Journal of Bioequivalence Studies
Volume 7 | Issue 1
ISSN: 2575-551X

Research Article Open Access

Chemical Characterization, Antimicrobial-Antifungal Activity on Probiotic Micro organisms and Genotoxicity–Cytotoxicity Effects of *Lamiaceae Family* Essential Oils from Different Plants

Cimik A*

Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey

***Corresponding author:** Cimik A, Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey, E-mail: alpercimik@anadolu.edu.tr, a.cimik@hotmail.com

Received Date: April 12, 2021 **Accepted Date:** May 14, 2021 **Published Date:** May 17, 2021

Citation: Cimik A (2021) Chemical Characterization, Antimicrobial - Antifungal Activity on Probiotic Microorganisms and Genotoxicity – Cytotoxicity Effects of Lamiaceae Family Essential Oils from Different Plants. J Bioeq Stud 7(1): 102

Abstract

Pipermint, thyme, rosemary, sage and basil species which have major characteristic specialities of *Lamiaceae* family, have antimicrobial activities on pathogene microorganisms. Probiotic microorganisms have valuable effects on human body and inhibition of probiotics causes many diseases. In this present study, it was aimed to determine indicate probiotic resistance against natural antimicrobial agents (as essential oils) compare to pathogenes in previous studies. Analysis of essential oils (Eos) from were analyzed by GC-FID and GC/MS, analysis of Eos antimicrobial and antifungal activity from were analyzed by Microdilution test. Genotoxicity and cytotoxicity results were found on *Salmonella typimurium* T4 and *Allivibrio fischeri* ATCC 7744 with Ames test. Menthole (%43.56), Menthole (%47.48), Carvacrol (%66.49), 1,8-Sineol (%46.29), Camphora (%30.52), Linalyl acetate (%62.63) and Thymol (%39.52) were found as major compounds of EOs respectively. All essential oils have antimicrobial activity without *Salvia sclarea* essential oil and also Mentha piperita, Ocimum basilicum and Satureja montana essential oils have antifungal activities on probiotic microorganisms. All essential oils were found non-mutagenic and non-cytotoxic.

Keywords: Essential oil; *Lamiaceae*; Antimicrobial; Antifungal; Genotoxicity; Cytotoxicity

Introduction

Probiotic microorganisms are valuable resources fort he all humanity from 17th century. Before Alexander Ian Fleming found antibiotic which is "penicilin" from *Penicilium notatum* French Sciencist Luis Pasteur had isolated lactic acid from Lactobacillus sp. microorganisms. Not only isolation of lactic acid but also identification of first microorganisms is so important in this period. Because "Antibiotic Age" was created by Lactobacillus sp. probiotics. As known in the medicinal science probiotics are using in Alzheimer disease (Lactobacillus acidophilus), Parkinson's Disease (Lactobacillus plantarum, Lactobacillus casei), inflammatory bowel diseases (Example: Chron's Disease and *Clostridium difficile* diseases) and immunmodulatory system. This microorganisms also more resistant than the other patogen microorganisms and fungal microorganisms. In this experiment series you can see which microorganism is more resistant and use them wisely on your health. Don't worry, be healty with "The Power of Nature".

Lamiaceae family, also known as the mint family. Plants in this family, are herbs or shrubs often with an aromatic smell. They are common in Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Some examples from this family include basils, mints, rosemarys, sages, savorys and thymes [1].

