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Exploring the chemical composition and anti-cancer potential of *Matricaria recutita* L. essential oil

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Abstract: This study aims to investigate the chemical composition and assess the anti-cancer potential of *Matricaria recutita* L. essential oil against various cancer cell lines. The chemical profile of the essential oil was determined through gas chromatography-mass spectrometry (GC-MS), identifying major constituents such as (Z)-*β*-farnesene, *α*-bisabolol oxides, and chamazulene. The anti-cancer activity was evaluated using *in vitro* cytotoxicity assays (MTT assay) on human lung adenocarcinoma (A549), human hepatocellular carcinoma (HepG2), human breast adenocarcinoma (MDA-MB-231), and mouse fibroblast (L929) cell lines. The experiments were performed in triplicate, and data were analyzed using ANOVA to determine statistical significance. The main constituents of the essential oil included (Z)-*β*-farnesene (33.90 %), *α*-bisabolol oxide B (11.60 %), and others. The oil demonstrated considerable cytotoxic effects across all tested cancer cell lines, with IC_{50} values indicating strong anti-proliferative activity, particularly at higher concentrations and longer exposure times. The results showed dose-dependent reductions in cell viability, with significant decreases in cell proliferation observed especially at concentrations over 7.81 µg/mL. These findings support its potential as a natural, complementary agent in cancer therapy, warranting further *in vivo* studies for clinical application.

Keywords: Bulgarian chamomile essential oil; chemical composition; cytotoxicity

1 Introduction

Matricaria recutita L. (syn. *Matricaria chamomilla* L., *Chamomilla recutita* L. Rauschert) commonly known as true chamomile or German chamomile, is indigenous to Central and Southern European regions but has spread to other continents like America, Africa, and Australia. This aromatic plant yields an essential oil renowned for its distinctive properties [1–3]. German chamomile is a medicinal herb renowned for its therapeutic benefits, particularly in the form of essential oil. Extracted from the flowers of this plant, chamomile essential oil possesses a diverse array of pharmacological properties, protective activities, and therapeutic uses that have been recognized and utilized across various medical and wellness practices.

The essential oil (MEO), a dense blue liquid with a potent aroma and a bitter-aromatic taste, undergoes a fascinating transformation in colder temperatures, solidifying into a mass reminiscent of a basement. The blue hue of the oil owes itself to chamazulene, a compound derived from the dehydrogenation of proazulenes present in the raw material. Chamazulene typically constitutes 1 %–18 % of the oil, occasionally reaching up to 24 % [1, 2, 4–10].

Scientific research has validated the biological efficacy of chamomile essential oil, showcasing its antimicrobial, antioxidant, and anti-inflammatory characteristics [4, 5, 9–18]. As a result, it finds widespread application in medicine, cosmetics, and the food industry [1–3, 9, 14].

Medicinal plants have long been utilized for treating various types of cancer complications, and their use has gradually increased due to the side effects associated with chemical medications [19, 20]. According to the World Health Organization (WHO), nearly 65 % of the global population relies on traditional medicine for primary healthcare [21, 22].

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The aim of the present study is to determine chemical composition and anticancer activity of *Matricaria recutita* L. using different cancer cell lines.

2 Materials and methods

2.1 Materials

The chamomile essential oil was provided by an essential oil manufacturer located in Southern Bulgaria. The oil is a dense blue liquid with a characteristic chamomile smell.

2.2 Methods

2.2.1 GC/MS analysis

A gas chromatography (GC) analysis of the chamomile oil was performed using an Agilent 7890A (Santa Clara, CA 95051, USA) gas chromatograph, HP-5 column MS (30 m × 250 µm × 0.25 µm), temperature: 35 °C/3 min, 5 °C/min to 250 °C for 3 min, 49 min in total, helium as carrier gas, 1 mL/min constant speed, 30:1 split ratio. A gas chromatography–mass spectrometric (GC/MS) analysis was carried out on an Agilent 5975C mass spectrometer, with helium as a carrier gas, column, and temperature the same as in the GC analysis. The identification of the chemical compounds was made by comparison to their relative retention time and library data [23]. Components were listed according to their retention (Kovat's) indices, calculated using a standard calibration mixture of C_8–C_{40} n-alkanes in n-hexane. Compound concentration was computed as a percentage of the total ion current (TIC).

