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Assessment of the phytotoxic, cytogenotoxic, antibacterial and antibiofilm properties of *Salvia sclarea* essential oil

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Synthetic herbicides have adverse effects on the environment and human health. Understanding allelopathic interactions can provide alternatives for weed control without using these synthetic chemicals. This study investigated the impact of three concentrations (10, 20, and 30 µg/mL) of *Salvia sclarea* essential oil (EO) on the *Matricaria chamomilla*, *Leucanthemum vulgare*, and *Trifolium repens* germination process, as well as its toxicological and antimicrobial profile. Our results showed that *S. sclarea* EO, particularly at higher concentrations, affected the germination and growth parameters of the tested species, with *T. repens* being the most sensitive. A statistically significant increase in the value of the mitotic index was observed for all three tested concentrations of *S. sclarea* EO compared to the control group. A significant increase in the number of acentric fragments at between 10 and 30 µg/mL of *S. sclarea* EO compared to the control group, as well as an increase in the frequency of aneuploidy at between 10 µg/mL of *S. sclarea* EO compared to the control was detected. Minimum inhibitory concentration of EO was 500 µL/mL for all tested bacteria. Impact on the bacterial biofilm formation was species-specific and concentration dependent. Our findings suggest that *S. sclarea* EO possesses certain allelopathic, proliferative, genotoxic, and antibacterial potential.

Keywords: allelopathy; antibacterial potential; bacterial biofilms; toxicological effects.

Introduction

Weeds are a serious problem in agricultural production today (Kubiak et al., 2022) due to crop losses. Synthetic herbicides provide good results in weed control, but they harm human health and the environment. Namely, their intensive use can lead to the development of herbicide-resistant weeds, soil erosion, and soil and groundwater contamination (Parven et al., 2025). Therefore, weed management is a key factor in most agricultural systems, and research on alternative approaches to complement chemical weed management is currently intensifying (Kubiak et al., 2022). Using natural compounds that have allelopathic effects on weeds is an alternative to overcome human health and environmental problems (Khamare et al., 2022). Allelopathy involves chemical interactions between two plants as well as chemical communication between plants and other organisms, including bacteria, insects and mammals (Šućur et al., 2015). Allelochemicals are products of secondary metabolism in plants and can affect the growth and development of organisms. Allelochemicals involved in plant-plant interactions are represented by phenolic compounds (simple phenols, flavonoids, coumarins and quinones), terpenoids (monoterpenes, sesquiterpenes, diterpenes, triterpenes and steroids), alkaloids and nitrogen-containing chemicals (non-protein amino acids, benzoxazinoids, cyanogenic glycosides) and many other chemical groups (Macías et al., 2019). Most of these allelochemicals inhibit seed germination or seedling growth, although some also exhibit stimulatory effects, such as strigolactones, which trigger seed germination in root-parasitic plants. However, allelochemicals involved in plant-microbe or plant-insect interactions are categorized as molecular signals for establishing symbiotic relationships or plant defence (Kong et al., 2019).

Bacterial antimicrobial resistance (AMR) is one of the major threats to global health, and urgent actions are essential to combat this issue (Ho et al., 2025). Microbial biofilms are polymer-dipped cell communities responsible for several chronic diseases, characterized by extremely high resistance to antimicrobial agents. To overcome these issues, natural plant compounds are in the focus of research due to their antimicrobial and antibiofilm potential, and they are continuously evaluated as an accessory or alternative to conventional antibiotics (Kuźma et al., 2007).

Salvia sclarea L. (Lamiaceae), known as clary sage, is native to Southern Europe. Today it is cultivated worldwide for commercial purposes (Kačániová et al., 2023) because it is a rich source of essential oils (EOs), which have found wide application in aromatherapy (as a relaxant for stress, asthma and digestive problems), in medical and pharmaceutical purposes (antimicrobial, antioxidant, antimutagenic, anticancer, anti-inflammatory, etc.) (Aćimović et al., 2022), in the food industry (flavouring agent) (Schmiderer et al., 2008). Oxygenated monoterpenes (linalool acetate, linalool), sesquiterpene hydrocarbon ((E)-caryophyllene), monoterpene hydrocarbon (p-cymene) and monoterpene ester (geranyl acetate) are the main compounds identified in the *S. sclarea* EO (Kačániová et al., 2023).

In this study, *S. sclarea* EO was tested for allelopathic activity against three weed species, cytogenotoxicity in peripheral human lymphocytes, as well as for antibacterial and antibiofilm activity.

