177 <sup>d</sup>	1179	(E)-3-Penten-2-ol	369.8±24.7	437.4±5.0	*	RI,MS,Std
2	1195 <sup>a</sup>	1190	Limonene	63.9±2.8	>LOQ	*	RI,MS,Std
3	1261 <sup>e</sup>	1253	3-Octanone	240.2±15.5	>LOQ	*	RI,MS,Std
4	1313 <sup>f</sup>	1310	2-Hexanol	92.7±4.4	123.3±2.0	*	RI,MS,Std
5	1320 <sup>g</sup>	1315	3-Methyl-2-buten-1-ol	38.3±5.4	64.2±1.9	*	RI,MS,Std
6	1390 <sup>h</sup>	1397	Nonanal	47.9±3.1	>LOQ	*	RI,MS,Std
7	1396 <sup>e</sup>	1394	3-Octanol	62.7±5.2	143.9±9.2	*	RI,MS,Std
8	1437 <sup>e</sup>	1430	(E)-2-Octenal	26.4±0.7	>LOQ	*	RI,MS,Std
9	1439 <sup>e</sup>	1439	(Z)-furan Linalool oxide	>LOQ	93.9±1.7	*	RI,MS
10	1463 <sup>i</sup>	1459	(E)-Limonene oxide	>LOQ	62.0±1.3	*	RI,MS
11	1451 <sup>b</sup>	1458	1-Octen-3-ol	44289.4±349.7	>LOQ	*	RI,MS,Std
12	1540 <sup>h</sup>	1537	Linanol	>LOQ	40.8±1.4	*	RI,MS,Std
13	1579 <sup>b</sup>	1579	1-Octanol	869.6±42.2	11.5±0.4	*	RI,MS,Std
14	1635 <sup>c</sup>	1630	γ-Butyrolactone	62.2±2.0	>LOQ	*	RI,MS,Std
15	1620 <sup>b</sup>	1620	(E)-2-Octen-1-ol	4985.7±220.1	>LOQ	*	RI,MS,Std
16	1767 <sup>h</sup>	1761	2 (5H) Furanone	51.4±3.3	>LOQ	*	RI,MS,Std
17	1770 <sup>h</sup>	1770	(Z)-pyran Linalool oxide	>LOQ	25.4±0.7	*	RI,MS
18	1788 <sup>j</sup>	1795	1-Phenylethanol	66.8±4.5	>LOQ	*	RI,MS,Std
19	1828 <sup>e</sup>	1830	Hexanoic acid	20.9±1.6	>LOQ	*	RI,MS,Std
20	2050 <sup>e</sup>	2041	(Z)-Nerolidol	>LOQ	54.0±0.8	*	RI,MS,Std
21	2051 <sup>h</sup>	2048	Methyl cinnamate	29074.1±1193.2	3495.7±18.2	*	RI,MS,Std
22	2182 <sup>k</sup>	2182	2-Octenoic acid	40.4±2.0	>LOQ	*	RI,MS
23	2268 <sup>g</sup>	2270	Decanoic acid	20.4±1.3	>LOQ	*	RI,MS,Std
24	2479 <sup>l</sup>	2474	Dodecanoic acid	119.2±1.0	>LOQ	*	RI,MS
25	2500 <sup>m</sup>	2495	Drimenol	25.3±1.2	77.2±0.8	*	RI,MS
26	-	2520	1-H-indole, 2,5-Dimethyl	>LOQ	3715.0±59.6	*	RI,MS,Std
27	-	2544	Linoleic acid	10268.3±341.2	405.8±69.3	*	RI,MS,Std
28	2729 <sup>k</sup>	2720	Pentadecanoic acid	679.8±8.9	>LOQ	*	RI,MS,Std
29	2882 <sup>m</sup>	2886	Hexadecanoic acid	2721.6±113.1	190.4±2.2	*	RI,MS,Std
30	3136 <sup>n</sup>	3130	Octadecanoic acid	303.6±3.4	>LOQ	*	RI,MS
31	3173 <sup>n</sup>	3167	Oleic acid	1573.4±19.6	>LOQ	*	RI,MS

RI: Retention indices calculated on DB-Wax capillary column; LRI: Literature retention indices on DB-WAX column reported from literature a[13], b[14], c[21], d[23], e[24], f[25], g[26], h[27], i[28], j[29], k[30], l[31], m[32], n[33]; ±Standard deviation; ID: Identification; MS: Mass spectrometry; Std: Chemical standard; LOQ: Limit of quantification; Sig.: Significance at which means differ as shown using analysis of variance \*p<0.05 level.

Terpenes are important volatile components concerning the volatile composition of mushrooms. While six terpene compounds were found in *T. caligatum*, only two components were detected in *T. anatolicum*. Limonene was the most abundant terpene compound in *T. anatolicum* (71.6%) and it was not recorded in *T. caligatum*. Different values have been reported on limonene perception threshold value in the literature, which are reported as 10 µg/L, 60 µg/L and 200 µg/L [43] and it has fresh, sweet, citrus notes [44]. (Z)-Furan linalool oxide was found to be most abundant terpene compound in *T. caligatum* and followed by drimenol and (E)-limonene oxide. Linalool is

another important terpene compound which has refreshing, floral notes and it was only detected in *T. caligatum*. Odour threshold value of linalool is 15  $\mu\text{L}$  [45], and it exceeded its threshold value. Drimenol was detected in both species; *T. anatolicum* and *T. caligatum*.

