FIMEK

Journal of Agronomy, Technology and Engineering Management

Article

GC-MSD Analysis of the *Melaleuca Alternofolia* Chemical Composition

Milena Đorđević[1], Tijana Stojanović[1], Vojislava Bursić[1], Gorica Vuković[2,*], Bojana Špirović Trifunović[2], Aleksandra Petrović[1], Dušan Marinković[1] and Snežana Tanasković[3]

[1] University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

[2] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

[3] University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32102 Čačak, Serbia

* Correspondence: goricavukovic@yahoo.com

Received: 18 April 2021; Accepted: 03 June 2021

Abstract: In recent decades *Melaleuca alternifolia* has gained increasing attention in scientific circles due to its broad-spectrum germicidal and antimicrobial effects. The aim of this study was to analyse its essential oil by the GC-MSD. The main constituent was terpinen-4-ol with 38.5% in content, followed by α-pinene (18.4% in content) and γ-terpinene (14.0% in content). Beside the mentioned components 24 other constituents were detected which in total make less than 30.00% of the studied essential oil.

Keywords: Tea tree; GC-MSD; terpinen-4-ol; α-pinene; γ-terpinene.

1. Introduction

Essential oils are secondary metabolites which consist of volatile and semi-volatile compounds. These compounds are extracted by distillation from different parts of the plant (flowers, fruits, buds, seeds, leaves, twigs, roots, etc.) [1]. These oils are mixtures of natural components with different concentrations, with major components being responsible for the biological activity of the extracted essential oil [2]. The extraction of essential oils and their biological activity depend on the phenological state of the plant, environmental conditions and the extraction method [3]. Genus *Melaleuca* belongs to the *Myrtaceae* family and comprises more than 230 species. *Melaleuca alternifolia* (Maiden&Betche) commonly referred to as the „tea tree" is woody plant which naturally occurs in Australia [4]. Bundjabung Aborigines have been using this plant for several millennia as medicine for bruises, insect bites or skin infections. When European colonists recognized the therapeutic properties, they began to distil oil from its leaves. In 1920s it became a topical antiseptic and characterized like essential oil with more effective activity than phenol. From that time, tea tree oil (TTO) became a standard antiseptic for dental surgery. Later, the British Pharmaceutical Codex reported about germicidal properties of the TTO. In the view of the above, a long-standing use of this oil has been recognized since 1930 internationally and since 1933 in the European Community. In addition to the traditional use of TTO, there are pharmacological or clinical data which confirm the antibacterial, antifungal and antiviral activity under the controlled conditions. Little evidence about the developing of the induced tolerance and resistance indicate the even more significant efficiency of TTO. In adition to the clinical data, TTO is useful in skin hygiene and protection. If the TTO is not used in the right dosage it can lead to the toxicity. The use of relatively high dosages of TTO (10-25 mL) results in symptoms like depression of Central Nervous System and muscle weakness. When we talk about the derma iritation, these adverse events have mostly local characters. The allergic reactions are reported in the literature ranges between 0.6% and 2.4% (mean 1.6%). Considering the toxicity of TTO reported in the literature no observed adverse effect level (NOAEL) was estimated for the whole TTO, which amounts to 330 mg/kg b.w. [5]. To a great extent *M. alternifolia* owes medicinal value to its main constituent terpinen-4-ol, by virtue of the activity of this monoterpene which inhibits bacterial respiration. *Melaleuca alternifolia* is a woody plant up to 7 m high which is dominant in streams and the swampy areas. The leaves have a linear shape and they are rich in bioactive components [6].