Materials and methods

The seeds of chamomile (*Matricaria chamomilla* L.), daisy (*Leucanthemum vulgare* Lam.) and white clover (*Trifolium repens* L.), commercially produced (manufacturer S.i.p.a.s, Via Emilia 1810/A, Longiano (FC), Italy) were used.

The clary sage (*Salvia sclarea* L.) EO was commercially produced and purchased from the manufacturer Biohalilović d.o.o., Obala 27, 71380 Ilijaš BiH.

Phytotoxicity analysis. The EO of *S. sclarea* was evaluated at three concentrations: 10, 20, and 30 µg/ml. The stock solution of the EO was prepared by diluting the oil in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). This stock solution was then diluted with distilled water to achieve the final concentration for testing. Chamomile, daisy, and white clover seeds (25 seeds per Petri dish) were cultivated for seven days in a climate chamber at 24 °C and 16 h photoperiod. The seeds were placed on two layers of filter paper and treated with 5 ml of the clary sage EO solution at each of the three different concentrations. The experiment included four repli-

cates for each oil concentration, along with four control replicates containing distilled water. Seeds were considered to have germinated when the radicle appeared (2 mm). The germination index (AOSA, 1983), percentage of seed germination (Scott et al., 1984), percentage of germination inhibition (Cayuela et al., 2007), and phytotoxicity (Rusan et al., 2015) were determined. Phytotoxicity values range from 0 to 1, with a lower value indicating a stimulatory effect of the applied EO and a higher value indicating toxic effects (Rusan et al., 2015).

Chromosome aberration analysis. Microscopic preparations for the cytogenotoxicity testing of *S. sclarea* EO were obtained using a modified lymphocyte cultivation method according to Moorhead et al. (1960). The method involved the cultivation of 0.5 mL of whole peripheral blood in a complete RPMI-based medium containing fetal bovine serum, L-glutamine, and phytohemagglutinin (GIBCO™ PB-MAX™ Karyotyping Medium, Invitrogen, California, USA). Peripheral blood lymphocytes were incubated for 72 hours at 37 °C. After 48 hours of incubation, the cultures were treated with *S. sclarea* EO at three test concentrations: 10, 20 and 30 μg/mL. The cultures were then incubated for the remaining 24 hours. In parallel, cultures containing untreated peripheral blood (control group) were established. For each test concentration and control group, two replicates were performed. Two hours before the end of the incubation period, ColcemidTM solution (Merck KGaA, Darmstadt, Germany, CAS number: 477-30-5) was added, which allowed cytogenetic analysis of metaphase karyotypes. The incubation period was followed by the treatment with a hypotonic solution (0.075 M KCl). The fixation was carried out by adding methanol/glacial acetic acid fixative (3:1 v/v). The final step included spreading the cell content onto clean and cooled glass slides, which were then stained in a 10% Giemsa, washed and air-dried.

Cytogenetic analysis included the examination of a total of two preparations (two replicates) for each of the tested concentrations of *S. sclarea* EO, as well as the control group. Assessment of the mitotic index (MI) values was performed by determining the frequency of mitotic cells in a population of 1000 cells per preparation (replicate), i.e. a total of 2000 cells per each test concentration and the control group, ignoring broken and clumped cells, as well as cellular debris (Muehlbauer & Schuler, 2003). An examination of the morphology and number of chromosomes was conducted in 100 metaphases per replicate (200 metaphases per test concentration and control).

Assessment of the antimicrobial and antibiofilm activity. The antimicrobial capacity of *S. sclarea* EO was evaluated by determining the minimum inhibitory concentration (MIC) in the broth microdilution method and the antibiofilm activity through the tissue culture plate (TCP) method. The investigation encompassed eight Gram-positive and Gram-negative bacteria, including the multidrug-resistant (MDR) strains. Tested Gram-positive strains were: *Bacillus spizizenii* ATCC 6633, *Staphylococcus aureus* NCTC 12393, *S. aureus* ATCC 25923, and *S. aureus* ATCC 33591 (methicillin-resistant *S. aureus*, MRSA); while Gram-negative strains were: *Esherichia coli* ATCC 14169, *Esherichia coli* ATCC 25922, *Salmonella abony* NCTC 6017, and *Klebsiella pneumoniae* ATCC BAA-1705 (carbapenemase producer, KPC). The bacterial strains are obtained from the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC). Inoculums were prepared following the EUCAST's recommendation (2017). After the overnight incubation in Mueller Hinton medium (Sigma Aldrich) at 37 ºC strains were dissolved in sterile saline solution to the final turbidity of 0.5 McFarland standard, corresponding with the bacterial cell concentration of 1.5×10^8 CFU/mL.