Herbs and spices have been used since ancient times to improve the sensory characteristics of food, to act as preservatives and for their nutritional and healthy properties. Herbs and spices are generally recognized as safe (GRAS) and are excellent substitutes for chemical additives. Essential oils are mixtures of volatile compounds obtained, mainly by steam distillation, from medicinal and aromatic plants (Table 1). They are an alternative to synthetic additives for the food industry, and they have gained attention as potential sources for natural food preservatives due to the growing interest in the development of safe, effective, natural food preservation. *Lamiaceae* is one of the most important families in the production of essential oils with antioxidants and antimicrobial properties. Aromatic plants are rich in essential oils and are mainly found in the Mediterranean region, where the production of such oils is a profitable source of ecological and economic development. The use of essential oils with antimicrobial and antioxidant properties to increase the shelf life of food is a promising technology, and the essential oils of the Lamiaceae family, such as rosemary, thyme, and sage, have been extensively studied with respect to their use as food preservatives. Regarding the new applications of essential oils, this review gives an overview of the current knowledge and recent trends in the use of these oils from aromatic plants as antimicrobials and antioxidants in foods, as well as their biological activities, future potential, and challenges [2].

Essential oil	Inhibited Pathogene Microorganisms
Mentha arvensis L.	*Pseudomonas aeruginosa* ATCC27853, *Proteus mirabilis* NCIM2241, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC11778, *Alcaligenes faecalis* ATCC8750 [3]
Mentha piperita L.	*Candida albicans MMA (8 mg/L), Escherichia coli* ATTC 35 218 (19.4 mg/L), *Escherichia coli* ATTC 25 922 (19 mg/L), *Salmonella enteritidis* IPH (17.9 mg/L), *Sarcina lutea* ATTC 9 341 (14.4 mg/L), *Staphylococcus epidermidis* ATTC 12 228 (19.2 mg/L) and *Bacillus cereus* ATTC 10 707 (17 mg/L) [4]
Origanum vulgare L.	*Staphylococcus aureus* CCT2740 (1 mg/mL), *Enterococcus faecium* (0,40 mg/mL), *Salmonella choleraesuis* CCT4296 (0.60 mg/ml), *Candida albicans* ATCC 10231 (2 mg/L) [5]
Rosmarinus officinalis L.	*Escherichia coli* ATCC 11775 (1.85 mg/ml), *Bacillus subtilis* ATCC 6633 (2.15 mg/mL), *Pseudomonas aeruginosa* ATCC 10145 (1.85 mg/ml), *Staphylococcus aureus* ATCC 25923 (0.5 mg/mL), *Candida albicans* ATCC 60193 (1.75 mg/mL), *Aspergillus niger* ATCC 16404 (2.25 mg/mL) [6]
Salvia lavandifolia L.	*Bacillus subtilis* ATCC 6633 (3.42 mg/mL), *Enterococcus faecalis* ATCC 19433 (6.93 mg/mL), *Micrococcus luteus* ATCC 10240 (4.62 mg/mL), *Staphylococcus aureus* ATCC 6538 (6.93 mg/mL), *Streptococcus mutans* ATCC 25175 (9.25 mg/mL), *Escherichia coli* ATCC 25922 (3.42 mg/mL), *Klebsiella pneumoniae* ATCC 13883 (2.87 mg/mL), *Salmonella choleraesuis* ATCC 10708 (2.31 mg/mL),, *Serratia marcescens* ATCC 13880 (2.87 mg/mL), *Shigella flexneri* ATCC 12022 (3.21 mg/mL) [7]