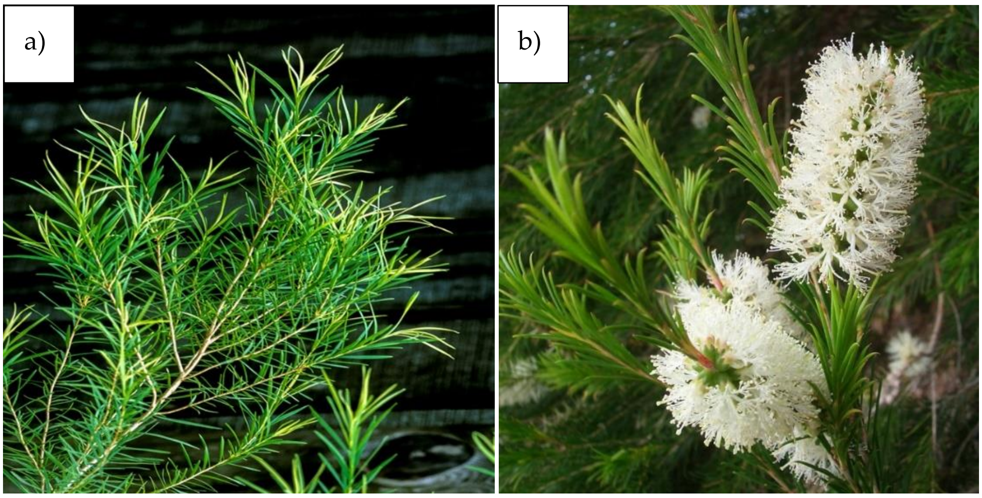

Figure 1. The plant (a) and the flower (b) of tea tree ((a) https://abc.herbalgram.org (b) https://herbaethylacini.com).

2. Materials and Methods

The TTO has been extracted from the commercially available dried plant material by the supercritical fluid extraction (SFE).

Conventional semi-continuous method was applied, using supercritical CO_2. The CO_2 with increased purity was heated through preheating coil. Forty grams of sample together with glass beads were put into the extraction cell with volume of 100 mL. As to prevent solid samples to enter the system, cotton wool was placed at the end of the cell. Heated CO2 was injected into the extraction cell, after which it was allowed to dissolve the essential oil by constant static extraction time. The essential oil left the cell by the constant flow rate of the CO_2, while being captured and harvested with 10 mL of ethanol solvent. Essential oil was kept in the refrigerator until the GC and GC-MSD analysis.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MSD) analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass spectrometer detector (MSD) and flame ionisation detector (FID) connected by capillary flow technology 2-way splitter with make-up. The HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60 to 300 °C at a rate of 3 °C min−1 and held for 15 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). An auto-injection system (Agilent 7683B Series Injector) was employed to inject 1 μL of sample. The sample was analysed in the splitless mode. The injector temperature was 300 °C, while the detector temperature was 300 °C. MS data were acquired in the EI mode with scan range of 30–550 m/z, source temperature of 230 °C and quadruple temperature of 150 °C; the solvent delay was 3 min. Identification of all compounds in the analyses was matched by comparison of their linear retention indices (relative to C8-C36 *n*-alkanes on the HP-5MSI column) and MS spectra with those of authentic standards from NIST (2011) and homemade MS library databases.

3. Results and Discussion

The chromatogram obtained by the GC-MSD analysis of the TTO is showed in Figure 2, while the detected components are presented in Table 1.