The broth microdilution method was used to determine the tested EO minimum inhibitory concentration, MIC (CLSI, 2018). The investigated EO was dissolved in 1% dimethyl sulfoxide ≥99% (DMSO) (Sigma Aldrich) to the final concentration of 1000 μL/mL. The amount of 100 μL of two-fold dilutions of EO in the range of 1000 to 1.95 μL/mL was added to a 96-well microtiter plate with the Mueller Hinton Broth (Sigma Aldrich). After that, the wells were inoculated with 10 μL of bacterial suspension. The pure bacterial culture was taken as the positive control, while the negative control was the uninoculated media. Experiments were performed in four replications, and the results were read after overnight incubation on the microplate reader (BioTek Epoch Microplate Spectrophotometer) at the wavelength of 595 nm.

The impact of the essential oil on bacterial biofilm formation was assessed through the TCP method in 96 well plates (Merritt et al., 2005) with tryptic soy broth, TSB (Sigma Aldrich) as the diluting medium. The initial concentration of investigated EO (1000 μL/mL) was twofold diluted in TSB to an end concentration of 1.95 μL/mL. The amount of 100 μL of such dilutions was added to each well, followed by the inoculation with 10 μL of the bacterial inoculum. The growth medium with bacteria was taken as the positive control, while the uninoculated TSB was used as the negative control. For the determination of biofilm formation, the adherence of bacteria only in the presence of TSB was used. After the overnight incubation, the content of the plates was decanted, the plates were washed in Phosphate Buffered Saline, PBS (Sigma Aldrich), and stained with 0.1% crystal violet solution for 10 minutes. Upon washing, each well was filled with 96% ethanol, and the results were read on the microplate reader (BioTek Epoch Microplate Spectrophotometer) at 595 nm. This procedure was performed in quadruplets, and results are given as mean values ± standard deviation (SD). According to Stepanović et al. (2007), the biofilm-forming category was determined. The optical density cut-off value (ODc) was calculated as three standard deviations above the mean OD of the negative control, while the biofilm-forming categories were determined as follows: $OD \leq ODc$: non-adherent, $ODc < OD \leq 2 \times ODc$: weakly adherent, $2 \times ODc < OD \leq 4 \times ODc$: moderately adherent, and $4 \times ODc$. The percentage of biofilm inhibition achieved by the activity of tested EO was calculated according to Jadhav et al. (2013).

Statistical analysis. Means and standard deviations were calculated for all analysed parameters. Determination of the presence of statistically significant differences in the frequency of the analysed markers between the tested substance (*S. sclarea* EO) and the control group was carried out using a one-way ANOVA test with a post-hoc multiple comparison test (LSD). Differences were considered statistically significant at $P \leq 0.05$ level.

Results

Phytotoxic effect of S. sclarea EO. The germination index of the tested plant species was affected by *S. sclarea* EO (Table 1). The inhibitory effect of EO increased with increasing concentration. The inhibition rates of the essential oil on the germination index of *L. vulgare* and *M. chamomilla* (except at the highest concentration) were generally lower than those of *T. repens*, where all three concentrations had a significantly strong effect. The results show that the highest germination percentage value for all three tested species was recorded in the control treatments. With increasing concentration of *S. sclarea* essential oil, the germination percentage decreased in all three tested species, and the lowest germination percentage was recorded in *T. repens* seeds at an essential oil concentration of 20 μg/mL (66.7%). Higher values of the percentage of inhibition indicate a higher allelopathic effect of the analysed treatment, i.e. the obtained values of the percentage of inhibition are directly proportional to the allelopathic effect of the given treatment. In *T. repens*, germination inhibition of 26.2% was recorded with the application of 20 μL and 23.6% with the application of 30 μL of essential oil (Table 1). Clary sage essential oil did not show a significant rate of inhibition of the germination of *L. vulgare*, where the lowest values were recorded. *L. vulgare* was least inhibited by treatment with 20 μL of sage essential oil. The obtained phytotoxicity index results were lower than 0 for *T. repens* and positive for *M. chamomilla* and *L. vulgare* (Table 1).

Cytogenotoxic effects of S. sclarea EO in human lymphocytes. The results of the cytotoxic and the genotoxic effects of *S. sclarea* EO in human peripheral blood lymphocytes are summarized in Table 2. A statistically significant increase in the value of the mitotic index (cytotoxic effect) was observed for all three tested concentrations of *S. sclarea* EO compared to the control group.

