

ARTICLE

CHEMICAL CHARACTERIZATION OF SOME COMMERCIALY AVAILABLE TEA TREE OILS

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**Abstract:** The essential oil obtained from *Melaleuca alternifolia* is widely used in complementary and alternative medicine due to its antioxidant, antimicrobial, and antifungal activity. The evaluation of the chemical composition of the essential oil available on the market is important especially when allergic potency is reported. The present study evaluated the chemical composition of seven tea tree essential oils by gas chromatography coupled with mass spectrometry. All the analysed samples exhibited a terpinen-4-ol chemotype.

**Keywords:** essential oils, tea tree oil, GC-MS, chemotype

## INTRODUCTION

Many natural dermato-cosmetic products and alternative medicinal products contain tea tree essential oil (TTO), which is known to have antioxidant capacity (Amarowicz and Pegg, 2019; Hartford and Zug, 2005; Yadav et al., 2017), antimicrobial (Brun et al., 2019; Cox et al., 2001; Melo et al., 2015; Nikolic et al., 2017; Pauli and Schilcher, 2016; Santos et al., 2014; Setzer et al., 2004; Sharifi-Rad et al., 2017), antifungal activity (da Silva et al., 2021; Noumi et al., 2011; Palmeira-De-Oliveira et al., 2009; Sevik et al., 2021), etc. Common examples, which are also available on the market, are: toothpaste, spray and solutions for treating calluses, acne or corns.

TTO is obtained from the leaves of tea tree (*Melaleuca alternifolia*) and is a mobile, colourless to pale yellow, clear liquid with a specific, pleasant odour due to terpenic, coniferous, camphor and mint scent. The yield of the essential oil varies and depends on parameters such as biomass (wild or cultivated shrubs, leaves only or leaves together with

terminal parts), variety, chemotype and/or method of production (commercial distillation or preparation by hydrodistillation in the laboratory using a Clevenger apparatus) (Bedini et al., 2020; Borotova et al., 2022; da Silva et al., 2021; de Figueiredo, 2006; de Groot and Schmidt, 2016; Hartford and Zug, 2005; Jammy et al., 2015; Lee et al., 2002; Noumi et al., 2011; Padalia et al., 2015; Sevik et al., 2021).

International Organization for Standardization (ISO) is the worldwide federation of national standards bodies. Certain characteristics of TTO, which belong to the chemotype terpinen-4-ol, are specified for the assessment of its quality. According to ISO, *Melaleuca* essential oil, the chemotype terpinen-4-ol, is obtained by steam distillation of the leaves and terminal parts of the plant *Melaleuca alternifolia* Cheel or *M. linariifolia* Sm (ISO 4730:2017) and should contain more than 30% terpinen-4-ol, 10-28%  $\gamma$ -terpinene, 5-13%  $\alpha$ -terpinene, less than 15% 1,8-cineole, less than 12% p-cymene, 1,5-8%  $\alpha$ -terpineol and small quantities of pinene, terpinolene, limonene, aromadendrene, as determined by gas

chromatography coupled with mass spectrometry: GC-MS (Carson et al., 2006). This chemotype for the TTO is accepted and used in commercial products. There are also other chemotypes for TTO: one terpinolene chemotype, and four 1,8-cineole chemotypes (Carson et al., 2006). The composition of TTO could change during storage, and usually the *p*-cymene levels increase and  $\alpha$ - and  $\gamma$ -terpinene levels decline in time. The stability of TTO is influenced also by light, exposure to air, heat, and moisture so that TTO should be stored in dark, dry, and cool conditions. The oxidation of TTO increased the allergenic potential (de Groot and Schmidt, 2016).

The incorporation of TTO in cream, ointment or gel for topical applications is possible due to its solubility on fats. Topical administration with products containing TTO were well described for diverse pathologies, such as for herpes labialis (Carson et al., 2001; Carson et al., 2008); to treat the ocular itching and ocular demodicosis (Gao et al., 2012); toenail infection; acne (Amer et al., 2020; Avonto et al., 2016); tinea pedis (Braun, 2007). The process of permeation and release kinetics of some TTO marker compounds (1,8-cineole, 4-terpineol and  $\alpha$ -terpineol) from diverse formulations (creams, ointments, gels) containing TTO was determined (Sgorbini et al., 2017). The results from the study suggests that gels make it possible to use less concentrated formulations with similar local/topical activity as compared with creams or ointments. The authors recommended that the TTO containing formulation as creams could be proposed for cosmetic applications, and gels could be more suitable for dermatological applications (Sgorbini et al., 2017). The inhalation and massage with TTO was reviewed in (Edris, 2007). The main component of the TTO, terpinen-4-ol, was demonstrated to be suitable as novel therapy for treating inflammatory bowel disease (IBD) (Yong et al., 2022).