Salvia sclarea L.	*Escherichia coli* ATCC 25922 (0,05 mg/L), *Staphyloccocus aureus* ATCC 25923 (0.05 mg/L), *Bacillus pumilus* ATCC 27142 (0.05 mg/L), *Klebsiella pneumonia* ATCC 13883 (0.05 mg/L), *Bacillus subtilis* IFO 3457 (0.05 mg/L), *Salmonella typhi*murium B11 (0.05 mg/L), *Pseudomonas aeruginosa* ATCC 27853 (0.05 mg/L) [8]
Salvia triloba L.	on *Bacillus subtilis* ATCC 6633 (6.93 mg/mL), *Enterococcus faecalis* ATCC 19433 (6.93 mg/mL), *Micrococcus luteus* ATCC 10240 (9.32 mg/mL), *Staphylococcus aureus* ATCC 6538 (9.32 mg/mL), *Streptococcus mutans* ATCC 25175 (3.42 mg/mL), *Escherichia coli* ATCC 25922 (3.42 mg/mL), *Klebsiella pneumoniae* ATCC 13883 (4.62 mg/mL), *Salmonella choleraesuis* ATCC 10708 (3.42 mg/mL),, *Serratia marcescens* ATCC 13880 (3.42 mg/mL), *Shigella flexneri* ATCC 12022 (3.42 mg/mL) [7]
Satureja montana L.	*S. aureus* ATCC 25923 (0.78 mg/L), *S. aureus ATCC P6538* (0.38 mg/L), *S. aureus* PGSA (0.78 mg/L), *E.coli* ATCC25922 (1.56 mg/L), *E.coli* PG19 (3.12 mg/L) *E.coli* PG32 (1.56 mg/L), *L. monocytogenes* ATCC 7644 (1.56 mg/L) , *L. monocytogenes* LM2 (3.12 mg/L), *L. monocytogenes* LM9 (3.12 mg/L) [9]
Ocimum basilicum L.	*Staphylococcus aureus* CCT2740 (0.70 mg/mL), *Enterococcus faecium* (1 mg/mL), *Salmonella choleraesuis* CCT4296 (2 mg/ml), *Candida albicans* ATCC 10231 (2 mg/L) [10]

Table 1: Antimicrobial Activity of Investigated Lamiaceae Essential oils on Pathogene Microorganisms in Previous Studies

Materials and Methods

Plant material

Pharmacopeae essential oils were used as standarts of Lamiaceae plants. EOs were selected from Anadolu University, Faculty of Pharmacy, Pharmacognosy Research Laboratory essential oil collection.

Microorganisms were bought from Christian Hansen©.

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MS system. Innowax FSC column (60 m, 0.25 mm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C /min, and kept constant at 220 °C for 10 min. Then, programmed to 240 °C at a rate of 1 °C /min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

The GC analysis was carried out using an agilent GC system. FID detector temperature was 300 °C to obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components

Characterization of the essential oil components was carried out by comparison of their retention times with those of authentic samples or by comparison of their Linear Retention Indices (LRI) to a series of n-alkanes. Computer matching against commercial Wiley GC/MS library (MacLafferty and Stauffer, 1989), MassFinder 3 Library (Koenig et al., 2004) and in house "Baser Library of

Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain and Koenig, 1998: ESO, 2000) was used for the identification.

Antimicrobial and Antifungal activities with Microdilution methods

This technique helps to determine MIC (minimal inhibitory concentration) and MLK (minimal lethal concentration) values of antimicrobial drugs. For this purpose, 2 or 10-fold dilutions of antimicrobial drug in Mueller-Hinton Broth are made and dilutions of dense concentrations of drugs are obtained. Ex. drug 256, 128, 64, 32, 16, 8, starting at 256 32g in 1 ml. 4, 2, 1, 0.5, 0.25, 0.12 /g / mL are gradually diluted in three layers. The isolated test is seeded in 100 µL of the 24-48 hours liquid culture culture of the microorganism and incubated at 37 °C for 24-48 hours. The reproduction in the tubes is evaluated by the eye. Thus, the final dilution without reproduction is accepted as MIC value. However, in order to be precise, it is appropriate to perform the test in three parallel. The average of the most recent results is the MIC or MLK obtained [11,12]. In this study we calculated MIC values as other MIC studies in the literature.

Ames Test (Mutagenity Test)

Ames test is the one of the *Salmonella sp.* mutagenity test and this test is frequently used in the detection of point mutations that occur in tumor suppressor genes in human and experimental animals in tumor formation and in the detection of antimutagenic and anticarcinogenic substances that eliminate the mutagenic and carcinogenic effects of chemicals by preventing the interaction of chemicals with DNA. In the Ames test, mutant strains of *Salmonella typhimurium* which contain various mutations in different regions of the histidine operon are used. The basis of this test is that the histidine synthesized strains of *S. typhymurium* have lost their ability to synthesize histidine (for example: *S. typhymurium* T4). Is based on the multiplication of the independent environment. Mutagenicity is determined by counting the spontaneously mutated colonies that cause them to reproduce in the histidine-free medium. If there is a positive mutagenic substance in the environment, the number of bacterial colonies proliferating back mutations increases statistically [13].