Table 1
Effect of different concentrations of *S. sclarea* EO on germination index, germination percentage, germination inhibition and phytotoxicity index of the tested plant species (n = 4, x ± SD)

Plant species	EO concentration, μg/mL	Index of germination	Germination, %	Germination inhibition, %	Phytotoxic index
M. chamomilla	0	22.46 ± 3.29	92.00 ± 7.30	–	–
	10	19.61 ± 4.58	83.00 ± 16.12	10.00 ± 5.49	0.02 ± 0.02
	20	15.23 ± 3.23	72.00 ± 16.97	21.98 ± 6.99	–0.34 ± 0.30
	30	14.91 ± 0.94*	76.00 ± 7.30	17.00 ± 1.32	–0.22 ± 0.08
L. vulgare	0	12.59 ± 0.59	96.00 ± 3.27	–	–
	10	10.60 ± 1.64	81.00 ± 6.00	15.56 ± 6.74	–1.05 ± 0.16
	20	11.08 ± 1.50	87.00 ± 6.83	9.39 ± 3.27	–0.78 ± 0.15
	30	10.05 ± 1.19	83.00 ± 6.00	13.43 ± 3.39	–0.82 ± 0.20
T. repens	0	22.34 ± 1.61	91.00 ± 8.25	–	–
	10	12.66 ± 2.88*	79.00 ± 7.57	13.08 ± 6.16	0.18 ± 0.00
	20	10.18 ± 4.41*	66.67 ± 24.44	26.22 ± 4.68	0.15 ± 0.04
	30	9.40 ± 2.50*	68.00 ± 19.04	23.63 ± 6.06	0.08 ± 0.02

Note: * – statistically significant differences as compared to the control group ($P \leq 0.05$).

As for the results of the genotoxic effects, a significant increase in the frequency of acentric fragments was noted in the test concentrations of *S. sclarea* EO (10 and 30 μg/mL) compared to the control group, as well as an increase in the frequency of aneuploidy in the test concentration of *S. sclarea* EO (10 μg/mL) compared to the control. Furthermore, the frequency of polyploid cells increased in all three tested concentrations of *S. sclarea* EO in comparison with the control group, but this increase was not statistically significant.

Table 2
The results of cytotoxic and genotoxic effects in human lymphocytes treated with different concentrations of *S. sclarea* EO (n = 4, x ± SD)

EO Concentration, μg/mL	Cytotoxic effects	Genotoxic effects		
	mitotic index, %	acentric fragments	aneuploidy	polyploidy
Control group	56.55 ± 0.65	4.50 ± 0.70	5.00 ± 1.41	0.50 ± 0.70
10	76.20 ± 0.35*	17.00 ± 7.07*	17.50 ± 7.77*	3.00 ± 1.41
20	82.95 ± 0.25*	11.50 ± 2.12	11.50 ± 2.12	1.50 ± 2.12
30	91.60 ± 0.40*	15.50 ± 2.12*	15.50 ± 2.12	2.50 ± 0.70

Notes: see Table 1.

Determination of the minimum inhibitory concentration and evaluation of the impact on bacterial biofilms. The minimum inhibitory concentration of *S. sclarea* EO was determined at 500 μL/mL for all tested bacteria, including the MDR strains of MRSA and KPC *K. pneumoniae*.

To evaluate the impact of tested EO on bacterial biofilm formation, the first step included determining the biofilm-forming category for all investigated strains. The category of bacteria with moderately adherent biofilm formations included *S. aureus* NCTC 12393 (SA1) and *S. aureus* ATCC 33591 (MRSA); weakly adherent biofilms were observed in the case of *B. spizizenii* ATCC 6633 (BS), *S. abony* NCTC 6017 (SAb), and *K. pneumoniae* ATCC BAA-1705 (KP); while *S. aureus* ATCC 25923 (SA2), *E. coli* ATCC 14169 (EC1), and *E. coli* ATCC 25922 (EC2) did not form biofilms in this experiment. Mean absorbance values (MAV) for positive and negative controls are presented as part of Table 3. The percentage of the biofilm inhibition was calculated at the concentration that caused a decrease in the biofilm-forming category or biofilm elimination.

