World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 2
Page: 257-266

DETERMINATION OF CHEMICAL COMPOSITION AND CYTOTOXIC ACTIVITY OF SELECTED ESSENTIAL OILS FROM MACEDONIAN ORIGIN

Iskra Davkova[1]*, Zoran Zhivikj[2], Ivana Cvetkovikj Karanfilova[1], Ana Trajkovska[1], Veronika Stoilkovska Gjorgievska[1], Gjose Stefkov[1], Tatjana Kadifkova Panovsk[1] and Marija Karapandzova[1]

[1]Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia.

[2]Institute of Applied Biochemistry, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia.

Article Received: 09 March 2024 || Article Revised: 01 April 2024 || Article Accepted: 22 April 2024

Corresponding Author: Iskra Davkova
Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia. **DOI:** 10.5281/zenodo.11141323

ABSTRACT

Determination of the chemical profile as well as cytotoxicity of the essential oils can be a very useful method for detecting substances that may provide particular health dangers to humans. GC/MS method was used for determination of the chemical composition of six essential oils. The most dominant compounds were linalool (33.73%) and linalool acetate (18.89%) in *Lavandulae*, *o*-cymene (38.60%) and thymol (35.06%) in *Thymi,* 1,8-cineol (27.42%) in *Rosmarini,* terpinen-4-ol (39.06%) and *γ*-terpinene (21.03%) in *Melaleucae*, *α*-pinene (26.49%) in *Helichrysi* and zonarene (13.28%), *α*-pinene (13.24%), viridiflorene (9.02%) and *γ*-cadinene (8.19%) in *Calendulae essential oil*. The cytotoxic potential was evaluated by Brine Shrimp Lethality Assay. After 24 hours, LC_{50} values had decreasing rate in the following order: 58.09 µg/mL, 57.95 µg/mL, 43.02 µg/mL, 19.38 µg/mL and 13.85 µg/mL, for *Lavandulae, Helichrysi, Melaleucae, Calendulae* and *Rosmarini essential oil*, respectively. There are no living shrimps in the 4th hour after application of *Thymi essential oil*, as the LC_{50} value (0.75 µg/mL) was exceedingly low in the first hour.

KEYWORDS: Essential oils, GC/MS, cytotoxicity, BSLA.

INTRODUCTION

Essential oils are natural, liposoluble and volatile compounds, composed of lipophilic and highly volatile secondary plant metabolites. The composition of essential oils is diverse, consisting of a wide range of chemical compounds. Some essential oils may contain up to 400 different components including various classes of compounds such as hydrocarbons (monoterpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers).[1,2] This diverse array of chemical components contributes to multifaceted biological activity of essential oils which led to their growing usage in recent years.[3] There are many

reports on the biological activity of the essential oils, commonly referred to synergism, antagonism, and additivity, as they represent complex mixtures of different constituents. The global focus on the use of natural products has pushed essential oils to become preferred antimicrobial and antioxidative agents in the place of the synthetic ones. The consumption of essential oils is increasing more and more in everyday life due to the fact that they are used as food additives and preservatives, as well as a part of the pharmaceutical industry, cosmetic and cleaning products, dentistry and aromatherapy and even for serious health issues, including chronic anxiety, pain and stress relief and skin disorders. Liposoluble substances like terpenes, which have demonstrated antibacterial and antioxidant properties, possess the ability to penetrate through the skin, more readily, due to its lipid composition. This facilitates enhanced bioavailability of the components, thereby improving absorption. Regarding this, it is necessary to revaluate carefully the toxicity of these compounds in order to detect substances that may provide particular health dangers to humans. When assessing the possible toxicity of a test sample, such as plant extracts or physiologically active chemicals that have been extracted from plants, cytotoxicity studies are helpful in first step, as they contribute to the timely identification of hazardous effects on living cells.[4] The brine shrimp lethality bioassay is an efficient, rapid and inexpensive test with small amount of test material being utilized and has proven to be an excellent choice for elementary toxicity investigations.[5]

Numerous studies have converged on the consensus that the cytotoxicity observed in certain essentials is predominantly linked to the presence and the activity of their main components. According to this, the aim of this study was to examine qualitative and quantitative chemical composition as well as cytotoxicity of six different essential oils (*Lavandulae, Thymi, Rosmarini, Melaleucae, Helichrysi and Calendulae essential oil)*, as there is no clear evidence for the cytotoxicity of these widely used essential oils.