Allivibrio fischeri Cytotoxicity Test

In this test, standards are applied starting from a concentration of 256 µg in 1mL on the microorganism as the microdilution method of the same antibiogram tests is applied. Half of the MTK (Minimum toxic concentration) value is EC50. Specimens are defined as log (1 / EC50) and Exp (log (1 / EC50)) by means of abscissa and ordinate. If the linear regression value (r^2) of the line formed as a result of the results obtained is between 0.8-1, these substances are considered non-toxic. If this value is lower than 0.8, the sample under right is cytotoxic [14].

Results and Discussion

As shown in Table 1, in total, 58 constituents were identified. The main components were menthole, carvacrol, 1,8-cineole, camphora and linalyl acetate in Esseintial Oils as %43.56-%47.48, %66.49, %46.28, %30.52 and %62.63 respectively. Menthone, p-cymene, camphora, 1,8-cineol and linalool were the second major component in EOs 21.83% - 22.15%, 10.16%, 10.95%, 24.78% and 24.04% resp. The third major component were isomenthone, menthyl acetate, linalool, β-pinene and germacrene D in EOs resp. The contents of these EOs show us β-pinene is most widely chemical compound in this study. These seconder metabolites may show antimicrobial, mutagenity and cytotoxic activity (Table 2).

Compound Name (EOs)	*M. arvensis*	*M. piperita*	*O. vulgare*	*R. officinalis*	*S. lavandifolia*	*S. sclarea*
α-pinene	-	-	-	10.58	6.54	-
linalyl acetate	**-**	**-**	**-**	**-**	**-**	**62.63**
β-pinene	**1.39**	**1.13**	**-**	**8.25**	**4.63**	**-**
sabinene	-	-	-	-	0.98	-
myrcene	-	-	-	1.09	2.07	-
caryophylene oxide	-	-	1.45	0.51	-	-
camphora	**-**	**-**		**10.95**	**30.52**	**-**
thymol	-	-	7.91	-	-	-
limonene	4.20	3.99	-	2.16	4.17	-
1,8-cineole	**10.22**	**3.83**	**-**	**46.28**	**24.78**	**-**
carvacrol	**-**	**-**	**66.49**	**-**	**-**	**-**
(Z)-β-ocimene	-	-	-	-	0.09	-
(E)-β-ocimene	-	-	-	-	0.05	-
p-cymene	**-**	**-**	**10.16**	**1.98**	**0.60**	**-**
terpinolene	-	-	-	0.13	-	-
methyl acetate	-	-	-	-	-	-
bicyclogermanilen	-	-	-	-	0.16	-
carvacrol	-	-	-	-	-	-
linalool	**-**	**-**	**3.16**	**0.71**	**-**	**24.04**
Δ-3-karnen	-	-	-	0.29	-	-
Δ-terpineol	-	-	-	0.38	-	
γ-muurolan	-	-	-	0.22	-	
γ-terpinene	-	-	-	0.13	0.22	-
bornyl acetate	-	-	-	1.22	0.91	-
geranyl acetate	-	-	-	-	0.19	1.50
terpinen-4-ol	-	-	-	0.84	0.44	-
β-caryophyllene	-	1.99	1.07	3.52	1.45	2.00
geranyl isobutirate	-	-	-	-	0.37	-
geraniol	-	-	-	-	0.27	1.28
p-cymene-8-ol	-	-	-	-	0.07	-
menthone	**21.83**	**22.15**	**-**	**-**	**-**	**-**
germacrene D	-	-	-	-	-	2.63
isomenthone	**10.66**	**4.15**	**-**	**-**	**-**	**-**
neomenthole	2.04	4.30	-	-	-	-
isopulegon	1.23	-	-	-	-	-
menthole	**43.56**	**47.48**	**-**	**-**	**-**	**-**
pulegon	1.11	1.00	-	-	-	-
menthofurane	-	0.76	-	-	-	-
isopulegol	-	0.34	-	-	-	-
camphene	-	-	0.98	4.39	5.27	-
α-kapaen	-	-	-	0.22	-	-
γ-terpinene	-	-	-	-	-	-
tricyclene	-	-	-	0.16	0.26	-
α-tuyen	-	-	-	-	0.10	-