The aim of our study was to determine the chemical composition of some TTO available on the market.

## MATERIALS AND METHODS

### *Essential oils*

Six samples of commercial tea tree essential oils were obtained from the European market and one sample was a gift received from Prof. Ülo Niinemets.

### *GC-MS analyses*

Chemical compounds in TTO samples were determined using a gas chromatograph (Shimadzu2010, Kyoto, Japan) coupled with a triple quadrupole mass spectrometer (TQ 8040, Shimadzu, Kyoto, Japan). The column used was an optimal IMS + WAX column (30 m x 0.25 mm i.d., 0.25  $\mu$ m film thickness, Macherey-Nagel, Duren, Germany) with helium as carrier gas and a  $\text{min}^{-1}$  flow of 1 ml. The oven temperature was initiated at 70 °C for 11 min and raised to 190 °C at a rate of 5 °C  $\text{min}^{-1}$  and then to 240 °C at a rate of 20 °C  $\text{min}^{-1}$ , where it was left for 5 min. The temperatures of the injector source and mass spectrometer sources were set at 250 °C and 200 °C, respectively. The 30% terpinen-4-ol, 10-28%  $\gamma$ -terpinene, 5-13%  $\alpha$ -terpinene, less than 15% 1,8-cineole, less than 12% *p*-cymene, 1,5-8%  $\alpha$ -terpineol and small quantities of pinene, terpinolene, limonene, aromadendrene injection volume was 1  $\mu$ l with a split ratio of 10:1. The constituent compounds of TTO were identified based on their mass spectra using mass spectra from the NIST 14 and Wiley 09 library.