Table 3
Absorbance values (mean ± standard deviation) in the assessment of the activity of *S. sclarea* EO on tested bacteria

Bacteria	Subinhibitory concentrations of *S. sclarea* EO, μL/mL							
	250.00	125.00	62.50	31.25	15.62	7.81	3.90	1.95
SA1	0.442 ± 0.037	0.546 ± 0.025	0.451 ± 0.034	0.483 ± 0.048	0.458 ± 0.058	0.444 ± 0.062	0.433 ± 0.050	0.442 ± 0.094
MRSA	0.394 ± 0.051	0.342 ± 0.042	0.330 ± 0.015	0.311 ± 0.023	0.350 ± 0.033	0.313 ± 0.032	0.281 ± 0.027	0.318 ± 0.067
BS	0.315 ± 0.015	0.124 ± 0.017	0.115 ± 0.007	0.105 ± 0.013	0.109 ± 0.012	0.104 ± 0.008	0.101 ± 0.009	0.098 ± 0.006
SAb	0.088 ± 0.010	0.078 ± 0.009	0.099 ± 0.008	0.106 ± 0.011	0.089 ± 0.010	0.087 ± 0.008	0.089 ± 0.005	0.087 ± 0.008
KP	0.166 ± 0.010	0.166 ± 0.080	0.163 ± 0.015	0.166 ± 0.015	0.162 ± 0.010	0.162 ± 0.014	0.157 ± 0.016	0.151 ± 0.002
SA2	0.182 ± 0.021	0.198 ± 0.003	0.196 ± 0.003	0.193 ± 0.005	0.197 ± 0.005	0.190 ± 0.011	0.198 ± 0.009	0.197 ± 0.011
EC1	0.161 ± 0.014	0.195 ± 0.021	0.211 ± 0.019	0.186 ± 0.018	0.171 ± 0.039	0.152 ± 0.032	0.161 ± 0.026	0.135 ± 0.030
EC2	0.075 ± 0.012	0.078 ± 0.007	0.075 ± 0.004	0.073 ± 0.006	0.077 ± 0.010	0.071 ± 0.006	0.072 ± 0.003	0.082 ± 0.009

Notes: SA1: *Staphylococcus aureus* NCTC 12393; MRSA: *Staphylococcus aureus* ATCC 33591; BS: *Bacillus spizizenii* ATCC 6633; SAb: *Salmonella abony* NCTC 6017; KP: *Klebsiella pneumoniae* ATCC BAA-1705; SA2: *Staphylococcus aureus* ATCC 25923; EC1: *Escherichia coli* ATCC 14169; EC2: *Escherichia coli* ATCC 25922. MAV ± STDEV (positive/negative control), SA1: 0.334 ± 0.041 / 0.080 ± 0.003; MRSA: 0.279 ± 0.043 / 0.075 ± 0.004; BS: 0.159 ± 0.018 / 0.104 ± 0.010; SAb: 0.129 ± 0.016 / 0.082 ± 0.010; KP: 0.136 ± 0.010 / 0.072 ± 0.004; SA2: 0.180 ± 0.003 / 0.105 ± 0.031; EC1: 0.250 ± 0.026 / 0.129 ± 0.030; EC2: 0.070 ± 0.000 / 0.078 ± 0.008. Bold values: biofilm elimination.

In the case of *B. spizizenii* ATCC 6633, positive controls showed weakly adherent biofilm, which was eliminated in most of the tested subinhibitory concentrations of EO. The percentage of biofilm inhibition was calculated for this strain, and the values ranged from 15.1% to 38.2%. Weakly adherent biofilm was also observed in *S. abony* NCTC 6017, and it was eliminated in all established subinhibitory concentrations. The inhibition percentage for *S. abony* biofilm formation by *S. sclarea* EO was 18.0% to 40.0%. MRSA strain formed moderately adherent biofilm in all replications of the positive control, which remained in that category up to the lowest subinhibitory concentration of EO (1.95 μL/mL). *K. pneumoniae* ATCC BAA-1705 formed a weakly adherent biofilm, and tested subinhibitory dilutions did not cause the change in the biofilm-forming category. *S. aureus* NCTC 12393 formed moderately adherent biofilm, but tested subinhibitory concentrations of EO induced the forming of the strongly adherent biofilm, which is observed through the increasing of the mean absorbance values (Table 3). Non-biofilm-forming strains in this experiment (*S. aureus* ATCC 25923, *E. coli* ATCC, and *E. coli* ATCC 25922) were not affected by the *S. sclarea* EO in terms of changing the biofilm-forming capacity.