Compound Name (EOs)	*M. arvensis*	*M. piperita*	*O. vulgare*	*R. officinalis*	*S. lavandifolia*	*S. sclarea*
terpineolene	-	-	-	-	0.26	-
trans- sabinene- hydrite	-	-	-	-	0.16	-
camphor	-	-	-	-	-	-
γ-terpineol	-	-	-	-	0.22	-
α-humulene	-	-	-	0.39	0.34	-
α-terpineol	-	-	-	1.76	1.04	3.05
α-terpinyl acetate	-	-	-	-	0.90	-
menthyl acetate	**-**	**4.56**	**-**	**-**	**-**	**-**
borneole	-	-	1.71	2.86	3.39	-
p-cymen-8-ol	-	-	-	-	-	-
bicyclogermanilene	-	-	-	-	-	-
Imalol	-	-	-	-	4.09	-
Imalil acetate	-	-	-	-	3.50	-
sabinyl acetate	-	-	-	-	1.89	-
Total %	**96,24**	**95,68**	**92,93**	**99,02**	**99,93**	**97,13**

Table 2: Chemical components of *Lamiaceae* essential oil

Antimicrobial Activity of Lamiaceae Essential Oils

Microorganism / Essential oil (mg/L)	La-5	La-14	L.reu.	L.rh.	L.fer	B.coa.	B.N.	B.cl.	S.sal.	S.ther.	S.b.	S.c.	BB-12
M.arvensis	0.25>	>128	2	36	2	64	64	48	1	48	>128	96	0.25
M.piperita	0.25>	0.25>	1	1	4	0.25>	1	0.5	>128	0.25>	>128	1	0.25
O.vulgare	64	64	48	96	1	64	2	4	>128	2	>128	4	2
R.officinalis	64	>128	>128	>128	0.25	>128	>128	0.25>	>128	0.25>	>128	>128	4
S.lavandifolia	>128	>128	>128	>128	2	>128	0.13	32	0.5	32	>128	48	1
S.sclarea	>128	>128	>128	48	32	72	80	48	64	64	>128	64	>128
S.triloba	32	48	>128	64	6	96	48	48	64	32	>128	64	0.5
S.montana	>128	>128	>128	>128	64	>128	2	4	>128	2	>128	4	>128
O. basilicum	>128	>128	>128	>128	-	>128	4	4	>128	4	>128	2	0.25
Ketoconazole	4	4	0.25	12	12	16	12	0.5	12	0.25	-	-	0.25
Chloramphenicol	-	-	-	-	-	-	-	-	-	-	1	1	-

La-5:*Lactobacillus acidophilus La-5*, **La-14**:*Lactobacillus acidophilus La-14*, **L.fer.:***Lactobacillus fermentum CECT-5716* **L.reu**.:*Lactobacillus reuteri DSM 17938*, **L.rh.:** *Lactobacillus rhamnosus GG*, **B.coa.:** *Bacillus coagulans SNZ 1969*, **B.cl.:** *Bacillus subtilis var. clausii ATCC9799*, **B.N.:** *Bacillus subtilis var. natto BN*, **S.sal.:** *Streptococcus salivarius K12*, **S.ther.:** *Streptococcus thermophilus TH-4*, **S.b.:** *Saccharomyces cerevisae var. boulardii ATCC – MYA976*, **S.c.:** *Saccharomyces cerevisae ATCC – MYA9763,* **BB-12:** *Bifidobacterium bifidum BB-12)*

