ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(2):
Pg. 518-527

Cytotoxicity Evaluation of *Origanum compactum*, *Melaleuca alternifolia* and *Cinnamomum camphora* Essential oils on Human Carcinoma Cells

MERIMA IBISEVIC[1]*, SASA PILIPOVIC[5], MAIDA SLJIVIC HUSEJNOVIC[1], ESMERALDA DAUTOVIC[1], ERMINA CILOVIC KOZAREVIC[1], AIDA SMAJLAGIC[2], ENIDA KARIC[1], EMIR HOROZIC[3], EKREM PEHLIC[4] and JASMIN TOROMANOVIC[4]

[1]Faculty of Pharmacy, University of Tuzla, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina.
[2]Faculty of Natural Sciences and Mathematics, University of Tuzla, Urfeta Vejzagića 4, 75000 Tuzla Department of Chemistry, Bosnia and Herzegovina.
[3]Faculty of Technology, University of Tuzla, Urfeta Vejzagića 4, 75000 Tuzla Department of Organic Chemistry, Bosnia and Herzegovina.
[4]Faculty of Health Studies, University of Bihac, Nositelja hrvatskog trolista 4, 77000 Bihac, Bosnia and Herzegovina.
[5]Control Laboratory of the Agency for Medicines and Medical Devices, Maršala Tita 9, 71 000 Sarajevo, Bosnia and Herzegovina.
*Corresponding author E-mail: merima.ibisevic@untz.ba

http://dx.doi.org/10.13005/ojc/410221

(Received: February 11, 2025; Accepted: April 08, 2025)

ABSTRACT

Origanum compactum, *Melaleuca alternifolia*, and *Cinnamomum camphora* essential oils are recognized for their therapeutic potential, including their selective cytotoxicity against cancer cell lines. Our research focused on examining the cytotoxic effects of these essential oils on three human carcinoma cell lines: lung carcinoma (H460), cervical adenocarcinoma (HeLa), and colorectal carcinoma (HCT116). The MTT-based cell viability assay was used to assess the cytotoxicity of essential oils. The results demonstrated that all three essential oils exhibited dose-dependent cytotoxic potential, with varying levels of growth inhibition across the cell lines. Notably, the highest sensitivity was observed in H460 cells, and the lowest sensitivity was found in HCT116 cells. *Origanum compactum* demonstrated the strongest cytotoxicity across all cell lines (GI50 73-154 nL/mL), making it the most promising candidate for further investigation, particularly for lung and cervical cancer treatment.

Keywords: MTT assay, Malignant cells, Antiproliferative potential, Bioactive components, Essential oils.

INTRODUCTION

Essential oils are complex mixtures of plant-derived substances that exhibit a wide range of pharmacological effects, such as the ability to neutralize free radicals, reduce inflammation, inhibit tumor growth, and combat microbial activity[1]. These natural products interact with a variety of biological targets, positioning them as promising candidates towards drug development.

Despite their widespread use and generally recognized safety, essential oils can cause adverse reactions such as sensitization, dermatitis, and neurotoxicity[2]. Therefore, a thorough understanding of essential oils' pharmacological and safety profiles is crucial for maximizing their benefits while minimizing health risks to humans.

Origanum compactum is an endemic Moroccan plant, where monoterpene phenols carvacrol and thymol serve as the main active components responsible for the therapeutic effect (Fig. 1). The phenolic group (-OH) in both carvacrol and thymol is responsible for their antimicrobial and antioxidant, antifungal and anti-inflammatory properties with great potential in combating resistance[3,4,5] as it interacts with cell membranes, neutralizes free radicals, and inhibits pro-inflammatory mediators. The isopropyl (-C_3H_7) and methyl (-CH_3) groups enhance their lipophilicity, aiding in cellular penetration and contributing to their bioactivity. The initiation of apoptosis, suppression of cell growth, as well as disruption of cell membranes serve as the fundamental mechanisms underlying their biological activity. Particularly extracts and essential oils of *O. compactum* (*O. compactum* EO) have shown selective cytotoxicity against various cancer cell lines, including cells originated from hepatocellular, mammary, and lung carcinoma[6].