Discussion

Interest in biological control methods has increased in recent years due to environmental pollution caused by chemical action and

herbicide-resistant weeds. Synthetic herbicides are slowly degraded (Katole et al., 2013), which is an additional problem, so in recent years, research has focused on natural ways to control their use (Bhadoria, 2011). Essential oils have proven to be very good in this regard because they are easily extracted, used in small quantities and often degrade quickly (Isman, 2000). They also cause fewer side effects than synthetic herbicides (Ohkawa et al., 2007). It has been observed that secondary metabolites are responsible for the allelopathic effect of essential oils. The main compounds identified in the essential oil of *S. sclarea* were oxygenated monoterpenes (linalool acetate, linalool), sesquiterpene hydrocarbon ((E)-caryophyllene), monoterpene hydrocarbon (p-cymene), and monoterpene ester (geranyl acetate) (Kačániová et al., 2023).

According to available literature, there are not many reports on the use of the allelopathic effect of *S. sclarea* on seed germination. We found only a few reports on the allelopathic effect of this oil. Bozok & Ulukanli (2016) observed that the essential oil of *S. sclarea* has dose-dependent significant phytotoxic effects against *Lepidium sativum*, *Lactuca sativa* and *Portulaca oleracea*. At the highest applied concentration, seed germination was inhibited from 50% in *P. oleracea* to 100% in *L. sativum*. Also, the oil inhibited the length of radicle and plumule by 100% in *L. sativum* to 81.0% in *P. oleracea*. Similar results were obtained for fresh and dry weight. Following these results, *S. sclarea* can be used as a pesticide of biological origin. This effect is attributed to the presence of terpenoid compounds (Bozok & Ulukanli, 2016; Kačániová et al., 2023). Linalool and borneol have also been found to be the main compounds in several essential oils, such as *S. sclarea* (Bozok & Ulukanli, 2016), *Origanum acutidens* (Kordali et al., 2008), *Nepeta flavida* (Bozok, 2018), *Salvia officinalis*, and *Ocimum basilicum* (De Almeida et al., 2010), which are all members of the Lamiaceae family. Accordingly, it is clear that the oxygenated monoterpenoids 1,8-cineole, linalool and borneol play a very important role as allopathic agents, and more research into their phytotoxic activity and phytotoxic mechanisms is recommended.

Šućur et al. (2015) observed that a lower concentration of *S. sclarea* aqueous extract causes lipid peroxidation in *Solanum nigrum*, however, the two applied concentrations (7 and 14 mL) did not show a phytotoxic effect on pepper and nightshade seedlings, but showed a toxic effect on greenhouse whitefly, with a mortality rate of 56.7%. In accordance with this, the authors concluded that *S. sclarea* can be investigated in the development of bioinsecticides and used for insect control.

Our results show that germination was influenced by *S. sclarea* EO. Increasing the essential oil concentration resulted in a slight decrease in the germination percentage and germination index. According to Luo et al. (2018), a substance is considered non-phytotoxic when the germination percentage is ≥ 83%, which in this case refers to an oil concentration of 10 µg/ml in *M. chamomilla* and concentrations of 20 and 30 in *L. vulgare*. On the other hand, the growth of the seedlings was not influenced by the essential oil. The highest phytotoxicity index was recorded in *T. repens* treated with concentrations of 10 and 20 µg/mL and was 0.18 and 0.15, respectively. The phytotoxic effect of EOs from various members of the Lamiaceae family has been previously confirmed with lower inhibition of germination (Cruz-Silva et al., 2017; Verdeguer et al., 2020; Yaman et al., 2024). Cruz-Silva et al. (2017) observed that sage oil in the concentrations tested in the study did not affect the germination of *Lycopersicum esculentum*, *Panicum maximum*, and *Salvia hispanica*. The allelopathic effect of the essential oil on seed germination may be absent, but it may affect other parameters, such as GSI and/or plant development (Ferreira, 2004).

The results of this work indicate that *S. sclarea* EO shows a certain proliferative potential. Analysis of the mitotic index revealed an increase in the value of the mitotic index with an increase in the tested concentration of *S. sclarea* EO. Likewise, the mitotic index values were significantly different between each of the three tested concentrations of *S. sclarea* EO and the control group. The mitotic index, as a marker of cytotoxicity, can also be used to assess the rate of cell proliferation. Namely, cell proliferation is closely related to the assessment of the cytotoxicity of the chemical and physical agents being studied. Cells with more chromosomal damage will either succumb to death before cell division or are less likely to enter this phase of cell growth (Purnami et al., 2020). Based on the all above, it is legitimate to suggest that *S. sclarea* EO shows a significant proliferative effect on human lymphocytes.

