

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *CITRUS SINENSIS*: INTERACTION WITH AMPICILLIN AND FLUCONAZOLE

Elif Ayşe Erdoğan ELIUZ^{a,*}, Erdal YABALAK^{b,*} and Yusuf SICAK^c

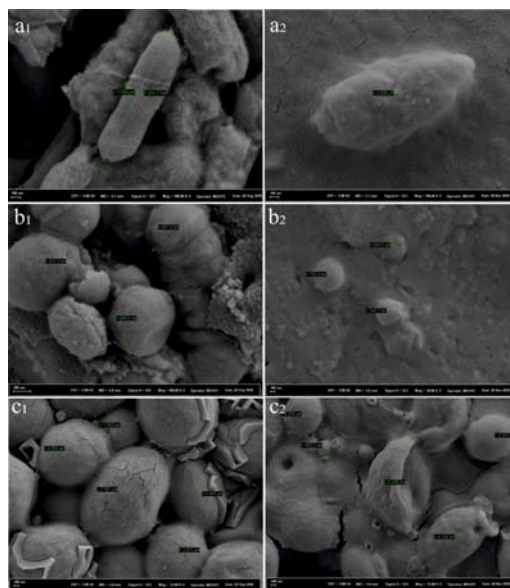
^a Mersin University, Technical Sciences Vocational School, Department of Food Technology, TR-33343, Mersin, Turkey

^b Mersin University, Faculty of Arts and Science, Department of Chemistry, Çiftlikköy Campus, TR-33343, Mersin, Turkey

^c Muğla Sıtkı Koçman University, Vocational School of Koycegiz, Department of Plant and Animal Production, Muğla, Turkey

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In the present study, the interactions of essential oil (EO) of *Citrus sinensis* L. Osbeck with two antibiotics (ampicillin and fluconazole), which are commonly used in the treatment of infections, were investigated. *C. sinensis* was hydrodistilled and the chemical composition of the obtained essential oil was identified by GC-MS (Gas Chromatography-Mass Spectrometry) analyses. The most representative compound of all obtained 18 compounds was *d*-limonene followed by the other major components such as linalool, α -terpineol, β -myrcene. The MICs (Minimum Inhibitory Concentration) of *C. sinensis* essential oil were 152.06 mg/mL, 20 mg/mL, 54.41 mg/mL and the IZs (Inhibition Zone) were noted as 3.4 mm, 3.3 mm, 8.3 mm for *E. coli*, *S. aureus* for *C. albicans*, respectively. Antimicrobial performance of antibiotics in combinations with the essential oil on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were determined using antimicrobial checkerboard method. According to this model, antagonist effect was observed as 14.29%, 57.14% and 0% for *S. aureus*, *E. coli* and *C. albicans*, respectively. As a result, the use of *C. sinensis* essential oil with antibiotics can be seen as a disadvantage as it may slow the effect of antibiotics. On the contrary, in another case, the using of *C. sinensis* essential oil with antibiotics may cause to delaying the resistance mechanisms of bacteria and turn of antagonism to advantage in clinical applications.



INTRODUCTION

Citrus sinensis (*C. sinensis*) is the botanical name of a well-known fruit, orange which belongs to the Rutaceae family.¹ Citrus varieties including orange, mandarins, kumquat and limes are the most widespread fruits with approximately 120000 thousand tons of global production.^{2,3} The largest citrus grown were belonged to *C. sinensis* with

70% of the total annual production and spread over temperate climatic zones. It is commonly 9–10 meters tall with large spines on branches and it has narrowly winged-petioles more than 3 mm wide. Leaves also vary from elliptical, bluntly toothed, oblong to oval, and especially their odour is characteristic because of the presence of copious oil leaves.⁴ The peel, leaves, roots, fruits and juice of *C. sinensis* is the rich sources of volatile

* Corresponding authors: eliferdogan81@gmail.com or yabalakerdal@gmail.com

compounds which provide great pharmacological importance to this plant.⁵ The essential oil of *C. sinensis* possesses antimicrobial activities against most prevalent species of gram-positive and gram-negative bacteria and fungal pathogens that are liable for many severe infections.⁶