A previous study showed that ethyl acetate extract of *O. compactum* showed antiproliferative effect on MCF-7 human breast tumor cells, A549 lung cancer cells, and SMMC-7721 hepatocytes[7]. Research by El Finou Hamza[8] highlights the antiproliferative effect of *O. compactum* against the skin cancer cell line A431 (epidermoid carcinoma), reducing cell viability by up to 20%.

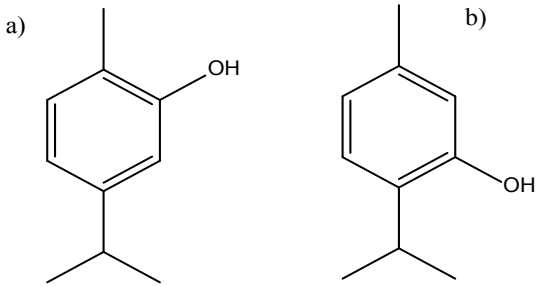


Fig. 1. Chemical structure carvacrol (a) and thymol (b) (ChemBioDraw Ultra 14.0)

Melaleuca alternifolia (tea tree) is a plant native to Australia and is highly valued for its medicinal and cosmetic uses, owing to its potent antiseptic, antimicrobial, antimycotic, and antiphlogistic properties[9]. The active components include various natural organic compounds terpenes, monoterpenes, and sesquiterpenes, with terpinen-4-ol and α-terpineol being central to its antimicrobial effects. The hydroxyl group (-OH) in their structure (Fig. 2) is primarily responsible for their biological activity, as it enables interactions with microbial cell membranes, neutralizes free radicals, and modulates inflammatory pathways.

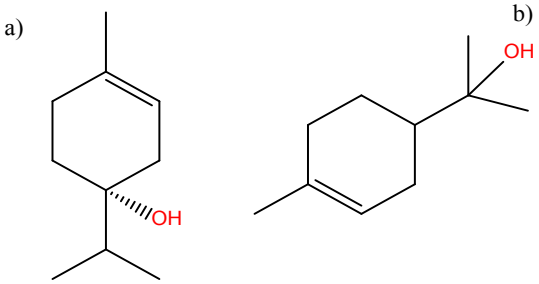


Fig. 2. Chemical structure terpinen-4-ol (a) and α-terpineol (b) (ChemBioDraw Ultra 14.0)

Tea tree oil has been found to initiate apoptosis in tumor cells, with terpinen-4-ol being particularly effective in activating the apoptosis pathway. Studies have demonstrated that this bioactive substance inhibits the cancer cell proliferation by increasing oxidative stress and promoting cell death[10]. Previous study showed that tea tree oil (TTO) from M. alternifolia reduces melanoma cell viability by triggering apoptosis[11]. Ireland *et al.,*(2012) showed that topically applied 10% M. alternifolia oil in DMSO decrease cell viability in subcutaneous tumor-bearing mice[12].

Cinnamomum camphora (camphor tree) of the Lauraceae family, is native to Asia, particularly China, Japan, and Taiwan[13]. The key compounds of its volatile oil are 1,8-cineole, limonene, alpha-pinene (Fig. 3), which demonstrate a broad spectrum of biological effects, including microbe neutralization, inflammation modulation, and tumor suppression growth. The biological activity of 1,8-cineole is primarily attributed to its epoxide functional group. The aromatic compound limonene owes its activity to the conjugated double bonds in its cyclohexene ring, enhancing its antioxidant and anti-cancer potential. Alpha-pinene exhibits antimicrobial and anti-inflammatory effects due to its reactive bicyclic structure and conjugated double bonds, which

interact with biological membranes and enzymes. Extracts and individual bioactive compounds from *C. camphora* have significant cytotoxic effects on various tumor cell types, induced by apoptosis, inhibition of cell proliferation, and modulation of signaling pathways involved in tumor growth. Previous research has indicated that certain compounds of *C. camphora* essential oil (Camphor EO) possess inhibitory and anti-mutagenic properties against several human cancer cell types[14].