2.2.2 Cytotoxicity assay, cell lines and culture conditions

2.2.2.1 Cell lines

Human lung adenocarcinoma epithelial cells (A549), human hepatocellular carcinoma cells (HepG2), mouse fibroblast cells (L929), and human breast adenocarcinoma cells (MDA-MB-231) were used to evaluate cytotoxic activity of chamomile essential oil. The cells were obtained from American Type Culture Collection (ATCC). A549, Hepg2 and L929 cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM). MDA-MB-231 cell line was cultured in RPMI-1640 medium. All cell lines were supplemented with 1 % penicillin and 10 % fetal bovine serum (FBS) at 37 °C in 5 % CO_2 and 95 % O_2 humidified cell incubator. When the growth of cell monolayer reached 70–80 % confluence, 0.25 % trypsin was used for digestion and passage.

Cells were seeded in 25 cm^2 culture flasks at 5×10^5/mL, harvested by trypsinization (0.05 % trypsin), and checked for reproduction daily up to 96 h. The cells were sub-cultured for three passages to check the consistency of growth, confluent stage, and viability. Every 3–4 days when the cells reached 70–80 % confluence sub-culturing was performed. To prepare the stock solution (100 mg/mL), the essential oil was dissolved in DMSO. Nine different essential oil concentrations (1,000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.9 µg/mL) were prepared by diluting the stock solution with cell culture media. DMSO was used as a vehicle for the essential oil and the concentrations of DMSO in the final OEO exposure solution was ≤ 1 %.

2.2.2.2 Cytotoxicity assay (MTT assay)

The cell viability was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Briefly, cells were seeded in 96-well plate each well 1 × 104 cells and allowed adhere for overnight at 37 °C with CO_2. The cells were treated with essential oil (3.9–1,000 µg/mL) over different incubation periods (24, 48, and 72 h). After incubation, 20 µL of MTT reagent (5 mg/mL MTT in PBS) was added to each well and the cells were incubated for 3 h at dark. Immediately after incubation, 100 µL of DMSO was added to each well. At the end of this period, the optical density of the each well was measured at 590 nm against the reference wavelength of 670 nm using a microplate reader (Thermo Scientific Multiskan GO). The cell viability was determined by accepting 100 % absorbance of the control group. Independent experiments were done in triplicate and repeated 3 times (n = 9).

2.2.2.3 Statistics

All measurements were carried out in triplicates. Data were analyzed using the IBM SPSS v27 software package. Multiple comparisons of treatments were performed using one-way ANOVA and Post Hoc test. Data are presented as mean ± standard deviation of three replicates. The results were expressed as mean ± SD and analyzed using MS Excel software. A difference was considered to have significance at $p < 0.05$.

3 Results

Table 1 presents the chemical composition of the essential oil extracted from flowers, revealing a total of 47 constituents that collectively account for 99.86 % of the oil's content. Among these constituents, several prominent compounds stand out, each comprising over 3 % of the oil's composition. The primary constituents include (Z)-β-farnesene, constituting

33.90 % of the oil, followed by *α*-bisabolol oxide B at 11.60 %, (E,E)-α-farnesene at 11.00 %, *α*-bisabolol oxide A at 6.79 %, germacrene D at 5.96 %, chamazulene at 5.04 %, *α*-bisabolone oxide A at 4.18 %, and *cis*-Spiroether at 3.16 %. Additionally, Table 1 outlines the distribution of major aroma substance groups within the oils. Sesquiterpene hydrocarbons emerge as the dominant group, constituting 64.05 % of the chamomile oil. Following closely are oxygenated sesquiterpenes at 28.79 %, aliphatic hydrocarbons at 3.17 %, monoterpene hydrocarbons at 3.08 %, oxygenated aliphatics at 0.40 %, phenyl propanoids at 0.22 %, oxygenated monoterpenes at 0.21 %, and furans at 0.08 %.

MTT assay was performed as three separate experiments to examine the cytotoxicity and the growth inhibitory potential of chamomile essential oil. The IC_{50} values of tested concentrations of chamomile essential oil are summarized in Table 2. It is clearly shown that essential oil showed nearly same cytotoxicity to all cancer cell lines.

Cytotoxic activity of chamomile essential oil in L929 cell lines is illustrated in Figure 1. All tested concentrations show reduced cell survival when compared with solvent control. This decrease was generally shown in 72 h exposure of 3.9, 7.81 and 15.62 µg/mL concentrations and all exposure times of 62.50 µg/mL and higher concentrations (Figure 1).

Figure 2 shows anti-proliferative activity of chamomile essential oil against human lung adenocarcinoma epithelial (A549) cell lines. All concentrations decreased the proliferation when compared with solvent control but the decrease was only found statistically significant levels at 72 h exposure of 31.25 µg/mL and all tested concentrations and exposure times of highest ones. The highest three concentrations showed nearly 100 % reduction in cell survival.