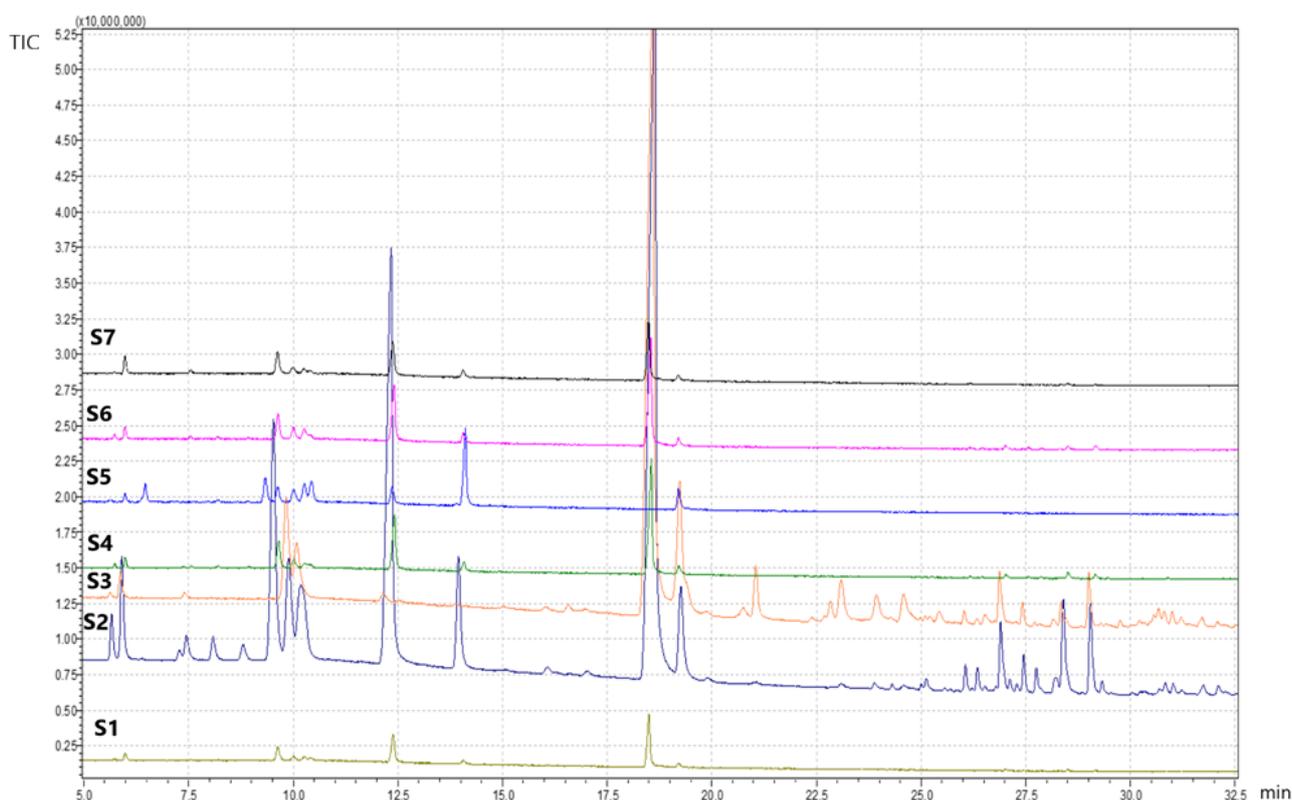
## RESULTS AND DISCUSSIONS

The recorded chromatograms for the essential oils, and the chemical composition are presented in Figure 1, and Table 1, respectively. 23 compounds were identified in the TTO, with 8 compounds being present in all the analysed samples.

The main component was terpinen-4-ol (31.40-61.09%), followed by different compounds: 4.20-23.42%  $\gamma$ -terpinene, 0.48-10.05%  $\alpha$ -terpinene, 1.67-7.96 1,8-cineole, 3.16-9.49% *p*-cymene, 2.42-8.68%  $\alpha$ -terpineol and small quantities of  $\alpha$ -pinene (0.32-8.22%),  $\alpha$ -terpinolene (0.65-4.18%), limonene (1.07-9.14%), aromadendrene (n.d.-1.69%). The values of the concentration found in the present study for the major compounds are in the domain found in other studies that determined

the chemical composition on more than ninety five TTO samples: terpinen-4-ol (6.2–44.9%), terpinolene (0.04–45.7%),  $\gamma$ -terpinene (3.1–23.0%), *p*-cymene (0.3–19.4%), *cis*-sabinene hydrate (trace–19.4%), 1,8-cineole (0.5–18.3%),  $\alpha$ -terpinene (2.3–11.7%),  $\alpha$ -pinene

(1.8–9.2%),  $\beta$ -phellandrene (trace–5.2%), and  $\alpha$ -terpineol (1.9–4.2%) (de Groot and Schmidt, 2016), except for sample S5 that revealed a 61.09% terpinen-4-ol (Table 1.)



**Figure 1.** Overlapped chromatograms of *Tea tree* essential oils recorded for the analysed samples: S1-lime green; S2-indigo; S3-orange; S4-green; S5-blue; S6-pink; S7-black

The total percentage of terpenes was found to be from 20.94% in S5 to 56.67% in S1, while the total percentage of alcohols was found to be from 42.36% in S1 to 73.37% in S5. The total percentage of sesquiterpenes was found to be from 0.00% in S3 to 9.54% in S6 (Table 1).