Antibiotics are among the most powerful tools to deal with infections. However, microorganisms have a remarkable genetic ability to develop resistance to antimicrobial compounds.⁷ Recent advances in medical biotechnology have not been able to handle the rapid emergence of resistant microorganisms which resulted in a considerable public health threat influencing humans worldwide.⁸

Recent works suggest that the different types of synergic or antagonistic interactions have contributed considerably to the understanding of drug resistance. Although the synergistic effect is beneficial in rapid treatment, it has been stated that it causes the development of drug-resistance. In particular, a growing number of laboratory studies indicate that antagonistic drug combinations merit further study as therapeutic options; some researchers emphasize that antagonism among antibiotics will support the development of treatment strategies specifically aimed at delaying the emergence of resistance.^{9,10}

Therefore, there is a need for the development of novel agents or combinations to be used as effective antimicrobial agents. Due to the antimicrobial effect of essential oils, their use in combination with antibiotics might lead to new products with enhanced antimicrobial efficiency. Despite the proven antimicrobial activity of essential oil of *C. sinensis* (*CsEO*), no study on the antimicrobial effectiveness of *CsEO* in combination with ampicillin or fluconazole has been reported. Hence, the present study aimed to assess the susceptibility of *E. coli*, *S. aureus* and *C. albicans* to single and combinations of *CsEO* to detect a synergistic effect (SynE), indifferent effect (IndE) and/or antagonistic effect (AntE) and to correlate the chemical profile of the oil to the antimicrobial activity. Besides, the damaged parts of microorganisms' cell surfaces were determined by SEM (Scanning Electron Microscopy) at the end of the 24-h incubation with *CsEO*. Thus, it was examined in nanoscale how the subjected microorganisms were damaged by the synergistic effect of antibiotics and *CsEO*.

Considering the potential effects of the compounds that exist in the essential oils on human health, and the fact that the components they

contain have fewer side effects than the synthetic counterparts, determining the chemical composition of essential oils is of utmost importance. Thus, the determination of the composition of essential oil of *C. sinensis* and the identification of the effects of the identified compounds extended the scope of this study.

EXPERIMENTAL

1. Materials and Instruments

Chemical analysis of *C. sinensis* essential oil was performed using Agilent brand GC-MS. Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Broth (SDB), Tryptic Soy Broth (TSB) were supplied from Merck (Darmstadt, Germany). Ampicillin (AMP) and Fluconazole (FLC) and Dimethyl Sulfoxide (DMSO) were purchased from Sigma. *E. coli* (ATCC 25293), *S. aureus* and *C. albicans* were purchased from Refik Saydam Hıfzıssıhha Centre (Ankara/Turkey). The 96-well microtiter plates and sterile Petri were purchased from Labkon Ltd. Eliza spectrophotometer (Thermo Scientific, MULTISKAN GO) was used for antimicrobial measurements and the microscopic imaging, the samples were dried using critical point dryer, EMITECH K850 and were platinum covered by spraying Quorum 150R ES and examined with the SEM (Scanning Electron Microscopy ZEISS SUPRA 55).

2. Plant Material and Essential Oil Extraction

C. sinensis were collected from Köyceğiz region of Muğla, Turkey in 2017. It was identified and confirmed by comparing it with the specimen located at the Herbarium of Biology, Faculty of Science, A Application and Research Center by GC-MS 7890A-(5975C inert MSD) instrument equipped with an Agilent 19091S-433 column (30m X 250 µm film X 0.25 µm thickness). Helium was used as a carrier gas. The sample was eluted for 64 minutes of retention time using the following temperature programme. After the initial temperature of 60°C for 5 min, it was gradually raised to 150°C by an increase of 3°C/min for 2 min, then by 3 °C/min to 200°C and by 4°C/min to 240°C. Characterization of *CsEO* components was performed based on the mass spectra library (Wiley Registry 9th/NIST 2011 database, W9N11.L).