MATERIALS AND METHODS

Plant materials: *Lavandulae*, *Rosmarini* and *Calendulae* essential oils were obtained by steam distillation from dried *Lavadulae flos*, *Calendulae flos* and *Rosmarini folium* supplied by Alkaloid AD Skopje.

Essential oil: *Thymi*, *Melaleucae* and *Helichrysi essential oil* were purchased in the markets in North Macedonia.

Chemicals: Dimethylsulfoxide was purchased from Sigma-Aldrich (Steinheim, Germany), sodium chloride from Merck (Darmstadt, Germany), anhydrous sodium sulfate from Kemica (Zagreb, Croatia), xylene from Alkaloid (Skopje, R. Macedonia) and potassium dichromate from Sigma-Aldrich (Steinheim, Germany).

Essential oil isolation: Essential oil isolation was made by steam distillation in a special all-glass Clevenger type apparatus, according to the European Pharmacopeia 11.0 (2.2.13).

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS): Essential oil samples were analyzed on an Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C Mass Quadrupole detector, as well as capillary flow technology, which enables simultaneous analysis of the samples on both detectors. For that purpose, a HP-5ms capillary column (30 m x 0.25 mm, film thickness 0.25 μm) was used. Operating conditions were as follows: oven temperature at 60°C (0 min), 3°C/min to 240°C (1 min) and 10°C /min to 280°C (1 min); helium as carrier gas at a flow rate of 1mL/min; injector temperature 220°C and FID 270°C. 1μL of each essential oil sample dissolved in hexane to obtain a 1 μL/mL oil solution was injected at a split ratio 1:1. The mass

spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230°C, transfer line temperature 280°C and mass range from 50-500 Da. The MS was operated in scan mode.

Identification of the components present in the essential oils was made by comparing mass spectra of components with those from NIST, Wiley and Adams mass spectra libraries and by literature Kovat′s (retention) indices.[6] The percentage ratio of essential oils components was computed by the normalization method of the GC/FID peak areas without any correction factors. Average values were taken into further consideration (n=3).

Brine shrimp lethality assay (BLSA)

The cytotoxic potential of the essential oils was evaluated by Brine Shrimp Lethality Assay *in vivo*[7] based on the number of dead *Artemia salina nauplii* after 1, 2, 4, 6 and 24 hours of exposure to the essential oils. Each analysis contains 5ml of solution with the appropriate concentration of the essential oil and 10 shrimps. From the basic (stock) essential oil solution prepared sodium chloride as a solvent (initial concentration of 10 mg/mL), the rest of the tested essential oil samples were prepared in the following concentrations: 5, 3, 1, 0.5, 0.1 and 0.01 mg/mL. Sodium chloride was used as solvent during the preparation procedure. A positive control containing only $K_2Cr_2O_7$ and negative control containing only sodium chloride in the same concentration range were also prepared in parallel. The percentage of mortality for each essential oil concentration was calculated based on the number of the dead shrimps.

Final results were expressed as LC_{50} values using probit regression analysis[8], which represent the concentration of each essential oil needed to elicit mortality in 50% of the tested population of brine shrimps.

Based on the obtained LC_{50} values, essential oils were classified according to two scales of toxicity: Meyer's scale and Clarkson's scale.[7,9] Both scales classify essential oils as toxic if their LC_{50} values were below 1000 μg/mL, while Clarkson's scale additionally categorizes them as oils with high (0-100 μg/mL), moderate (100-500 μg/mL) and low (500-1000 μg/mL) toxicity.

Brine shrimp larvae were successfully hatched in artificial seawater prepared with a sodium chloride (NaCl) solution at a concentration of 38 mg/mL and a pH level of 9. The controlled environment included constant aeration, and temperature regulation maintained within the range of 25°C to 28°C. After a continuous 48-hour exposure to light, the *Artemia* larvae underwent successful hatching, developing into instar stage 3. This specific stage is considered optimal for conducting the Brine Shrimp Lethality Assay (BSLA), making the larvae suitable for further experimental investigations.[10,11]

RESULTS

Using GC/MS analysis, total of eighty-nine components were identified. Chemical composition of each essential oil is given in Table 1. The essential oil with the smallest spectrum of distinct components was *Thymi essential oil* (fifteen components), while the essential oil with the largest spectrum of different identified compounds was *Calendulae essential oil* (forty-five components). The total of eighteen, twenty, twenty-five and twenty-eight chemical compounds were identified in *Melaleucae, Rosmarini*, *Lavandulae* and *Helichrysi* essential oils, respectively, which correspond to the 97.35%, 93.65%, 92.99%, 90.35%, 90.33%, and 90.15% of the identified *Lavadulae, Melaleucae, Rosmarini, Calendulae, Helichrysi* and *Thymi* essential oil constituents, respectively. The most dominant components in *Lavandulae essential oil* were linalool (33.73%), linalool acetate (18.89%), bornyl acetate (7.28%), *β*-E-ocymene