Ester group was another chemical class with one compound identified. Methyl cinnamate was determined both in *T. anatolicum* (30.2%) and in *T. caligatum* (39.1%). Methyl cinnamate is one of the major volatile compounds detected in *Tricholoma* species. This cinnamic derivative compound has fruity-balsamic notes and it is also found to be a very important flavour compound [36, 37].

Among nine volatile acids detected in *T. anatolicum*, linoleic acid was the most abundant one and followed by hexadecanoic acid and oleic acid, respectively. This is in agreement with the results reported by Riberio *et al.* [46]. The volatile fatty acids (such as linoleic acid) are important compounds in mushroom, because they are the precursor to aliphatic compounds with eight carbons such as 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 3-octanone which are the main compounds responsible for the characteristic mushroom-like odour [12, 36]. In *T. caligatum*, only linoleic acid and hexadecanoic acid were detected. Normally, linoleic acid represents 63–74% of the fatty acids in mushrooms [36].

An Indole derivative compound; 2,5-dimethyl, 1-H-indole was the major component (41.5%) found in *T. caligatum* and this compound was not detected in *T. anatolicum*. Indole derivatives of *Tricholoma* species were previously reported in a study performed by French scientists [37].

The results indicate that major volatile compositions of *T. anatolicum* and *T. caligatum* are significantly different. While 1-octen-3-ol was the most abundant volatile in *T. anatolicum*, indole derivative compound; 2,5-dimethyl, 1-H-indole was the major compound in *T. caligatum*. *Tricholoma anatolicum* appears more similar to *T. matsutake* in terms of its volatile compounds. The phylogenetic analyses have also showed that they are very close each to other in a genetic manner. In a recent phylogenetic study, *T. anatolicum* has been nested in “Calicata” clade together with *T. matsutake*, *T. magnivelare*, *T. caligatum*, *T. fulvocastaneum*, *T. ilkkæ*, *T. dulciolens*, *T. bakamatsutake* and *T. anatolicum* resolves as a sister of *T. matsutake* [47]. In fungi identification based on morphological features may sometimes be misleading due to the temporal changes in the phenotype with the effect of environmental conditions. Besides, the developmental stage of wild mushrooms is one of the important factors for morphotaxonomic identification, thus making sometimes identification of too undermature and overmature collections challenging. As to overcome misidentification issues, we used DNA sequencing technique to verify species identification, moreover when sequences of studied materials are deposited in public genetic databases, future reassessments of published chemical data, driven by taxonomic changes could be easily performed. In recent years, molecular systematic and chemical content studies have started to be evaluated together.

The characteristic aroma-active compounds of raw and cooked *T. matsutake* were investigated by aroma extract dilution analysis using gas chromatography-olfactometry [13]. 1-Octen-3-one (mushroom-like) found to be the major aroma-active compound was followed by ethyl 2-methylbutyrate (floral and sweet), linalool (citrus-like), methional (boiled potato-like), 3-octanol (mushroom-like and buttery), 1-octen-3-ol (mushroom-like), (E)-2-octen-1-ol (mushroom-like), and 3-octanone (mushroomlike and buttery) in the raw samples. Methional, 2-acetylthiazole (roasted), an unknown compound (chocolatelike), 3-hydroxy-2-butanone (buttery), phenylacetaldehyde (floral and sweet) and C8 compounds were detected as the major components in the cooked samples. Volatile profile of ectomycorrhizal mushroom species may be affected by host trees. *T. matsutake* tends to make ectomycorrhiza with *P. densiflora*. *P. densiflora* (red pine) is one of the common forest tree species in the Far East of Asia such as Korea, Japan and China [48, 49]. In a study carried out in Korea [48], volatile components of the needles of *P. densiflora* were identified by simultaneous distillation–extraction using gas chromatography–mass spectrometry. At the end of the study;  $\alpha$ -pinene,  $\beta$ -phellandrene, germacrene D,  $\beta$ -caryophyllene and myrcene were determined as major compounds. While (Z)-3-Hexenal (green/apple) and bornyl acetate (pine/herbaceous) were detected as important aroma-active compounds,  $\alpha$ -pinene (pine/fresh) was found to be the most intense aroma-active compound in the aroma extract dilution analyses. In another study performed again in Korea [49], volatile composition of needles of *P. densiflora* were determined by comparing six different extraction methods using gas chromatography-mass selective detector (GC-MSD). The researchers found 65 compounds classified into six categories: 25 hydrocarbons, 16 alcohols, 9 carbonyls, 6 esters, 7 acids and 2 ethers. The compounds having high or low boiling point showed different response in the six