Regarding the genotoxic effects of *S. sclarea* EO, significant differences in the frequency of acentric fragments between *S. sclarea* EO (10 and 30 µg/mL) and control, as well as in the frequency of aneuploidy between *S. sclarea* EO (10 µg/mL) and control group were found. A recent study demonstrated that *S. fruticosa* leaf extract increased sister chromatid exchange, as well as the frequency of chromosomal aberrations and micronuclei in human peripheral lymphocytes (Sevindik & Rencuzogullari, 2013). However, it has been demonstrated that *S. sclarea* EO did not induce significant DNA damage in HEL 12469 cells *in vtro* (Puškárová et al., 2017). Acentric fragments represent independent chromosome fragments without centromeres that arise as a result of chromosome breakage accompanied by subsequent deletion (Katavic et al., 2023). Acentric fragments represent good biomarkers for disease prognosis in humans and their advantage is reflected in the possibility of separate observation from all other centromeric chromosomes (Bonassi et al., 2004). The connection between chromosomal aberrations and cancer risk is based on the thesis that genetic damage in lymphocytes is reflected in a very similar way as damage in cancer cells (Handa et al., 2006). One study described that cancer risk can be predicted based on the frequency of chromosomal aberrations in peripheral blood lymphocytes of healthy individuals (Güzel Bayülken & Ayaz Tüylü, 2019). A recent study showed that acentric fragments are associated with an increased risk of cancer in humans exposed to ionizing radiation (Fucic et al., 2016). Additionally, Hille et al. (2010) demonstrated that individuals suffering from prostate cancer have increased spontaneous chromosomal instability, where the number of acentric fragments was significantly increased. All the above mentioned suggests that *S. sclarea* EO, through increase in the frequency of acentric fragments, could influence the onset or development of the human malignant phenotype. Numerical aberrations of chromosomes, which are designated as aneuploidies, represent a major contributor to the development and progression of cancer (Sdeor et al., 2024). Genomic instability is a hallmark of cancer cells that facilitates the acquisition of mutations that confer aggressive or drug-resistant phenotypes during cancer evolution. Chromosomal instability is a form of genomic instability that involves frequent cytogenetic changes that lead to changes in the number of chromosome copies (aneuploidy). In fact, aneuploidy, chromosomal instability, and genomic instability together promote a vicious cycle of genomic chaos (Giam & Rancati, 2015). It has been proposed that the transition from a stable diploid to an unstable aneuploid cell is the primary cause of genomic instability in cancer cells, while the mutations are a secondary. Namely, aneuploidy destabilizes the karyotype, and thus the species, independent from mutations because it disrupts highly conserved groups of proteins that segregate, synthesize and repair chromosomes (Duesberg et al., 2004). Therefore, it is reasonable to assume that *S. sclarea* EO, by causing an increased frequency of aneuploidy, could lead to genomic instability and consequently to negative effects on human health.

The results of the broth microdilution method revealed that all investigated bacterial strains were sensitive to the clary sage EO, with MIC values at 500 µL/mL. The previous investigation of the antibacterial potential of *S. sclarea* EO (Pierozan et al., 2009) observed the activity against several bacteria such as *E. coli*, *K. pneumoniae*, *Salmonella enterica*, *Serratia marescens*, *Shigella flexneri*, *Bacillus subtilis*, *Enterococcus faecalis*, *Micrococcus luteus*, *S. aureus*, and *Streptococcus mutans* with MIC values from 2.87 to 6.93 mg/mL. There are some data about the very strong antibacterial activity of *S. sclarea* EO against various Gram-positive and Gram-negative strains (Kuźma et al., 2009), but in comparison to our study, higher MIC values were observed. In the investigation of Ovidi et al. (2021), *S. sclarea* EO performed lower antibacterial activity in comparison to other tested samples. In our study, Gram-positive and Gram-negative bacteria did not differ in determined MIC values. Kačániová et al. (2023) investigated the antimicrobial activity of *S. sclarea* EO and

reported weak to moderate activity against tested bacteria, except for *B. subtilis*, where strong activity was observed in the disc diffusion method. In further evaluation, MIC values were determined, and they suggested the best antimicrobial activity against *S. aureus* and *Pseudomonas fluorescens*. In research by Ben Akacha et al. (2023), *S. sclarea* EO exhibited bactericidal and bacteriostatic activity, with the Gram-positive strains being more susceptible, probably as the result of the diffusion restriction through the outer membrane of the Gram-negative bacteria and peptidoglycan being less resistant to hydrophobic compounds. Together with the increased cell permeability caused by the hydrophobicity of EO, toxic effects on membrane structure and function are discussed. Average MIC values were in the range of 3.7–11.2 mg/ml. On the contrary, Aćimović et al. (2022) noted that Gram-negative bacteria exhibit more sensitivity to *S. sclarea* EO when compared to Gram-positive bacteria. A possible explanation for the inconsistencies between results could be found in the reports of Cui et al. (2015), where some factors were listed: a difference between species, differences in the extraction methods of EOs, and differences between strains of the same origin, resulting from a long adaptation to the environment, artificial selection and crossbreeding.