(7.07%), terpinen-4-ol (6.00%), borneol (5.20%) and caryophyllene E (4.88%). Predominant components in *Thymi essential oil* were: *o*-cymene (38.60%), thymol (35.06%), while less dominant was *α*-pinene (6.03%). 1,8-cineol (27.42%) was the most abundant component in *Rosmarini essential oil* followed by camphor (15.35%) and *α*-pinene (14.49%). On the other hand, less dominant components in the same essential oil were: myrcene (5.49%) and camphene (4.99%). *Melaleucae essential oil* had the highest percentage of terpinen-4-ol (39.06%) and *γ*-terpinene (21.03%), while *Δ*-3-carene (9.89%) and *o*-cymene (4.76%) were the less dominant components. *α*-Pinene was the most dominant component (26.49%) in *Helichrysi essential oil* followed by neryl acetate (9.19%), *ar*-curcumene (7.99%), *β*-selinene (7.94%), *γ*-curcumene (7.22%) and *α*-selinene (4.95%). Although *Calendulae essential oil* had large spectra of different compounds, the most dominant compounds were zonarene (13.28%), *α*-pinene (13.24%), viridiflorene (9.02%) and *γ*-cadinene (8.19%). Compound with lower percentage in this oil was cubeban-11-ol (5.04%). The most common identified component in all tested essential oils were *α*- and *β*-pinene, with value range from 0.24 to 26.49% and from 0.18 to 3.46%, respectively. These two monoterpenes were followed by *γ*-terpinene (0.17-21.03%) in all essential oils except in *Lavandulae essential oil*. *o*-Cymene (0.21-38.60%) and *trans*-E-Caryophyllene (0.10-4.88%) were identified in four of the tested essential oils, but not in *Rosmarini* and *Calendulae essential oil*, respectively.

According to the BSLA, the mortality of *Artemia* larvae was occured after 6 hours of applying *Helichrysi* and *Calendulae essential oil*, after 2 hours of applying *Lavandulae* and *Rosmarini essential oil*, and even 1 hour of applying *Thymi* and *Malelaucae essential oil*. The trend of mortality rate for the *Artemia nauplii* for each hour is demonstrated on Figure 1. LC_{50} values for the examined oils, after 24 hours, had decreasing rate in the following order: 58.09 µg/mL, 57.95 µg/mL, 43.02 µg/mL, 19.38 µg/mL and 13.85 µg/mL, for *Lavandulae*, *Helichrysi*, *Melaleucae*, *Calendulae* and *Rosmarini essential oil*, respectively. As shown, *Thymi* essential oil showed highest mortality (100%) in each hour at its lowest concentration (0.01 mg/mL). Moreover, there are no living shrimps in the 4th hour after application of *Thymi essential oil*, as the LC_{50} value (0.75 µg/mL) is exceedingly low in the first hour, thus indicating that this oil is extremely toxic. Based on their estimated LC_{50} values, essential oils were categorized using both Meyer's and Clarkson's toxicity scales. Therefore, after 24 hours of application, all analyzed essential oils were determined as cytotoxic (values below 1000 µg/mL) according to the Meyer’s scale or extremely cytotoxic (values form 0-100 µg/mL), according to Clarkson's toxicity scales.

Table 1: GC/MS profile of examined essential oil.

No.	KIL	Component	Lavandulae essential oil (%)	Thymi essential oil (%)	Rosmarini essential oil (%)	Melaleucae essential oil (%)	Helichrysi essential oil (%)	Calendulae essential oil (%)
1	921	Tricyclene	-	0.39	-	-	-	-
2	924	*α*-Thujene	0.11	-	-	0.22	-	-
3	932	*α*-Pinene	0.24	6.03	14.49	3.98	26.49	13.24
4	945	*α*-Fenchene	-	-	-	-	0.39	-
5	946	Camphene	0.26	2.24	4.99	-	-	-
6	961	Verbenene	-	-	-	-	-	0.58
7	969	Sabinene	-	-	-	0.17	-	0.35
8	974	*β*-Pinene	0.18	0.26	3.46	1.27	0.57	0.18
9	988	Myrcene	3.16	-	5.49	-	-	-
10	1008	Δ^3-Carene	0.51	-	1.75	9.89	-	-
11	1014	*α*-Terpinene	-	-	0.62	-	-	-
12	1022	*o*-Cymene	0.24	38.60	-	4.76	0.50	0.21
13	1024	Limonene	0.63	-	2.68	2.43	3.77	-
14	1026	1,8-Cineole	-	1.40	27.42	-	0.87	-