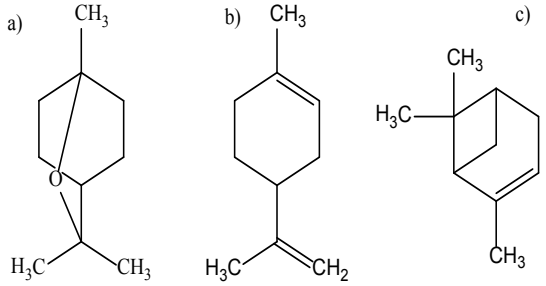


Fig. 3. Chemical structure of 1,8 cineole (a), limonene (b) and alpha-pinene (c) (ChemBioDraw Ultra 14.0)

This research intends to assess the cellular toxicity of *O. compactum*, *M. alternifolia*, and *C. camphora* essential oils on human lung carcinoma (H460 cells), cervical adenocarcinoma (HeLa cells), and colorectal carcinoma (HCT116 cells) by evaluation their impact on cell proliferation. There is evident lack of studies focused on the tumor-suppressive potential of these essential oils in the aforementioned cultured cells. The findings will contribute to understanding the efficacy of these plant extracts as natural anticancer agents. Therefore, this study may contribute valuable insights for future research.

EXPERIMENTAL

Essential oils

This study employed the following essential oils:

- *O. compactum* EO (Pranarom International, Belgium)
- Tea tree oil (BIOETERICA, Zagreb, Croatia)
- Camphora EO (Volimo prirodno, Mostar, Bosnia and Herzegovina)

Essential oils were prepared by dissolving them in Dulbecco's Modified Eagle Medium (DMEM) at concentrations of 0.01 µL/mL, 0.05 µL/mL, 0.1 µL/mL, 0.5 µL/mL, and 1 µL/mL. These stock solutions were used for subsequent experimental applications. The MTT test was performed using a microplate reader (Multiskan EX, Thermo Labsystems, Austria), and the standard chemotherapeutic agents used in the control of the MTT test were doxorubicin and etoposide (Sigma Aldrich).

Cell cultures

In this study, we utilized human malignant cell lines commonly employed in toxicological assessments: H460 epithelial lung carcinoma cells, HeLa epithelial cells derived from uterine and cervical adenocarcinoma, and HTC116 epithelial cells from colorectal cancer. These cell lines were selected for their relevance in evaluating the cytotoxic effects of the tested compounds.

Since the cell lines used in this study are adherent, they were cultured in Dulbecco's modified Eagle medium (DMEM), enriched with 2 mM L-glutamine, 10% fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 U/mL penicillin, at 37°C with 5% CO_2. Upon reaching 80-90% confluence, cells were detached using 0.25% trypsin and resuspended in fresh medium. All procedures were carried out under sterile conditions to avoid contamination.

MTT-based cell viability assay

On the initial day, three study-defined cell lines were plated separately into 96-well microtiter plates at a density of 1.5×10 cells/mL. Essential oils were introduced into the wells at five defined concentrations. Following a 72-h incubation, cell proliferation was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, which assess the metabolic activity via dehydrogenase in viable cells. This colorimetric assay is based on the measurement of the reduction of yellow tetrazolium salts by mitochondrial enzymes in metabolically active cells, leading to the formation of crystalline purple formazan[15]. After removing the basal medium, 40 µL of MTT reagent (0.5 µg/µL) was transferred to each well. Following a four-hour incubation, the formed precipitates were solubilized in 160 µL of dimethyl sulfoxide (DMSO), and absorbance readings were taken at 570 nm using a microplate reader (Multiskan EX, Thermo Labsystems, Austria), with the obtained absorbance

value being directly proportional to the viability of cancer cells. The percentage of cell growth (PG) for each treatment group was calculated using one of the formulas given bellow.