Chamomile essential oil decreased cell survival of human breast adenocarcinoma cells (MDA-MB-231) at all concentrations tested. The reduction was found statistically significant levels at especially over 7.81 µg/mL concentrations. The highest decrease was observed at 250, 500 and 1,000 µg/mL concentrations (Figure 3).

Concentrations of chamomile essential oil significantly reduced cell survival of human hepatocellular carcinoma cells (HepG2) at 72 h exposure of 15.62 and 31.25 µg/mL and all exposure time of 62.5 µg/mL and over concentrations (Figure 4).

4 Discussion

Based on its chemical composition, the studied chamomile essential oil can be classified as the *β*-farnesene chemotype, which distinguishes it from data in the literature. In our opinion, this difference may be attributed to soil and climatic

Table 1: Chemical composition of German chamomile essential oil.

Peak	RT[a]	RI[b]	Compounds	% of TIC[c]
1.	9.69	930	*α*-pinene	0.09 ± 0.0
2.	11.00	967	Sabinene	0.17 ± 0.0
3.	11.58	986	2-pentyl furan	0.08 ± 0.0
4.	12.69	1,022	*p*-cymene	0.22 ± 0.0
5.	12.81	1,024	Limonene	0.13 ± 0.0
6.	12.92	1,026	Eucalyptol	0.10 ± 0.0
7.	13.08	1,035	*β-trans*-Ocimene	0.19 ± 0.0
8.	13.42	1,043	*β-cis*-Ocimene	1.14 ± 0.0
9.	13.77	1,055	*γ*-terpinene	0.92 ± 0.0
10.	14.64	1,090	Terpinolene	0.44 ± 0.0
11.	22.25	1,342	7-epi-Silphiperfol-5-ene	0.10 ± 0.0
12.	23.02	1,370	*α*-copaene	0.15 ± 0.0
13.	23.20	1,380	Silphiperfol-6-ene	0.13 ± 0.0
14.	23.37	1,390	*α*-isocomene	0.63 ± 0.0
15.	23.97	1,410	*β*-isocomene	0.08 ± 0.0
16.	24.20	1,420	*β*-caryophyllene	0.44 ± 0.0
17.	24.66	1,434	Aromadendrene	0.16 ± 0.0
18.	25.12	1,440	(*Z*)-*β*-farnesene	33.90 ± 0.30
19.	25.21	1,456	allo-Aromadendrene	0.18 ± 0.0
20.	25.62	1,475	*γ*-muurolene	0.42 ± 0.0
21.	25.78	1,488	Germacrene D	5.96 ± 0.05
22.	25.91	1,492	*cis-β*-guaiene	1.14 ± 0.01
23.	25.97	1,494	*β*-selinene	0.31 ± 0.0
24.	26.01	1,497	Viridiflorene	0.45 ± 0.0
25.	26.13	1,501	Bicyclogermacrene	2.98 ± 0.0
26.	26.32	1,505	(*E*,*E*)-*α*-farnesene	11.00 ± 0.10
27.	26.37	1,508	*β*-bisabolene	0.17 ± 0.0
28.	26.52	1,515	*γ*-cadinene	0.15 ± 0.0
29.	26.64	1,520	*δ*-cadinene	0.56 ± 0.0
30.	27.60	1,535	*cis*-Nerolidol	0.12 ± 0.0
31.	28.04	1,581	Spathulenol	0.50 ± 0.0
32.	28.25	1,588	Globulol	0.35 ± 0.0
33.	28.46	1,594	Viridiflorol	0.27 ± 0.0
34.	29.89	1,662	*α*-bisabolol oxide B	11.60 ± 0.10
35.	30.45	1,683	*α*-bisabolone oxide A	4.18 ± 0.04
36.	30.50	1,688	*α*-bisabolol	1.40 ± 0.01
37.	31.53	1721	Chamazulene	5.04 ± 0.05
38.	31.94	1747	*α*-bisabolol oxide A	6.79 ± 0.06
39.	33.76	1848	Hexahydrofarnesyl acetone	0.38 ± 0.0
40.	34.61	1882	*cis*-Spiroether	3.16 ± 0.03
41.	35.44	1907	Methyl hexadecanoate	0.23 ± 0.0
42.	36.77	1969	Ethyl hexadecanoate	0.17 ± 0.0
43.	38.64	1991	Geranyl linalool	0.11 ± 0.0
44.	42.28	2,298	Tricosane	0.18 ± 0.0
45.	45.51	2,500	Pentacosane	0.52 ± 0.0
46.	49.51	2,702	Heptacosane	0.18 ± 0.0
47.	51.02	2,801	Octacosane	2.29 ± 0.02
			Aliphatic hydrocarbons,%	3.17
			Oxygenated aliphatics,%	0.40
			Monoterpene hydrocarbons,%	3.08
			Oxygenated monoterpenes,%	0.21
			Sesquiterpene hydrocarbons,%	64.05
			Oxygenated sesquiterpenes,%	28.79
			Furans,%	0.08
			Phenyl propanoids,%	0.22

[a]retention time, min; [b]retention (Kovat's) index; [c]total ion current.