15	1044	*β*-E-Ocymene	7.07	-	-	-	-	-
16	1054	*γ*-Terpinene	-	1.13	1.15	21.03	0.17	0.42
17	1086	*α*-Terpinolene	0.20	1.33	1.33	4.07	-	-
18	1095	Linalool	33.73	1.07	3.05	-	1.09	-
19	1124	Chrysanthenone	-	-	0.11	-	-	-
20	1128	*allo*-Ocymene	0.66	-	-	-	-	-
21	1135	*trans*-Pinocarveol	-	-	-	-	-	-
22	1141	Camphor	0.76	0.33	15.35	-	-	-
23	1158	*iso*-Menthone	-	-	-	-	-	1.03
24	1160	Pinocarvone	-	-	-	-	-	0.35
25	1165	Borneol	5.20	0.13	3.43	-	-	-
26	1167	Menthol	-	-	-	-	-	2.72
27	1174	Terpinene-4-ol	6.00	-	-	39.06	-	1.47
28	1183	Cryptone	0.44	-	-	-	-	-
29	1186	*α*-Terpineol	2.27	1.45	-	4.31	-	-
30	1199	*γ*-Terpineol	-	0.43	-	-	-	-
31	1204	Verbenone	-	-	3.24	-	-	0.45
32	1227	Nerol	-	-	-	-	0.64	-
33	1233	Pulegone	-	-	-	-	-	0.28
34	1239	Carvone						0.23
35	1254	Linalool acetate	18.89	-	-	-	-	-
36	1282	E-Anethole	-	-	-	-	-	0.54
37	1284	Bornyl acetate	7.28	-	1.66	-	-	0.15
38	1288	Lavandulyl acetate	1.08	-	-	-	-	-
39	1289	Thymol	-	35.06	-	-	-	-
40	1294	Methyl acetate	-	-	-	-	-	0.67
41	1298	Carvacrol	-	-	-	-	-	0.38
42	1345	*α*-Cubebene	-	-	-	-	-	0.34
43	1359	Neryl acetate	-	-	-	-	9.19	-
44	1374	*α*-Copaene	-	-	-	-	2.22	1.22
45	1379	Geranyl acetate	2.14	-	-	-	-	-
46	1387	*β*-Cubebene	-	-	-	-	-	0.59
47	1405	Italicene	-	-	-	-	3.13	-
48	1409	*α* -Gurjunene	-	-	-	0.22	-	-
49	1411	*cis*-*α*- Bergamotene	-	-	-	-	1.19	-
50	1417	*trans*-E-Caryophyllene	4.88	0.32	2.14	0.10	3.57	-
51	1431	*β*-Gurjunene	-	-	-	-	-	0.38
52	1432	*trans*-*α*-Bergamotene	-	-	-	-	4.02	-
53	1437	*α*-Guaiene						0.34
54	1439	Aromadendrene	-	-	-	0.31	-	-
55	1440	Z-*β*-Farnesene	-	-	0.08	-	-	-
56	1451	Muurola-3,5,-diene	-	-	-	-	-	0.44
57	1452	*α*-Humulene	-	-	0.34	-	1.62	-
58	1454	E-*β*-Farnesene	1.10	-	-	-	0.78	-
59	1458	allo-Aromadendrene	-	-	-	0.12	-	0.22
60	1469	*β*-Acoradiene	-	-	-	-	0.28	-
61	1478	*γ*-Muurolene	-	-	-	-	-	0.56
62	1479	*ar*-Curcumene	-	-	-	-	7.99	-
63	1481	*γ*-Curcumene	-	-	-	-	7.22	-
64	1484	Germacrene D	0.15	-	-	-	-	-
65	1489	*β*-Selinene	-	-	-	-	7.94	-
66	1487	E-*β*-Ionone	-	-	-	-	-	0.98
67	1493	*trans*-Muurola-4(14),5-diene	-	-	-	-	-	1.55
68	1496	Viridiflorene	-	-	-	1.40	-	9.02
69	1498	*α*-Selinene	-	-	-	-	4.95	-
70	1500	*α*-Muurolene	-	-	-	-	-	2.51
71	1505	*β*-Bisabolene	-	-	-	-	0.13	-