Table 3: MIC table of Lamiaceae family Eos

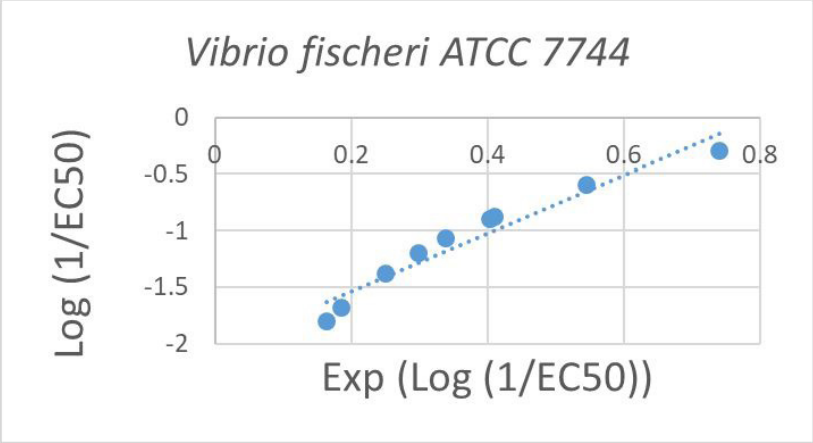
For these results, *Mentha arvensis*, *Mentha piperita*, *Origanum vulgaris* and *Salvia triloba* essential oils are most effective EOs against probiotic microorganisms. If they are used on gastrointestinal microflora directly, they can inhibit many microorganisms and cause many gastroinstestinal problems. All of the EOs in this study effect *Lactobacillus fermentum* CECT-5716. This microorganism isn't resistant against EOs. *Bifidobacterium bifidum BB-12* is resistant against *Satureja montana*. *Salvia sclarea* didn't show any antimicrobial activity against probiotic microorganisms. When compared all datas about this study and previous studies antimicrobial activities shows us pathogen microorganisms less resistant than probiotics. This study shows us probiotic microorganisms abilities to protect human body when natural antimicrobial compounds are taken (Table 3).

Cytotoxicity and Mutagenity Tests

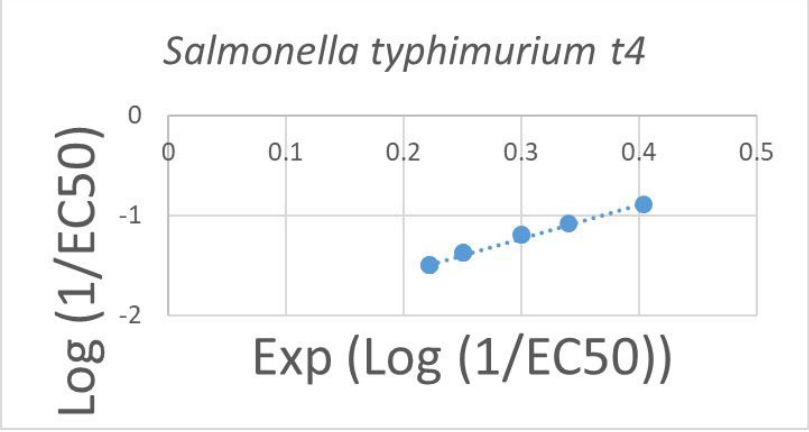
Salvia lavandulifolia and Salvia triloba essential oils have in vitro non-toxic results and Salvia sclarea essential oil was found to be more cytotoxic than other essential oils. Mentha arvensis and Origanum vulgare essential oils will have linear cytotoxicity values at increasing concentrations relative to other essential oils (Table 4).