3. Antimicrobial Screening

The inoculums of *E. coli* (ATCC 25293), *S. aureus* and *C. albicans* were prepared in 4 mL TSB for bacteria, 4 mL SDB for yeasts and incubated at 37 °C, overnight. After 24 hours, the culture suspensions were adjusted to 0.5 McFarland Standard Turbidity and stored at +4 °C until further use.

3.1. Disc Diffusion Assay

Microorganism cultures were spread onto MHA plates. Paper discs (6 mm in diameter) were impregnated on the agar to load 15 µL of *CsEO* (pure) and incubated at 37 °C for 24 hrs. The results were recorded in the zones of growth inhibition surrounding the disc using a digital calliper. Ampicillin and Fluconazole were used as positive controls.

3.2. Spectrophotometric Checkerboard Microdilution

The 50 μL of MHB medium were added into 96-well microtiter plates. Two-fold serial dilutions of 50 μL CsEO solution (3.5 mg/mL in 10% DMSO) was made (A1-H1) on the y-axis along of chequerdoard plate. Two-fold serial dilutions of 50 μL antibiotic dilution (starting concentration; AMP: 128 μg /and FLC: 5128 μg /mL) was made x-axis along from 2nd to 10th columns and CsEO solution (single concentration: 100, 50, 25, 12.5, 6.25, 3.12, 1.56 μl) was added to each line to make fraction and obtain the FIC final

concentrations. Columns 11 and 12 were used as negative and positive controls, respectively (Figure 1). Finally, 5 μL culture of microorganisms were inoculated on all wells except negative control. All plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours, the growth (turbidity) was measured at 600 nm and 415 nm for bacteria and yeast, respectively. For MIC analysis, the optical density was read both before incubation, t_0 , and after 24 hours-incubation, t_{24} . For each plate, MIC was calculated using the regression curve. The OD for each replicate at t_0 was subtracted from the OD for each replicate at t_{24} .

$$\text{Growth (\%)} = \left(\frac{OD_{test}}{OD_{control}} \right) \times 100 \quad \text{Eq. 1}$$

$$\text{Inhibition (\%)} = \left(1 - \frac{OD_{test\ well}}{OD_{corresponding\ control\ well}} \right) \times 100 \quad \text{Eq. 2}$$

For each row of the 96-well plate, the lowest concentration of test material (MIC) which results in 99.9% inhibition of growth were calculated using Eq. 1 and Eq. 2

For each plate, the sum of the FICs (Fractional Inhibitory Concentration) was calculated for each well using equation 3;

$$\Sigma\text{FIC} = \text{FIC}_{A(\text{antibiotic})} + \text{FIC}_{C(C.\ sinensis\ EO)} = (\text{MIC}_{(A+C)/A}) + (\text{MIC}_{(A+C)/C}) \quad \text{Eq. 3}$$

where MIC_A and MIC_C , present the MICs of antibiotics and the CsEO alone, in all wells corresponding to a MIC.¹¹ Fractional Inhibitory Concentration Index (FICI) were interpreted following the conventional model suggested by

Odds; According to that, a synergistic effect (SynE) is observed when FICI value ≤ 0.5 ; an indifferent effect (IndE) when $0.5 < \text{FICI} \leq 4$ and an antagonistic effect (AntE) when FICI value > 4 .¹²