72	1513	*γ*-Cadinene	0.18	-	-	-	0.40	8.19
73	1514	*β*-Curcumene	-	-	-	-	0.19	-
74	1522	*δ*- Cadinene	-	-	-	-	0.58	0.59
75	1528	Zonarene	-	-	-	0.10	0.27	13.28
76	1533	*trans*-Cadina-1,4-diene	-	-	-	-	-	0.40
77	1537	*α*- Cadinene	-	-	-	-	-	0.97
78	1544	*α*-Calacorene	-	-	-	-	0.17	0.78
79	1564	*β*-Calacorene	-	-	-	-	-	0.39
80	1567	Palustrol	-	-	-	-	-	0.35
81	1592	Viridiflorol	-	-	-	0.21	-	2.80
82	1595	Cubeban-11-ol	-	-	-	-	-	5.04
83	1602	Ledol	-	-	-	-	-	1.92
84	1618	1,10-di-*epi*-Cubenol	-	-	-	-	-	1.55
85	1627	1-*epi*-Cubenol	-	-	-	-	-	1.67
86	1638	*epi*-α-Cadinol	-	-	-	-	-	2.28
87	1644	*α*-Muurolol (Torreyol)	-	-	-	-	-	2.89
88	1652	*α*-Cadinol	-	-	-	-	-	5.86
89	1685	*α*-Bisabolol	-	-	0.24	-	-	-
		TOTAL	**97.35**	**90.15**	**92.99**	**93.65**	**90.33**	**90.35**

KIL - Kovat's (retention) index - literature data (24); (-) - not found; No. - ordinal number of the component according to its retention time