Table 2: IC_{50} values of samples showing anticancer activity against different cancer cell lines.

Cell lines	24 h	48 h	72 h
L929	76.9	74.35	50.84
A549	69.36	63.37	59.1
MDA-MB-231	67.27	43.76	28.97
HepG2	74.21	55.1	27.75

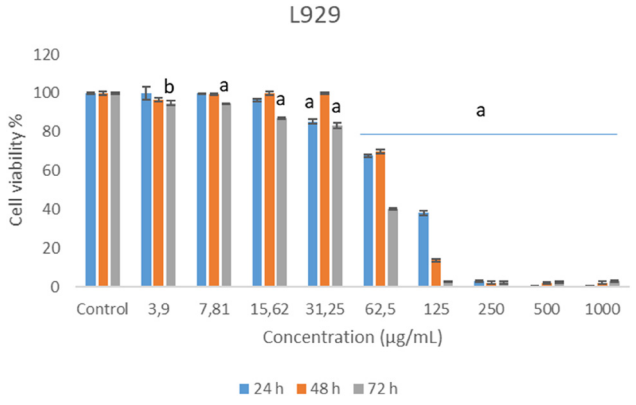

Figure 1: Cytotoxic effects of German chamomile essential oil in L929 cell lines.

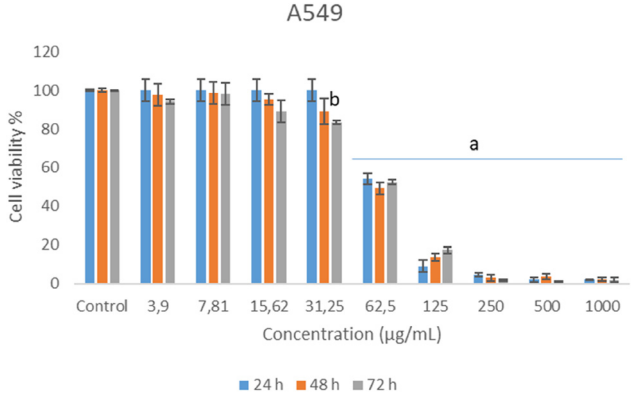

Figure 2: Cytotoxic effects of German chamomile essential oil in A549 cell lines.

conditions, as well as the various chemotypes of chamomile that grow in different regions of Europe [1].

Chamomile essential oil is rich in bioactive compounds that contribute to its pharmacological effects. One of its notable properties is its anti-inflammatory activity, attributed to constituents such as bisabolol oxide A and B. These compounds inhibit inflammatory pathways and reduce swelling, making chamomile oil effective in treating inflammatory conditions such as arthritis, dermatitis, and

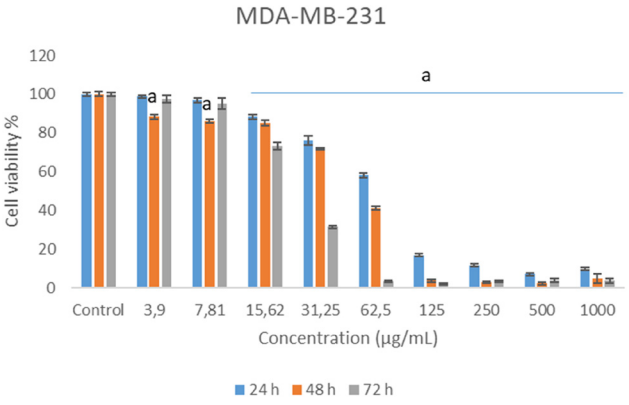

Figure 3: Cytotoxic effects of German chamomile essential oil in MDA-MB-231 cell lines.