**Table 1.** The chemical composition determined for the *Tea tree* essential oils analysed in this study.

Compound	Chemical formula	Type of compound	RT (min)	Concentration (%)						
				S1	S2	S3	S4	S5	S6	S7
$\alpha$ -Thujene	C <sub>10</sub> H <sub>16</sub>	T	5.73	n.d.	1.05±0.06	0.55±0.15	1.16±0.21	0.15±0.01	1.07±0.01	0.65±0.18
$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>	T	5.98	8.22±0.51	2.95±0.15	2.88±0.68	3.32±0.86	0.32±0.00	2.58±0.03	4.01±0.25
Sabinene	C <sub>10</sub> H <sub>16</sub>	T	7.45	n.d.	n.d.	7.59±0.78	0.46±0.13	n.d.	0.23±0.03	n.d.
$\beta$ -Pinene	C <sub>10</sub> H <sub>16</sub>	T	7.56	1.57±0.01	0.61±0.11	0.38±0.56	0.54±0.24	n.d.	0.69±0.04	n.d.
$\beta$ -Myrcene	C <sub>10</sub> H <sub>16</sub>	T	8.19	n.d.	0.66±0.11	0.56±0.13	0.65±0.02	n.d.	n.d.	n.d.
$\alpha$ -Phellandrene	C <sub>10</sub> H <sub>16</sub>	T	8.93	n.d.	0.39±0.12	11.07±0.36	0.44±0.10	n.d.	0.72±0.01	n.d.
$\alpha$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	T	9.63	14.65±0.58	9.34±0.38	6.10±0.66	10.05±0.50	5.59±0.02	0.48±0.04	10.89±0.25
<i>p</i> -Cymene	C <sub>10</sub> H <sub>14</sub>	T	10.00	4.28±0.06	4.32±0.33	6.22±0.27	3.42±0.12	8.19±0.31	9.77±0.15	3.16±0.31
1,8-Cineol	C <sub>10</sub> H <sub>18</sub> O	A	10.27	2.68±0.16	4.10±0.37	7.96±0.81	1.67±0.03	4.36±0.01	3.80±0.05	2.45±0.30
(+)-Limonene	C <sub>10</sub> H <sub>16</sub>	T	10.38	1.86±0.51	1.07±0.29	9.14±0.25	1.63±0.60	1.67±0.00	4.79±0.13	2.61±0.69
$\gamma$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	T	12.38	21.92±0.69	21.88±0.39	6.85±0.35	21.56±0.60	4.20±0.02	18.89±0.04	23.42±1.45
$\alpha$ -Terpinolene	C <sub>10</sub> H <sub>16</sub>	T	14.06	4.18±0.21	3.03±0.23	0.65±0.32	3.07±0.24	0.82±0.01	3.72±0.13	2.70±0.47
<b>Terpinen-4-ol</b>	C <sub>10</sub> H <sub>18</sub> O	A	18.49	<b>36.65±0.27</b>	<b>43.27±1.83</b>	<b>31.40±0.08</b>	<b>44.34±1.85</b>	<b>61.09±0.12</b>	<b>39.80±0.02</b>	<b>43.70±2.64</b>
$\alpha$ -Terpineol	C <sub>10</sub> H <sub>18</sub> O	A	19.20	3.03±0.14	2.42±0.23	8.68±0.30	2.78±0.09	7.92±0.01	3.75±0.27	3.18±0.15
$\alpha$ -Gurjunene	C <sub>15</sub> H <sub>24</sub>	ST	26.17	n.d.	0.29±0.06	n.d.	0.20±0.05	0.45±0.01	0.54±0.01	n.d.
Caryophyllene	C <sub>15</sub> H <sub>24</sub>	ST	26.46	n.d.	0.26±0.01	n.d.	0.19±0.13	0.20±0.02	0.46±0.10	n.d.
Aromadendrene	C <sub>15</sub> H <sub>24</sub>	ST	27.01	n.d.	1.16±0.18	n.d.	0.90±0.20	2.11±0.01	1.68±0.12	1.69±0.08
Longipinene	C <sub>15</sub> H <sub>24</sub>	ST	27.56	n.d.	0.41±0.01	n.d.	0.36±0.12	n.d.	0.78±0.12	n.d.
Isodene	C <sub>15</sub> H <sub>24</sub>	ST	27.86	n.d.	0.34±0.12	n.d.	0.29±0.21	0.80±0.00	0.57±0.04	n.d.
Ledene	C <sub>15</sub> H <sub>24</sub>	ST	28.47	0.97±0.16	1.08±0.13	n.d.	1.88±0.26	2.13±0.01	2.95±0.29	0.93±0.43
$\delta$ -Cadinene	C <sub>15</sub> H <sub>24</sub>	ST	29.16	n.d.	1.44±0.01	n.d.	1.15±0.00	5.59±0.01	2.33±0.01	0.64±0.40
Cubenene	C <sub>15</sub> H <sub>24</sub>	ST	29.32	n.d.	n.d.	n.d.	n.d.	n.d.	0.25±0.01	n.d.
Globulol	C <sub>15</sub> H <sub>26</sub> O	A	30.84	n.d.	n.d.	n.d.	n.d.	n.d.	0.20±0.01	n.d.
	Total	T		56.67	45.27	51.97	46.27	20.94	42.92	47.43
		A		42.36	49.78	48.03	48.79	73.37	47.54	49.32
		ST		0.97	4.96	0.00	4.95	5.69	9.54	3.26

n. d. : not determined; T: terpene; ST: sesquiterpene; A: alcohol. The values are expressed in mean ±standard deviation.

## CONCLUSIONS

The determination of the chemical composition of tea tree essential oil (TTO) available on the market was carried out using a gas chromatograph apparatus equipped with a mass spectrometer as a detector. We carried out this study as our initial hypothesis was that the identification of the TTO chemotype and the minor compounds found in different products from the market is important and probably could be displayed on the product label more widely, to help the consumer in choosing the most suitable product for personal use, to minimize the occurrence of allergic reactions as side effects. It was shown that all the TTO samples investigated showed a terpinen-4-ol chemotype, the compound found in the highest concentration.

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