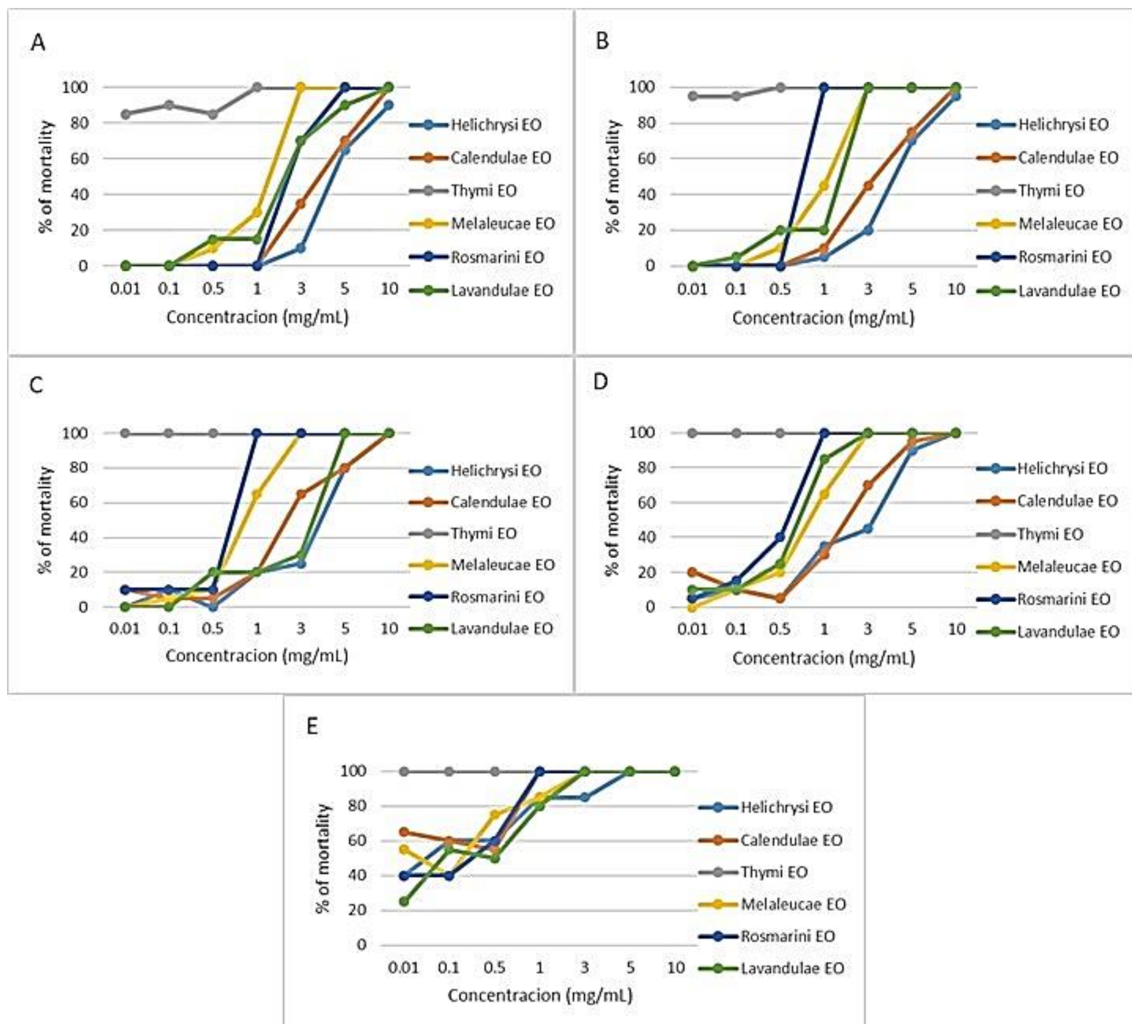

Figure 1: Mortality trend for *Artemia salina* against concentration for essential oil in A) 1 h, B) 2 h, C) 4 h, D) 6 h, E) 24 h.

DISCUSSION

The widespread use of essential oils requires an accurate understanding of the relationship between chemical composition of the essential oil and their cytotoxicity. Rigorous investigations, spanning various disciplines, consistently point towards a cause-and-effect relationship between the essential components and the observed cellular toxicity.

In this way, Alves et al.[12], Bajracharya et al.[4], and Niksic et al.[13], have confirmed cytotoxicity of some essential oils using BLSA test. Alves et al.[12], confirmed cytotoxicity of *Ocimum basilicum essential oil* with obtained LC_{50} below 90 µg/mL, and declared that cytotoxicity may be in relationship with linalool as one of the main components. In this order, linalool cytotoxicity can be explained through different mechanism. As described by Kladniew et al.[14], linalool has capacity to easily react with other molecules in HepG2 cells. On the other side, Chang et al.[15], declared that linalool can have cytotoxic effects by inducing cells to undergo apoptosis, triggering cell death. According to these studies, the cytotoxicity of *Lavandulae essential oils* is probably due to the cytotoxic activity of linalool. Niksic et al.[13], analyzed *Thymi essential oil* and accomplished LC_{50} of 60.38 µg/mL, after 24h. This result is opposite from our findings as *Thymi essential oil* showed high toxicity with LC_{50} of 0.75 µg/mL in the first hour. Furthermore, Niksic et al.[13] identified thymol and *p*-cymene as the predominant components probably responsible for their cytotoxicity of the oil. The cytotoxicity of *Rosmarini essential oil* was confirmed by Bajracharya et al.[4], using the same test with obtained LC_{50} of 12.71 µg/mL, which is similar to our obtained LC_{50} value (13.85 µg/mL) for the same essential oil. Futhermore, Miladi et al.[16], confirmed cytotoxicity of *Rosmarini essential oil* using MTT on the human respiratory epithelial cell line (A549) and claimed that anticarcinogenic activity of rosemary is due to the major bioactive compounds such as 1,8-cineole, camphor, and *α*-pinene. The cytotoxicity of 1,8-cineole was also seen in this component's ability of suppressing cell proliferation and DNA fragmentation in Molt 4B and HL-60 leukemia cell lines. The researchers determined that the observed changes in leukemia cell lines were caused by this compound's specific apoptosis stimulation.[1] The cytotoxicity of 1,8-cineole was additionally explained by Mobarakain et al.[17], as 1,8-cineole, may function through common pathways and mediate its cytotoxic effects through targeting p53 and its acetylation in SF-9 cells.

Satooka et al.[18], pointed out thymol toxicity at high doses on B16 melanoma cells and its primary mechanism appears to be related to oxidative stress. This oxidative stress-induced toxicity is a prominent factor in the adverse effects associated with thymol exposure. Interestingly, the cytotoxicity of thymol or thyme oil is not organism-specific; it manifests across various organisms, including bacteria as well as fungi. The cytotoxicity of thymol was also explained by Slamenova et al.[1] Three different thyme extracts (*Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis*) were examined on THP-1 monocyte-derived human macrophages activated by oxidized LDL. The researchers discovered that thyme extracts significantly reduce proinflammatory mediator synthesis and gene expression, suggesting that these extracts may have anti-inflammatory capabilities. Moreover, the toxicity of thymol and carvacrol, against human laryngeal cancer cells was also confirmed as they decreased the viability and proliferation of Hep-2 cells in a dose-dependent manner.[1] The cytotoxicity of *p*-cymene, as one of the most dominant components in *Thymi essential oil*, was also confirmed by Blahbib et al.[19], on four human carcinoma cells: MCF-7, MDA-MB-453, SW-480 and IM9. According to the studies done on different cell lines that confirmed cytotoxicity of thymol, it may be considered that the cytotoxicity of *Thymi essential oil* is largely due to the presence of its main component thymol as well as *p*-cymene.