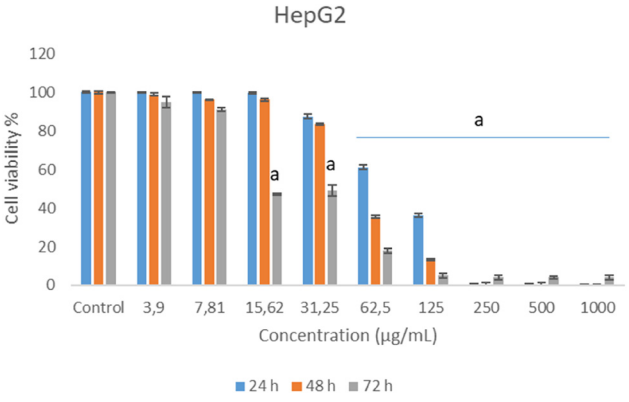

Figure 4: Cytotoxic effects of German chamomile essential oil in HepG2 cell lines.

gastrointestinal inflammation. In this study, we determined chemical composition of chamomile essential oil and tried to figure out anti-carcinogenic profile using human lung adenocarcinoma epithelial cells (A549), human hepatocellular carcinoma cells (HepG2), mouse fibroblast cells (L929), and human breast adenocarcinoma cells (MDA-MB-231). Our results clearly show that CEO has strong anti-proliferative capacity to all used cell lines especially at higher concentrations and treatment period.

The potential beneficial effects of chamomile essential oil were also noted in several studies. The essential oil demonstrated a dose-dependent reduction in cell viability, with an IC_{50} of approximately 300 µg/mL. Furthermore, it significantly inhibited the levels of important angiogenesis markers in both HepG2 cells and ex vivo, as reported by [24]. Sak et al. [25] investigated the potential anticancer effects of extracts from German chamomile on human melanoma and epidermoid carcinoma cells. Methanolic extracts from chamomile flowers were evaluated using the sulforhodamine B assay to measure cytotoxic activity. The IC_{50} value of

chamomile flower extract against melanoma cells (40.7 µg/mL) was approximately twofold higher than that against epidermoid carcinoma cells (71.4 µg/mL).

Shaaban et al. [26] have shown that the (Z)-*β*-farnesene component in *M. chamomilla* extracts has significant anticancer potential, inhibiting Caco-2 colon cancer cell migration. The (Z)-*β*-farnesene component in essential oil obtained from the leaves of *Cedrelopsis grevei*, an aromatic and medicinal plant from Madagascar, has shown a significant correlation with anticancer activity (on human breast cancer cells MCF-7) [27]. The anticancer effects and mechanisms of *α*-bisabolol in numerous types of cancer including, pancreatic, endometrial, breast and liver have been demonstrated in many experimental studies [28, 29]. Additionally, upregulating the expression of *bcl-2* and suppression of *bax, P53, APAF-1, caspase-3* and *caspase-9* activity indicates the anti-apoptotic effects of *α*-bisabolol [28]. The molecular mechanisms underlying anti-apoptotic effects of *α*-bisabolol were proven in different study models. Based on those research studies, upregulating the expression anti-apoptotic *bcl-2* expressions and suppression of the *bax, P53, APAF-1, caspase-3* and *caspase-9* activity indicates the anti-apoptotic effect *α*-bisabolol. It is noteworthy that, two probable molecular mechanisms for anticancer effects of *α*-bisabolol appear to be through NF-κB and PI3K/PDK1/Akt signaling pathways [29].

Santos et al. [30] evaluated the effects of combining the antineoplastic drug 5-fluorouracil (5-FU) with *Matricaria recutita* flower extract (MRFE) in treating mice transplanted with sarcoma 180. They assessed tumor inhibition, variations in body and visceral mass, as well as biochemical, hematological, and histopathological parameters. The study found that both isolated 5-FU and combinations of 5-FU with MRFE at doses of 100 mg/kg/day and 200 mg/kg/day reduced tumor growth. Notably, the 5-FU + MRFE at 200 mg/kg/day demonstrated a more significant reduction in tumor size compared to 5-FU alone. These findings suggest that the combination of 5-FU with MRFE at 200 mg/kg/day may enhance antitumor activity while reducing chemotherapy-induced loss of body mass and minimizing toxicity.

5 Conclusions

In this study, we explored the chemical composition and anticancer potential of *Matricaria recutita* L. essential oil from Southern Bulgaria. The detailed analysis revealed that the essential oil primarily belongs to the *β*-farnesene chemotype, a distinction influenced by the specific soil and climatic conditions of the region. This chemotype variation emphasizes the importance of geographic and environmental factors in defining the chemical profiles of essential oils. Our investigation into the anti-cancer properties demonstrated promising results, particularly against select cancer cell lines. The bioactive compounds identified within the oil, including chamazulene, *α*-bisabolol, and apigenin, have shown significant cytotoxic effects, supporting the potential of chamomile essential oil as a complementary approach in cancer treatment. These findings contribute to the growing body of evidence suggesting that natural products, particularly those derived from *Matricaria recutita* L., could play a valuable role in cancer treatment.

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