In case of $(A_t - A_0) \geq 0$ the calculation was performed by using the expression:

$$PG(\%) = 100 \cdot \frac{A_t - A_0}{A_c - A_0}$$

In case of $(A_t - A_0) < 0$ the calculation was performed by using the expression:

$$PG(\%) = 100 \cdot \frac{A_t - A_0}{A_0}$$

where:

- A_0 represents the mean absorbance before the exposure,
- A_t represents the mean absorbance after 72 hours of exposure, and
- A_c represents the mean absorbance of untreated cells after 72 hours.

Results were presented as concentration-response curves, with negative values indicating cytotoxic effects. A-100% value corresponds to the total loss of cell viability at the given concentration. Additionally, the growth inhibition (GI_{50}) was also calculated, reflecting the concentration at which 50% of cell growth was inhibited.

Statistical analysis

The normality in data distribution was assessed with the Shapiro-Wilk statistical test, while further verification was carried out via Skewness and Kurtosis analysis, as well as graphical methods, including the Q-Q plot and histogram. Given that the data did not follow a normal distribution, non-parametric statistical approach was employed for subsequent analyses. Specifically, the Kruskal-Wallis test was utilized to evaluate differences among groups, while Spearman's rank correlation analysis was applied to evaluate the associations between variables.

A p-value of less than 0.05 was considered significant. Statistical analyses were carried out using software provided by IBM SPSS, V21.0.

RESULTS AND DISCUSSION

The concentration-dependent variation of PG(%) for *O. compactum* EO, TTO, and Camphor EO on H460, HeLa, and HCT116 cell cultures are given below.

Citotoxicity of *Origanum compactum* essential oil

To evaluate the cytotoxic activity of *O. compactum* EO MTT assay was performed. Cell viability was evaluated in three cell lines H460, HeLa, and HCT116 cells following exposure to the *O. compactum* EO at five different concentration values (0.1–1 µL/mL), allowing for a dose-dependent assessment of cytotoxic effects. According to the Kruskal-Wallis test, a statistically significant difference in cell viability distribution across concentration groups ($p = 0.014$) was found indicating that increasing concentrations of *O. compactum* essential oil significantly affect cell survival.

The treatment of H460, HeLa, and HCT116 cells with increasing concentrations of *O. compactum* essential oil led to a pronounced, dose-dependent decline in cell viability (Fig. 4). Notably, at the highest concentration (1 µL/mL), fewer than 30% of H460 and HeLa cells remained viable, whereas HCT116 cells exhibited greater resistance, maintaining approximately 60% viability (Table 1). The increase in HCT116 cells resistance to *O. compactum* essential oil compared to H460 and HeLa cells can be attributed to several factors. These include complex mechanisms of drug resistance in colon cancer cells with enhanced antioxidant defense and increased expression of p-glycoprotein transporter. Additionally, HCT116 cells harbor TP53 mutations, which can impair apoptosis, and may have slower proliferation rates or metabolic adaptations that reduce susceptibility to cytotoxic agents[16,17].

Table 1: Dose-response profile for *O. compactum* Essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

Concentration (µL/mL)	H460	HeLa	HCT116
0.01	101.4	107.7	85.4
0.05	87.2	79.6	62.6
0.1	62.6	53.4	28.5
0.5	-82.0	-43.0	-42.2
1.0	-74.8	-72.8	-38.1