It is believed that the toxicity not only of *Rosmarini essential oil*, but also of *Helichrysi* and *Calendulae essential oil* may be due to their main component *α*-pinene. As described by Tukrez et al.[20], *α*-pinene demonstrated a significant increase in LDH levels in cultured human blood cells in a time-dependent manner. LDH is an enzyme present in cells, and its concentration can serve as an indicator of cell necrosis. In instances of cell death, such as necrosis, the LDH enzyme is released into the extracellular space, including the serum and tissues. This release occurs due to the disintegration of cell membranes and subsequent leakage of cellular contents, including LDH. Moreover, as described by Santana et al.[21], the cytotoxicity of *α*- and *β*-pinene may due to the presence of double bond in their structure. The cytotoxicity of *α*-pinene, *β*-pinene, germacrene D and *α*-terpinol was also confirmed in Salihu et al.[22] study, using MTT assay on different cancer cell lines: HeLa, CaCo-2 and MCF-7. The cytotoxic activity of *β*-caryophyllene may occurs because of the apoptosis effect of this compound which was already reported by Najar et al.[23] This compound's cytotoxicity is also confirmed with MTT method on 4 human cancer cell lines, breast (MCF7), melanoma (SKMEL-19), gastric (AGP01), colon (HCT116), and a non-malignant human lung fibroblast cell (MRC5).[24] The cytotoxicity of essential oils can often result not only from one chemical component, but also by the collaborative actions of the other constituents present in the essential oil. According to Mennai et al.[25], *Melaleucae essential oil* cytotoxicity may owe to the cytotoxic activities of *o*-cymene, terpinen-4-ol and *γ*-terpinene. As it is described in that study by Jie et al.[26], the cytotoxic activity is mainly due to the presence of the mono and sesquiterpenes.

CONCLUSION

The essential oil seemed to be rich both in mono- and sesquiterpenes. As a source of bioactive compounds, the results demonstrated that essential oils have a cytotoxic effect. *Lavandulae essential oil* had showed the lowest cytotoxic activity with the LC_{50} value 58.09 µg/mL while the most cytotoxic essential oil was *Thymi essential oil* as the LC_{50} value 0.75 µg/mL was established in the first hour. This effect may result from the major compounds as *α*- and *β*-pinene, *p*-cymene, 1,8-cineole, *α*-terpineol, *γ*-terpinene, terpinen-4-ol, linalool, thymol, carvacrol and germacrene D, as well as from the synergistic effects of the complex mixture. This should be elucidated by future designed studies as the recognition of specific components as central players in inducing cytotoxicity opens avenues for targeted research and applications. In summary, both mono- and sesquiterpenes look promising for possible anticancer activity.

REFERENCES

1. Slamenova D, Horvathova E. Cytotoxic, anti-carcinogenic and antioxidant properties of the most frequent plant volatiles. Neoplasma, 2013; 60(4): 343-354.
2. Bakali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils--a review. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 2008; 46(2): 446-475.
3. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines, 2016; 3(4): 25.
4. Bajracharya GB, Tuladhar SM. Brine-shrimp Bioassay for Assessment of Anticancer Property of Essential Oils from Species. Nepal Journal of Science and Technology, 2011; 12: 163-170.
5. Piaru SP, Mahmund R, Ismail S. Studies on the Phytochemical Properties and Brine Shrimp Toxicity of Essential Oil Exctracted from Myristica fragnans Houtt. (Nutmeg). Journal od Essential Oil Bearing Plants, 2012; 15(1): 53-7.