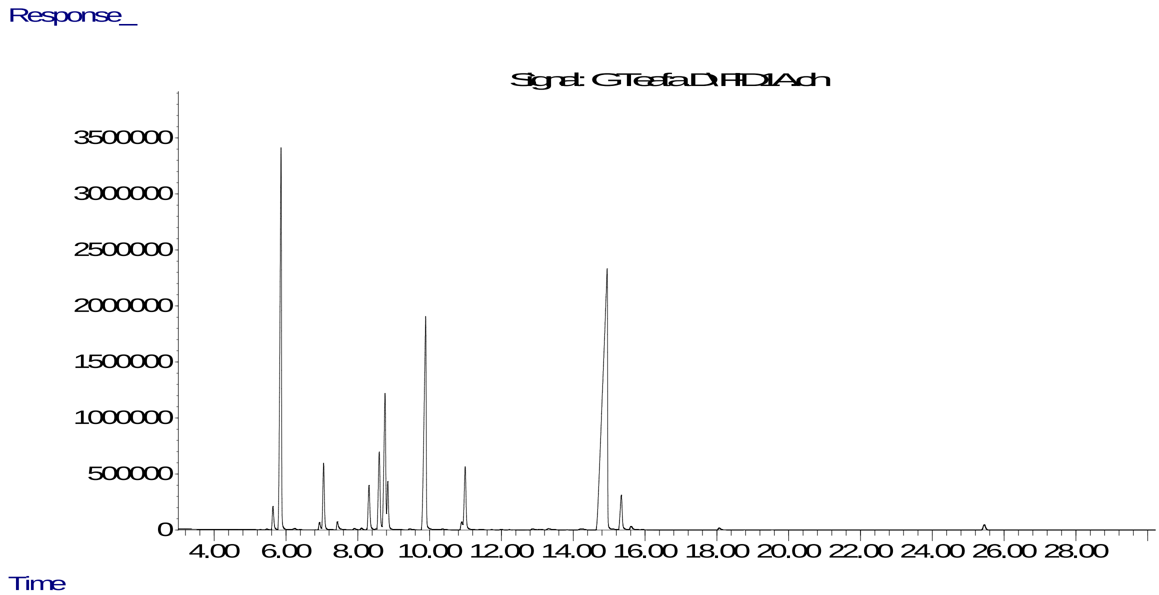

Figure 2. Chromatogram of the *Melaleuca alternifolia* essential oil.

Due to the biological effect of essential oils, they are very interesting as potential agents in pest control. They are considered to be the by-products of plant metabolism and regarded to as evaporable secondary metabolites of plants which are the mixture of mono and sesquiterpenes [7].

Table 1. Constituents of the *E. obliqua* essential oil.

Peak	RI	RI NIST	R.T. min	peak area	% max.
Thujene<alpha->	922	924	5.636	6188846	1.1%
Pinene<alpha->	930	932	5.862	10378780	**18.4%**
Camphene	945	946	6.241	426350	0.1%
Sabinene	970	969	6.932	1975732	0.3%
Pinene<beta->	974	974	7.047	17997415	3.2%
Myrcene	988	988	7.428	2559567	0.5%
Phellandrene<alpha->	1004	1002	7.9	512471	0.1%
Carene<delta-3->	1009	1008	8.098	496416	0.1%
Terpinene<alpha->	1015	1014	8.311	13269800	2.3%
Cymene<para->	1023	1020	8.598	24281043	4.3%
Limonene	1027	1024	8.758	42642222	7.6%
Cineole<1,8->	1029	1026	8.835	12167349	2.2%
Ocimene<(E)-beta->	1046	1044	9.439	439293	0.1%
Terpinene<gamma->	1058	1054	9.89	79094378	**14.0%**
NI	1070	0	10.352	263406	trace
Mentha-2,4(8)-diene<para->	1085	0	10.891	2151577	0.4%
Terpinolene	1088	1086	10.991	20119762	3.6%
NI	1113	0	11.988	158926	trace
NI	1133	0	12.861	463767	0.1%
NI	1138	0	13.063	322634	0.1%
NI	1143	0	13.303	673893	0.1%

Borneol	1164	1165	14.24	791807	0.1%
Terpinen-4-ol	1180	1174	14.944	217593646	**38.5%**
Terpineol<alpha->	1190	1186	15.34	12173870	2.2%
Terpineol<gamma->	1196	1199	15.606	1180981	0.2%
NI	1251	0	18.06	796333	0.1%
Caryophyllene(E-)	1419	1417	25.448	2155826	0.4%

*NI – not identified

Based on the obtained results the conclusion is that terpinen-4-ol is the main constituent of the *Melaleuca alternifolia* essential oil with 38.5% in content. It chemical structure is showed in Figure 3.

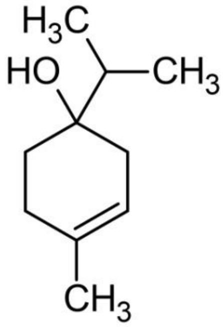

Figure 3. Chemical structure of terpinen-4-ol.

Other major components were defined as the α-pinene (18.4% in content) and γ-terpinene (14.0% in content). Beside the mentioned components 24 other constituents were detected which in total make less than 30.00% of the studied essential oil. The chemical structures of α-pinene and γ-terpinene are showed in Figure 4.