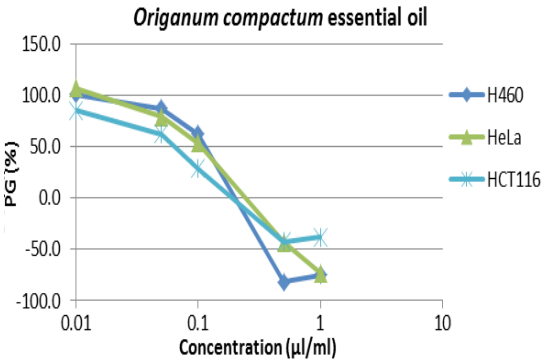
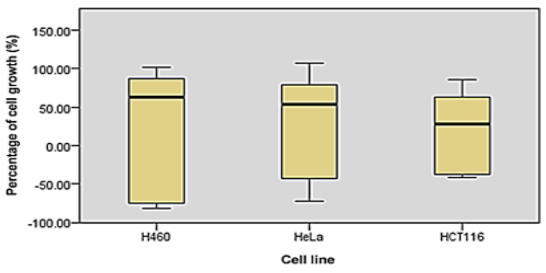


Fig. 4. Dose-response profile for *O. compactum* Essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

Although HCT116 cells appeared more resistant to *O. compactum* essential oil as a potential cytotoxic agent, statistical analysis did not confirm this observation. No statistically significant difference in cell viability among the three examined cell lines ($p = 0.993$) was found by the Kruskal-Wallis test indicating that the distribution of percentage of cell growth (%) was consistent across all groups. Furthermore, Spearman's rank correlation analysis demonstrated a strong negative correlation ($\rho = -0.912$, $p = 1$), suggesting an inverse association between the tested variables. These findings indicate that despite variations in cell lines, no significant difference in cytotoxicity was found, while the observed correlation reflects a strong negative trend without statistical significance (Figure 5).



Total N	15
Test statistic	0.015
Degrees of freedom	2
Asymptotic Sig. (2-sided test) p	0.993

Fig. 5. Comparison of cell growth distribution across three cell lines exposed to *O.compactum* essential oil

Citotoxicity of *Melaleuca alternifolia* essential oil

Recent studies have explored the potential anticancer effects of *M. alternifolia* essential oil. Topically applied, it demonstrates direct cytotoxicity against subcutaneous tumors in mice, suggesting its potential as a topical anticancer treatment[12]. In vitro studies showed that *M. alternifolia* essential oil exhibites strong cytotoxicity against human melanoma and squamous carcinoma cell lines, inducing apoptosis and cell cycle arrest[18]. Furthermore, TTO which contains terpinen-4-ol, synergistically enhance the effectiveness of targeted melanoma therapies by activating apoptosis[11]. These observations highlight the utility of TTO as an anticancer agent, particularly for skin cancers. However, there is evident lack of research focusing on other carcinoma cell lines.

In the current study, MTT assay of H460, HeLa, and HCT116cell lines following 72-h incubation with five different concentrations of *M. alternifolia* essential oil in a microtiter plate demonstrated a dose-dependent response. A significant increase in cytotoxicity was observed with increasing oil concentration, with the most pronounced effect occurring when the concentration levels were adjusted from 0.5 to 1 µL/mL (Fig. 6). According to the Kruskal-Wallis test, a notable difference was detected in cell viability across the concentration groups ($p = 0.017$), indicating that increasing concentrations of tea tree oil markedly influence cell survival.

The *in vitro* cytotoxicity results of *M. alternifolia* essential oil on H460, HeLa, and HCT116 cancer cells are outlined in Table 2. At concentrations between 0.01 and 0.1 µL/mL, the essential oil had no significant effect on cell viability. However, exposure to 0.5 µL/mL induced a cytotoxic response in all three cell lines, with HCT116 exhibiting the highest sensitivity, while H460 was the least sensitive, respectively. After incubation with 0.5 µL/mL of *M. alternifolia* essential oil, the percentage of cell growth in H460, HeLa, and HCT116 cells was 78.4%, 56.3%, and 42.8%, respectively. Cell viability declined further with increasing concentrations, with the highest concentration (1 µL/mL) exhibiting the strongest cytotoxic effect across all three cell lines.