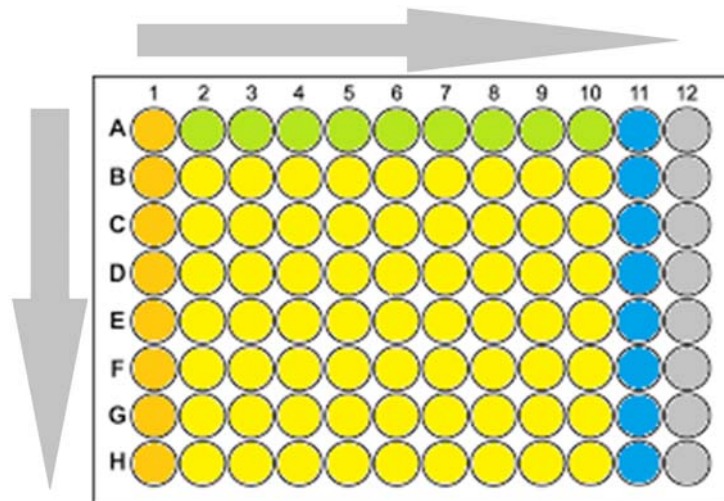


Fig. 1 – Design of the FICI experiment on a multiwell (ELISA) plate; orange wells: CsEO dilution and microorganism; green wells: antibiotic dilution and microorganism; yellow wells: antibiotic and CsEO combinations and microorganism; blue wells: bacteria growth control; gray wells: media growth control.

4. Cell Surface Analysis Using SEM

After 24 hours incubation of CsEO with *E. coli*, *S. aureus* and *C. albicans*, cultures in MIC wells were collected and centrifuged in a 1.5 mL Eppendorf at 15000 rpm for 5 min and the pellet was resuspended in 1.5 mL of 2.5% glutaraldehyde

solution for 4 h. Then the samples were dehydrated by successive 10-min incubations in 35%, 50%, 70%, 95%, 100% ethanol and allowed to dry on aluminium foil. Finally, before microscopic imaging, the specimens were dried using a critical point dryer. The samples were platinum covered by spraying (Quorum 150R ES) and examined with the SEM.¹³

5. Statistical Analysis

Statistical analyses and significance were measured by LSD test and Tamhane's T2 in one-way analysis of variance for MICs using SPSS 25. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Chemical composition

The components of the essential oil of *C. sinensis* were determined by GC-MS analysis using mass spectral libraries. Kováts indices (KI), which is a frequently used method to prevent the effect of GC analysis conditions on retention times, were calculated for each compound and obtained indices were compared to the ones reported in literature.¹⁴⁻¹⁶ In total, 18 compounds were identified with the quality values above 80% and were given in Table 1 along with their values such as retention time (t_r), peak area (%), peak quality (%), KI and Kováts index obtained from literature (RIL). In this manner, the obtained compounds were evaluated for their bioactivity properties such as antimicrobial activities in the following.

α -Pinene was obtained by 0.32% of peak area with 94% of the peak quality and the calculated KI was in good compatibility with the reported one.¹⁷ Also, Vujisić *et al.* reported that α -pinene was found to be 1.5% in the essential oil of *A. ruthenicum*.¹⁷ Saraglou *et al.* detected α -pinene and sabinene as the components of essential oils of *Hypericum* species in their work, in which they

specified the antimicrobial activity of the mentioned essential oils.¹⁸ β -myrcene was detected at a peak area of 1.42 with 93% peak quality. It was observed that the calculated KI value of β -myrcene (987.67) was quite consistent with the value in literature (992).^{19,20} In the reported works, β -myrcene, α -pinene, sabinene, linalool, 4-terpineol, α -terpineol and carvacrol were found in the essential oils of some *Thymus* and *Origanum* species in different levels. The total peak area of these compounds was found as 6.08% in this work. They mentioned that the existence of the mentioned compounds along with the others and their levels may have affected the antimicrobial and antioxidant activities.^{20,21}

The most remarkable components found in the essential oil of *C. sinensis* is undoubtedly *d*-limonene, which was found at a peak ratio of 88.9% in this study. *d*-Limonene was followed by linalool (2.02%), α -terpineol (1.43%), and β -myrcene (1.42%). Similarly, the main compounds in the essential oil of *C. sinensis* peel were reported to be limonene (96.62%), β -myrcene (1.72%), β -pinene (0.53%), α -pinene (0.47%), citral Z (0.31%) and citral E (0.34%).²² Singh *et al.* depicted that *d*-limonene (90.66%), linalyl acetate (2.80%) and β -myrcene (1.71%) were the major components in *C. sinensis* oil according to GC-MS analyses.²³ Furthermore, *d*-limonene and *d*-carvone have strong bioactivity profiles as stated by Aggarwal *et al.* in their research on antimicrobial activity of the enantiomers of these compounds found in the essential oils of *Mentha spicata* and *Anethum sowa*.²²