methods tested. In addition to;  $\beta$ -phellandrene,  $\beta$ -myrcene,  $\alpha$ -pinene,  $\beta$ -pinene and limonene were detected as major compounds similar to the previous study [48]. *Tricholoma anatolicum* is linked to prefers *C. libani* in ectomycorrhizal relationship. Volatile composition of *C. libani* from the ancient Lebanese forests were identified and compared with the ones grown in Jerusalem using GC/MS [50]. While  $\alpha$ -pinene,  $\beta$ -pinene, myrcene and limonene were detected as major aroma compounds in the needles of *C. libani*, sesquiterpens  $\alpha$ - and  $\beta$ -himachalene were determined in the wood. Manool, atlantones,  $\beta$ -himachalene oxide and longiborneol were identified to be main oxygenated components of Lebanese Cedar wood extract. Chemical characterization of *C. libani* tar obtained by the traditional method and Jenkner Retort was carried out in roots and fallen branches using FID-GC and GC-MS [51]. The tar yield was found to be 60% and 30-40% in the Jenkner Retort and traditional method, respectively. Totally, 41 compounds were detected and  $\beta$ -himachalane,  $\alpha$ -himachalane and longifolene were assessed as the main components in the all tars. Some compounds known with antifungal and insecticidal effects such as deodarone and E-( $\alpha$ )-atlantone were just recorded in tars of roots.

Volatile studies on different *Tricholoma* species such as *T. equestre*, *T. ustaloides*, *T. sulphureum* and *T. terreum* have been carried out by different researchers. Volatile and semi volatile components of eleven wild edible mushroom species including *T. equestre* collected under *P. pinaster* were detected by using HS-SPME and GC-MS [15]. The eleven mushroom species were evaluated in three groups and *T. equestre* was nested in the second group described as “hay-herb, nutty and mushroom like” and found to be rich in 1-octen-3-ol and 3-octanol. Volatile aroma composition of *T. terreum* collected from Turkey was determined by using HS-SPME/GC-MS [17]. 1-octen-3-ol (37.08%), (E)-2-octen-1-ol (19.68%), hexanal (16.00%), 3-octanone (3.36%), acetic acid (2.63%) and (E)-2-octenal (2.27%) were found to be major aroma compounds among 17 components obtained. Volatile organic compounds (VOS) of seven wild inedible macrofungi species including *T. ustaloides* and *T. sulphureum* collected from the Mediterranean Basin were investigated by using HS-SPME and GC-MS [16]. Totally 72 different compounds including 5 esters, 17 alcohols, 14 aldehydes, 9 ketones, 18 terpenes and 9 other compounds were identified. With 28 compounds, the maximum number of VOCs was determined in *T. sulphureum*. In terms of C8 derivatives such as 3-octanol, 3-octanone, 1-octen-3-ol, trans-2-octenal and 1-octen 3-one, *T. sulphureum* was found to be rich together with *Hygrophorus cossus*, *Mycena pura* and *Clitocybe odora*. Especially, 1-octen-3-ol was recorded to have highest amount in *T. sulphureum* with 81.2%. The studies carried out in *Tricholoma* species have clearly shown that C8 components have been found most species belonging to the genus *Tricholoma* at different quantity. The number and amount of C8 derivatives were high in *T. anatolicum* as in most other species of the genus *Tricholoma*. However, most C8 compounds could not be detected in *T. caligatum*. Although this result seems surprising at first glance, similar data were obtained in the study conducted by the French group [37]. However, as it was already mentioned above, aroma composition of mushrooms is influenced by different factors such as environment, the stage of maturity and part of the mushroom fruitbodies used. Unlike mushrooms that cannot be cultured, it is easier to optimize such factors in mushroom species, obtained in culture. In mushrooms that cannot be cultured, it may be difficult to find collections in the same stage and maturity for study. For all these reasons, aromatic studies on *T. caligatum* should be continued by analyzing samples from different localities and at different stages of maturity.

#### 4. Conclusions

In mushrooms species identification by macromorphological and microscopic diagnostic methods may be sometimes challenging due to variety of intrinsic and extrinsic factors. Therefore, molecular methods, especially based on DNA sequencing, are able to aid the process significantly. Despite all these difficulties in species identification, some mushroom species can be recognized due to their typical odour, morphology and ecology. Many collectors or researchers claim that *T. anatolicum* can easily be separated from other *Tricholoma* species by its odour. The results of this research clearly show that *T. anatolicum* is rich in terms of aroma components which are important for mushroom flavor such as 1-octen-3-ol, 3-octanol, 3-octanone and 1-octen 3-one. All these important ingredients strengthen the odour of this species. Among *Tricholoma* species, the food quality of *T. matsutake* is considered high because of its intense flavor, glance, which is appreciated by consumers. For this reason, this species is sold at high prices and it is highly valued, especially on the Japanese

market. The present study revealed that *T. anaticum* shows aromatic profile similar to *T. matsutake*, supplemented by tar odour apparently derived from its mycorrhizal host tree *C. libani*.

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