Table 2: Dose-response profile for *M. alternifolia* Essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

Concentration (µL/mL)	H460	HeLa	HCT116
0.01	101.1	101.5	99.1
0.05	95.5	100.6	87.6
0.1	92.0	100.9	84.9
0.5	78.4	56.3	42.8
1.0	-36.7	-34.3	-44.5

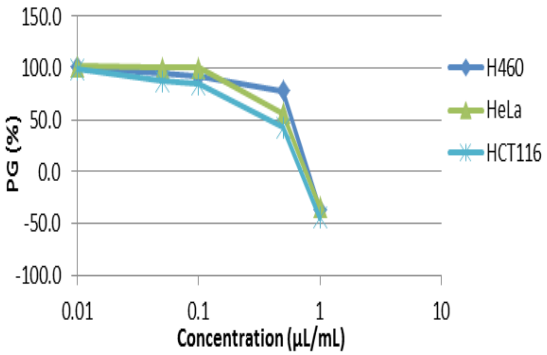


Fig. 6. Dose-response profile for *M. alternifolia* essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

The three cell lines demonstrated similar sensitivity to *M. alternifolia* essential oil, as supported by data interpretation. The Kruskal-Wallis test suggested no considerable difference in cell viability across the cell lines (p = 0.482), implying that the distribution of cell growth percentages was uniform among the groups. Additionally, Spearman's rank correlation analysis showed a very weak negative correlation (ρ = -0.187, p = 1), suggesting a slight inverse relationship between the variables, though the correlation was not statistically significant (Figure 5).

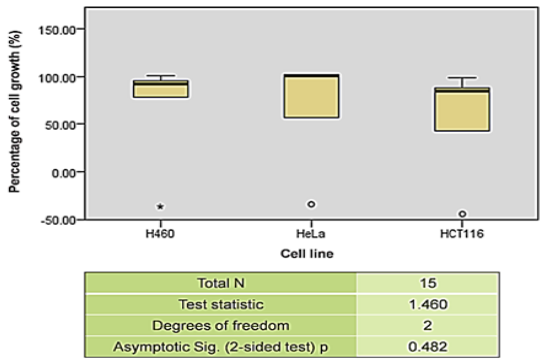


Fig. 7. Comparison of cell growth distribution across three cell lines exposed to *M. alternifolia* essential oil

Citotoxicity of *Cinnamomum camphora* essential oil

Cell proliferation was evaluated with the MTT colorimetric protocol, where the reduction of MTT to formazan reflects viable cell metabolism following exposure to *C. camphora* essential oil. The results of the *in vitro* MTT assay on H460, HeLa, and HCT116 cancer cell lines are shown in Table 3 and Fig. 8. The essential oil reduced cell viability in a dose-dependent manner. The percentage of cell growth was similar for HeLa and HCT116 cells, while it differed for H460 cells. Cytotoxicity of *C. Camphora* essential oil was evaluated at five different concentrations. At lower concentrations (0.01-0.1 µL/mL), all three cell lines exhibited similar sensitivity, but concentrations above 0.5 µL/mL induced significantly higher cytotoxic effects in H460 cells compared to HeLa and HCT116 cells. Researchers have shown that essential oils demonstrate cytotoxic effects on various cancer cell lines, with different cell types showing varying sensitivities[19,20,21]. H460 cells, in particular, exhibit increased sensitivity due to the complex interplay of cellular defense mechanisms, oxidative stress, and apoptosis. The findings from Wu *et al.,*(2010), Lu *et al.,*(2011) further highlight the intricate mechanisms that contribute to the H460 cells' heightened sensitivity to cytotoxic agents, including essential oils[22,23]. As shown in Table 3, more than 70% of H460 cells did not survive the 72-h incubation with 1 µL/mL *C. camphora* essential oil, while the percentage of cell growth for HeLa and HCT116 cells was -3.9% and 2%, respectively.