Microorganisms/ EOs	*Vibrio* fischeri *(ATCC 7744)*	*Salmonella typhimurium t4*
Mentha arvensis aetheroleum	48 µg/mL	64 µg/mL
Salvia sclareae aetheroleum	32 µg/mL	48 µg/mL
Salvia lavandulifoliae aetheroleum	128 µg/mL	64 µg/mL
Salvia trilobae aetheroleum	16 µg/mL	32 µg/mL
Origanum vulgare aetheroleum	24 µg/mL	24 µg/mL
Satureja montana aetheroleum	96 µg/mL	48 µg/mL
Rosmarinus officinalis aetheroleum	2 µg/mL	16 µg/mL
Mentha piperitae aetheroleum	0.13 µg/mL	64 µg/mL
Ocimum basilicum aetheroleum	4 µg/mL	32 µg/mL

Table 4: Minimum Toxic Concentration (MTK/ EC100) values of Eos



Graph 1: Vibrio fischeri ATCC 7744 cytotoxicity graph of some Lamiaceae essential oils



Graph 2: Salmonella typhimurium t4 genotoxicity graph of some Lamiaceae essential oils

According to the results of *Salmonella typhimurium* t4, all essential oils were found on the linear graphs 1 and 2 and their cytotoxicity increased at increasing concentrations. Salvia triloba essential oil, which is slightly lower than the line, has a minimum cytotoxicity and no essential oil is genotoxic.

Special Thanks

Thanks Prof. Dr. Betul Demirci and Prof. Dr. Fatih Demirci for their GC/MS supports.

References

1. Zgórka G, Głowniak K (2001) Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. Journal of Pharmaceutical and Biomedical Analysis 26: 79-87.

2. Nieto G (2017) Biological activities of three essential oils of the Lamiaceae family. Medicines 4: 63.

3. Nair R, Chanda S (2007) Antibacterial activities of some medicinal plants of the western region of India. Turkish Journal of Biology 31: 231-6.

4. Mimica-Dukić N, Božin B, Soković M, Mihajlović B, Matavulj M (2003) Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta medica 69: 413-9.

5. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, et al. (2004) Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology 35: 275-80.

6. Santoyo S, Cavero S, Jaime L, Ibanez E, Senorans FJ, et al. (2005) Chemical composition and antimicrobial activity of Rosmarinus officinalis L. essential oil obtained via supercritical fluid extraction. Journal of Food Protection 68: 790-5.

7. Pierozan MK, Pauletti GF, Rota L, Santos ACAD, Lerin LA, et al. (2009) Chemical characterization and antimicrobial activity of essential oils of Salvia L. species. Food Science and Technology 29: 764-70.

8. Cui H, Zhang X, Zhou H, Zhao C, Lin L (2015) Antimicrobial activity and mechanisms of Salvia sclarea essential oil. Botanical studies 56: 1-8.

9. Vitanza L, Maccelli A, Marazzato M, Scazzocchio F, Comanducci A, et al. (2019) Satureja montana L. essential oil and its antimicrobial activity alone or in combination with gentamicin. Microbial Pathogenesis 126: 323-31.

10. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, et al. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology 35: 275-80.

11. National Committee for Clinical Laboratory Standards (2002) Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard, NCCLS document M27-A2 (2nd Edn) Wayne: National Committee for Clinical Laboratory standards, Turkey.

12. National Committee for Clinical Laboratory Standards (2006) Methods for dilution microbial susceptibility tests for bacteria that grow aerobically. Approved Standard, NCCLS document M7-A7 (7th Edn) Wayne: National Committee for Clinical Laboratory standards, Turkey.

13. Şekeroğlu ZA, Şekeroğlu V (2011) Genetic toxicity tests [Genetik toksisite testleri]. TÜBAV Bilim Dergisi 4: 221-9.

14. Lee J, Bang SH, Kim YH, Min J (2018) Toxicities of four parabens and their mixtures to Daphnia magna and Aliivibrio fischeri. Environmental Health and Toxicology 33.