6. Adams RP. Identification of essential oil components by gas chromatography/ mass spectrometry, 4th Ed., USA: Allured Publishing Corporation, IL: 2007, pp. 9-31.
7. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica, 1982; 45(5): 31-34.
8. Finney DJ. Probit analysis: a statistical treatment of the sigmoid response curve. 2nd Ed., Cambridge University Press, Cambridge, 1952.
9. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, Bhagwandin N, Smith PJ, Folb PI. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of ethnopharmacology, 2004; 92(2-3): 177-191.
10. Sorgeloos P, C. Remiche-Van Der Wielen C, Persoone G. The use of Artemia nauplii for toxicity tests—A critical analysis. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY, 1978; 2(3-4): 249-255.
11. Vanhaecke P, Persoone G, Claus C, Sorgeloos P. Proposal for a short-term toxicity test with Artemia nauplii. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY, 1981; 5(3): 382-387.
12. Alves PB, Filho PSF, Moraes VRS, Blank AF, Filho JLSC, Arrigonei-Blank MF, Olivia G, Thiemann OH. Chemical Composition of Essential Oil from Seven Ocimum basilicum L, Accesions, Brine Shrimp Lethality Bioassay and Inhibitory Activiies Against GAPDH and APRT. Journal of Essential Oil Research, 2007; 19(1): 89-92.
13. Niksic H, Becic F, Koric E, Gucis I, Omeragic E, Muratovic S, Miladinovic B, Duric K. Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Sci Rep, 2021; 11: 13178.
14. Kladniew BR, Castro A, Starkel P, Saeger C, Bravo MG, Crespo R. Linalool induces cell cycle arrest and apoptosis in HepG2 cells through oxidative stress generation and modulation of Ras/MAPK and Akt/mTOR pathways. Life Sciences, 2018; 199: 48-59.
15. Chang MY, Shen YL. Linalool Exhibits Cytotoxic Effects by Activating Antitumor Immunity. Molecules, 2014; 19(5): 6694-6706.
16. Miladi H, Slama RB, Mili D, Zouari S, Bakhrouf A, Ammar E. Essential oil of Thymus vulgaris L. and Rosmarinus officinalis L.: Gass chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and bacterial activities against foodborne pathogens. Natural Sciene, 2013; 5(6): 729-739.
17. Mobarakian M, Dastjerdi MN, Shakarami J, Abtahi SM. Mechanisms of Cytotoxicity-Inducing Effect of 1,8-cineole, a Plant Terpenoid, on Lepidopteran (Spodoptera frugiperda) Cell Line. Journal of Applied Biotechnology Reports, 2021; 8(1): 32-36.
18. Satooka H, Kubo I. Effects of Thymol od B16-F10 Melanoma Cells. Journal of Agricultural and Food Chemistry, 2012; 60(10): 2746-2752.
19. Balahbib A, Omari NE, Hachafi NE, Lakhdar F, Menyiy NE, Salhi N, Mrabti HN, Bakrim S, Zengin G, Bouyahya A. Health beneficial and pharmacological properties of p-cymene. Food and Chemical toxicology, 2021; 153: 112259.
20. Turkez H, Aydin E. In vitro assessment of cytogenetic and oxidative effects of α-pinene. Toxicology and Industrial Health, 2016; 32(1): 168-176.
21. Santana JS, Sartorelli P, Guadagnin RC, Matsuo AL, Figueiredo CR, Soares MG, da Silva AM, Lago JH. Essential oils from Schinus terebinthifolius leaves - chemical composition and in vitro cytotoxicity evaluation. Pharmaceutical biology, 2012; 50(10): 1248-1253.

22. Salihu MB, Schuster R, Hajdari A, Mulla D, Viernstein H, Mustafa B, Mueller M. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three Pinus spp. Pharmaceutical Biology, 2017; 55(1): 1553-1560.
23. Najar B, Shortrede JE, Pistelli L, Buhagiar J. Chemical composition and in vitro cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. Chemistry and Biodiversity, 2019; cbdv.201900478.
24. Jeronimo LB, da Costa JS, Pinto LC, Montenegro RC, Setzer WN, Mourao RHV, da Silva JKR, Maia JGS, Figueriedo PLB. Antioxidant and Cytotoxic Activities of Myrtaceae Essential Oils Rich in Terpenoids from Brazil. Natural Products Communications, 2021; 16(2): 1-136.
25. Mennai I, Lamera E, Slougui N, Benaicha B, Gasmi S, Samai Z, Rahmounia N, Bensouici C, Pinto DCGA. Chemical Composition and Antioxidant, Antiparasitic, Cytotoxicity and Antimicrobial Potential of the Algerian Limonium Oleifolium Mill. Essential Oil and Organic Extracts. Chemistry and Biodiversity, 2021; 18; e2100278.
26. Jie H, Tao S, Jun H, Shuangyang C, Xiqoqiang C, Guolin Z. Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of Photinia serrulata. Food Chemistry, 2007; 103: 355-358.