Table 3: Dose-response profile for *C. camphora* Essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

Concentration (µL/mL)	H460	HeLa	HCT116
0.01	98.3	100.0	100.6
0.05	82.2	96.6	98.4
0.1	73.0	88.9	85.1
0.5	31.7	78.2	67.4
1.0	-72.0	-3.9	2.0

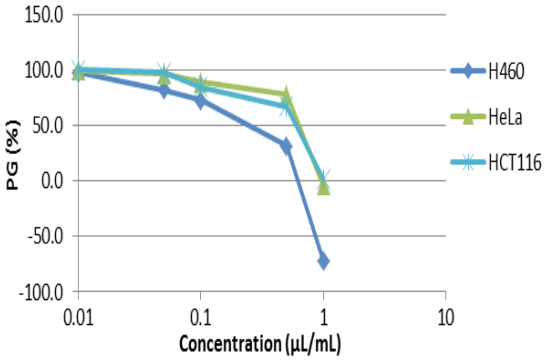
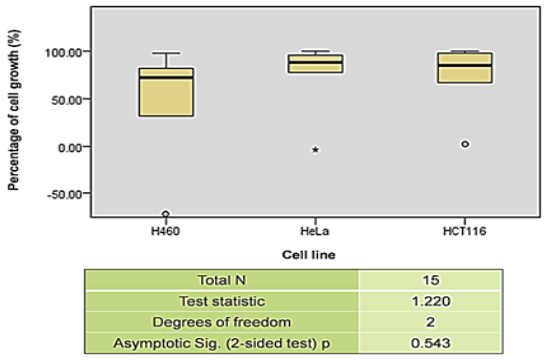


Fig. 8. Dose-response profile for *C. camphora* essential oil tested *in vitro* on H460, HeLa and HCT116 cell lines

As shown in Fig. 9, although the cytotoxicity of the essential oil was observed different for H460 cells, there was no statistical difference within the tree cell lines (p=0.543). The three cell lines exhibited similar sensitivity to *C. camphora* essential oil, as confirmed by statistical analysis. As shown in Fig. 9, while the cytotoxicity of the essential oil appeared different for H460 cells, the difference between the three cell lines was not statistically significant

(p = 0.543). Furthermore, Spearman's rank correlation analysis indicated a weak positive correlation (ρ = 0.265), suggesting a slight direct relationship between the variables, though this correlation was not statistically significant (p = 0.341).



Total N	15
Test statistic	1.220
Degrees of freedom	2
Asymptotic Sig. (2-sided test) p	0.543

Fig. 9. Comparison of cell growth distribution across three cell lines exposed to *C. camphora* essential oil

Growth inhibition effects of essential oils on H460, HeLa and HCT116

The GI_{50} values were determined for *M. alternifolia, O. compactum*, and *C. camphora* essential oils across H460, HeLa, and HCT116 cell lines. The findings revealed variations in the sensitivity of the cell lines to the Essential oils (Table 4).

For the H460 cells, the GI_{50} values were 646 ± 114 nL/mLfor M. alternifolia, 109 ± 34 nL/mLfor *O. compactum*, and 373 ± 131 nL/mLfor *C. camphora*. The *O. compactum* essential oil exhibited the lowest GI_{50}, suggesting that these cells were most sensitive to its cytotoxic effects, followed by *C. camphora* and *M. alternifolia*.

In the HeLa cells, the GI_{50} values were 432 ± 43 nL/mLfor M. alternifolia, 73 ± 23 nL/mLfor *O. compactum*, and 760 ± 210 nL/mLfor *C. camphora*. Notably, *O. compactum* again showed the lowest GI_{50}, indicating a higher potency compared to the other two essential oils. In contrast, *C. camphora* was the least effective in reducing HeLa proliferation, requiring the highest concentration for a 50% inhibition of cell growth.

For the HCT116 colorectal carcinoma cells, the GI_{50} values were 539 ± 16 nL/mL for *M. alternifolia*, 154 ± 76 nL/mL for *O. compactum*, and 750 ± 163 nL/mL for *C. camphora*. Here, *M. alternifolia* showed moderate cytotoxicity, while *O. compactum* was more potent than *C. camphora*, which again showed the weakest inhibition at higher concentrations.