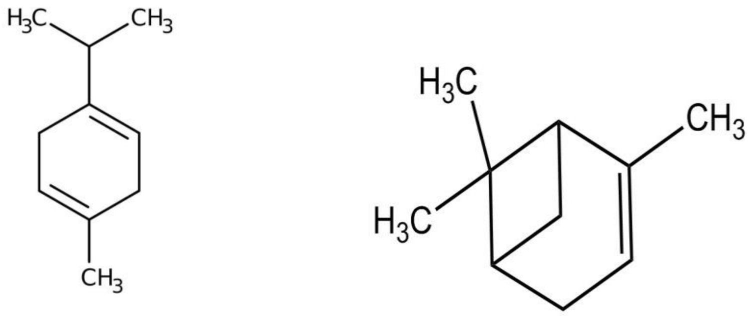

Figure 4. Chemical structure of α-pinene and γ-terpinene.

Similar results were obtained by Verghese et al. [8] who identifed the constituents of the tea tree of the Indian and Australian origin. Namely, the detected major compounds in the studied samples of tea tree were present as follows: terpinen-4-ol ranged between 36.4 and 45.4%, γ-terpinene ranged between 15.7 and 23.2% and α-pinene ranged between 2.1 and 3.4%.

4. Conclusions

The main constituent of the tea tree essential oil determined by the GC-MSD analysis in this paper was terpinen-4-ol with 38.5%, followed by α-pinene with 18.4% and γ-terpinene with 14.0% in content.

Beside the mentioned components 24 other constituents were detected which in total make less than 30.00% of the studied essential oil.

Since the main components are considered responsible for the biological activity of essential oils the conclusion is that the biological effect of *Melaleuca alternifolia* essential oil is affected by monocyclic and bicyclic monoterpenes (terpinen-4-ol, α-pinene and γ-terpinene).

Acknowledgments: The authors acknowledge the financial support of the Ministry of Science and Technological Development, Republic of Serbia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sankarikutty B.; Narayanan C.S. Essential oils/Isolation and Production. *Encyclopedia of Food Sciences and Nutrition* (Second Edition). *Academic Press* **2003**, 2185.
2. Bakkali S.; Averbeck S.; Averbeck D.; Idaomar M. Biological Activities of Essential Oils – A review. *Food and Chemical Toxicology* **2008**, 46, 446-475.
3. Stojanović T.; Tešević V.; Bursić V.; Vuković G.; Šućur J.; Popović A.; Petrović M. The chromatographic analysis of caraway essential oil as the potential biopesticide, Proceedings of the 23rd International Symposium on Analytical and Environmental Problems October 9-10, **2017**. Szeged, Hungary, Publisher: University of Szeged, Department of Inorganic and Analytical Chemistry. 15.
4. Baskorowati L.; Moncur M.W.; Cunningham S.A.; Doran J.C.; Kanowski P.J. Reproductive biology of Melaleuca alternifolia (Myrtaceae). *Australian Journal of Botany* **2010**. 58, 384-391.
5. EMA. Assessment report on *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*, aetheroleum **2013,** 8, 9, 72, 73.
6. Puvač, N.; Čabarkapa I.; Petrović A.; Bursić V.; Prodanović R.; Soleša D.; Lević J. Tea tree (*Melaleuca alternifolia*) and its essential oil: antimicrobial, antioxidant and acaricidal effects in poultry production. *World's Poultry Science Journal* **2019**. 75(2), 235-246.
7. Stojanović T.; Bursić V.; Vuković G.; Šuću, J.; Popović A.; Zmijanac M.; Kuzmanović B.; Petrović A. The chromatographic analysis of the star anise essential oil as the potential biopesticide. *Journal of Agronomy, Technology and Engineering Management* **2018**, 1, 65-70.
8. Verghese J.; Jacob C.V.; Kunjunni Kartha C.V. Indian Tea Tree (*Melaleuca alternifolia* Cheel) Essential Oil. *Flavour And Fragrance Journal* **1996**, 11: 219-221.
9. https://abc.herbalgram.org
10. https://herbaethylacini.com