Pierozan et al. (2009) reported the chemical characterization of several *Salvia* species EO, including *S. sclarea*, where the major compounds were linalol, linalyl acetate, and α-terpineol, while the thujones, camphor, and 1,8-cineole were detected in low concentrations. Keeping in mind that many components are present in the EO, the possible antibacterial activity of the sample could be the result of synergism, antagonism, or additive effects of the major chemical constituents. According to Kačániová et al. (2023), (E)-caryophyllene could also be related to the antibacterial impacts of clary sage EO. Cui et al. (2015) indicated that *S. sclarea* EO is an effective bacterial inhibitor and bactericide with a broad antibacterial spectrum. This EO can induce harmful effects on the bacterial cell morphology, causing the occurrence of deformed, incomplete, and pitted shapes in treated bacteria. Furthermore, changes in membrane integrity were also observed and correlated with the releasing of the intracellular components, from small ions to macromolecules as DNA. *S. sclarea* EO also reduces the ATP concentration in bacterial cells and possibly acts against bacterial enzymes such as ATPase.

In the evaluation of the impact of *S. sclarea* EO on bacterial biofilms, we noticed species-specific and dose-dependent effects. Literature is scarce regarding this issue. Srivastava et al. (2023) discussed the antimicrobial and anti-quorum sensing activity of *S. sclarea* against *Pseudomonas aeruginosa*, and Kačániová et al. (2023) presented the capacity of the clary sage EO to inhibit *P. fluorescens* biofilm formation. Specific compounds as diterpenoids salvipisone and aethiopinone from the hairy roots of *S. sclarea* were investigated against methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE), and the results show a decreased number of live biofilm cells and changes in the parameters of biofilm morphology (Walencka et al., 2007). In general, *B. subtilis* can form robust biofilms, but many laboratory-derived strains are unable to develop highly-structured biofilms due to the accumulation of mutations in a phenomenon known as "domestication" (Mielich-Süss & Lopez, 2015). In our study, *B. spizizenii* (which is a subspecies of *B. subtilis*) formed a weak biofilm, which was eliminated by the subinhibitory dilutions of tested EO. A similar situation was observed for *S. abony*, where the biofilm was eradicated in EO concentrations below MIC. Although *Salmonella* species are intestinal pathogens, they are characterized by improved survival in non-host environments, with biofilm formation being a major adaptation (Steenackers et al., 2012). In the study of El-Tarabily et al. (2021), essential oils are discussed as promising antimicrobial and antibiofilm agents. Nută et al. (2021) presented several mechanisms of the antibiofilm activity of essential oils, and highlighted the inhibition of the intercellular communication systems; induction of the changes in the substrate; alteration of the cytoplasmic membrane due to the hydrophobic constituents; inactivation of the bacterial enzymes; blockage of the quorum-sense system; inhibition of the transcription of flagellar genes and interference with bacterial motility; increase in oxidative stress, which causes damage to macromolecules and leads to apoptosis; enzymatic alteration etc.

Some of the tested strains in our research were not affected by the *S. sclarea* EO (MRSA and *K. pneumoniae*), and their determined biofilm-forming category was not reduced. Microbial biofilms represent structured communities of bacteria that adhere to surfaces and are surrounded by a matrix of extracellular polymeric substances (EPS). Different components present in a perplexed biofilm structure are responsible for the physiological and structural stability of such formation and are associated with unique traits of microbes involved in the biofilm formation (Saharan et al., 2024). Ultimately, cells in biofilms could have up to 1000 times greater resistance to antimicrobial agents (Mah & O'Toole, 2001). In addition, we noticed that clary sage EO increased the biofilm-forming capacity of *S. aureus* NCTC 12393 in subinhibitory concentrations. There are findings of the increase in biofilm formations after the application of particular plant compounds, explained as the specific response of bacteria to stressful conditions, mostly illustrated through the more matrix production (Abedini et al., 2020).

Conclusions

The results of the present study indicate that *Salvia sclarea* EO exhibit specific allelopathic, proliferative, genotoxic, and antibacterial activity. These findings significantly contribute to the increasing body of evidence regarding the allelopathic, toxicological, and antimicrobial effects of *S. sclarea* EO. Further research is needed to support and expand these findings.

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