These results clearly show that the essential oils demonstrated varying degrees of growth inhibition across different cancer cell lines. The highest growth inhibition (lowest GI_{50}) was observed in HeLa cells treated with *O. compactum* (GI_{50} = 73 ± 23 nL/mL), while the lowest growth inhibition showed *C. camphora* (GI_{50} = 760 ± 210 nL/mL). In general, *O. compactum* exhibited the strongest cytotoxic effects across multiple cell lines, while *M. alternifolia* and *C. camphora* showed variable effectiveness depending on the cancer type.

Table 4: GI_{50} values for Essential oils

Cell lines	*O. compactum* GI_{50} (nL/mL)	*M. alternifolia* GI_{50} (nL/mL)	*C. camphora* GI_{50} (nL/mL)
Lung carcinoma (H460)	109±34	646±114	373±131
Cervical adenocarcinoma (HeLa)	73±23	432±43	760±210
Colorectal carcinoma (HCT116)	154±76	539±16	750±163

Table 5: GI_{50} for standard chemotherapeutics (doxorubicin and etoposide)

Standard chemoterapeutic agent	H460 (ng/mL)	HCT116 (ng/mL)	HeLa (ng/mL)
Doxorubicin	5.18	22.03	1.74
Etoposide	324.70	1294.76	82.40

Although doxorubicin and etoposide were used as controls in the MTT assay, they can also serve for comparing the results of essential oils. Doxorubicin is the most effective of all the tested compounds in all cell lines (with the lowest GI_{50} values), confirming its high effectiveness as a chemotherapy drug. Among all the essential oils, O. compactum stands out as particularly effective, especially in H460 and HeLa cells, with GI_{50} values comparable to or even lower than those of doxorubicin.

Essential oils are typically used in low concentrations; however, they are often combined with other compounds that share similar mechanisms of action in pharmaceuticals, cosmetics, and food products, potentially increasing human exposure and health risks. Substances targeting the same cellular pathways may exhibit additive, synergistic, potentiating, or even antagonistic effects, emphasizing the need for an integrative approach in cytotoxicity assessment[24,25]. Future research should investigate interactions between active phytochemicals in essential oils and conventional anticancer drugs to optimize combination therapies while minimizing toxicity[26]. Additionally, environmental factors such as oxidative stress, inflammation, and microbiome composition may influence the bioactivity and metabolism of these compounds, underscoring the importance of comprehensive studies to ensure both therapeutic efficacy and safety.

CONCLUSION

This study demonstrated that the cytotoxic effects of *O. compactum, M. alternifolia*, and *C. camphora* essential oils vary across different cancer cell lines, highlighting their potential as natural anticancer agents. *O. compactum* exhibited the strongest inhibitory effects, particularly in cervical (HeLa) and lung (H460) cancer cells, suggesting its potential as a promising candidate for further research. It is evident that aromatic compounds like carvacrol and thymol exert a strong influence and are of considerable significance. *M. alternifolia* showed moderate cytotoxicity, indicating potential therapeutic applications, although with lower efficacy compared to *O. compactum*. Conversely, *C. camphora* demonstrated the weakest cytotoxic effects, which may limit its use as a standalone treatment but suggests potential as an adjuvant in combination therapies.

To fully assess the clinical potential of these essential oils, further toxicological studies are necessary to establish safe and effective dosing regimens while minimizing risks of irritation and systemic side effects, especially for topical, rectal, or vaginal applications. Additionally, future research should explore the possibility of combining these essential oils with conventional anticancer treatments to enhance therapeutic efficacy through potential synergistic interactions.

ACKNOWLEDGMENT

Our research was funded by the Ministry of Education and Science of Tuzla Canton, Bosnia and Herzegovina (no.10/1-11-21343-5/23). The authors would like to thank the Rućer BoškovićInstitute from Zagreb (Croatia) for their help with the experimental part of the work.

Conflicts of interest

The mentioned authors have no conflicts of interest regarding the publication of this paper.

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