Table 1

Chemical composition of *C. sinensis* essential oil

C. No	Compound Name	Chemical Formula	Molecular Weight (g/mol)	t_r (min)	Peak Area (%)	Quality (%)	KI	RIL	Ref. No
1	α -Pinene	C ₁₀ H ₁₆	136.24	9.30	0.32	94	886.41	890	17
2	Sabinene	C ₁₀ H ₁₆	136.23	11.00	0.21	96	958.33	953	17
3	β -myrcene	C ₁₀ H ₁₆	136.23	11.76	1.42	93	987.67	992	19
4	octanal	C ₈ H ₁₆ O	128.21	12.36	0.28	80	990.33	993	17
5	<i>d</i> -limonene	C ₁₀ H ₁₆	136.24	13.96	88.9	98	1017.71	1018	17
6	1-octanol	C ₁₀ H ₁₈ O	130.23	15.64	0.32	90	1072.63	1065	17
7	Linalool	C ₁₀ H ₁₈ O	154.25	16.99	2.02	97	1107.72	1104	21
8	citronellal	C ₁₀ H ₁₈ O	154.14	19.43	0.13	97	1155.96	1158	19
9	4-terpineol	C ₁₀ H ₁₈ O	154.25	20.61	0.52	96	1180.58	1177	21
10	α -Terpineol	C ₁₀ H ₁₈ O	154.25	21.29	1.43	91	1192.62	1190	21
11	Decanal	C ₁₀ H ₂₀ O	156.20	21.82	0.37	91	1194.37	1198	17
12	<i>trans</i> -(+)-carveol	C ₁₀ H ₁₆ O	152.23	22.63	0.31	96	1214.64	1212	17

Table 1 (continued)

13	Nerol	C ₁₀ H ₁₈ O	154.25	23.02	0.62	86	1227.90	1229	25
14	Z-citral	C ₁₀ H ₁₆ O	152.24	23.55	0.46	90	1236.80	1238	26
15	d-carvone	C ₁₀ H ₁₄ O	150.22	23.73	0.13	97	1239.54	1241	25
16	Geraniol	C ₁₀ H ₁₈ O	154.25	24.22	0.32	96	1270.63	1267	26
17	Carvacrol	C ₁₀ H ₁₄ O	150.22	26.46	0.16	94	1298.65	1299	26
18	<i>p</i> -vinylguaiaicol	C ₉ H ₁₀ O ₂	150.18	26.94	0.26	96	1311.34	1315	27

RIL: Literature Kováts index, RI: Calculated Kováts index

α -Pinene, sabinene, myrcene, linalool, 4-terpineol, α -terpineol, citronellal, nerol, geraniol and carvacrol were detected in the composition of eleven essential oils from the different origin by Sacchetti *et al.*²⁸ They investigated the effects of these essential oils against some bacterial strains. Linalool and geraniol were reported to be found in the composition of essential oil of *C. citratus*, α -pinene, nerol was in the *T. citriodorus* and myrcene were in the both of them, which were reported as the most effective species against the tested strains.²⁸ Citral may have the same efficacy as geraniol considering the isomeric structure of them.²⁸ Besides, the obtained KI values of β -myrcene, 4-terpineol, α -Terpineol, nerol and carvacrol were in good accordance with the ones reported by Sacchetti *et al.*²⁸ Küçük *et al.* reported the moderate antimicrobial activity of the essential oil of *Teucrium chamaedrys* L. subsp. *chamaedrys*, *Teucrium orientale* var. *puberulens*, and *Teucrium chamaedrys* L. subsp. *lydium* against Gram-

positive and Gram-negative bacteria.²⁹ Decanal and *p*-vinylguaiaicol, which were found in the essential oil of *C. sinensis* in this work, were the compounds detected in the mentioned essential oils by Küçük *et al.*²⁹

2. Antimicrobial Activity and Antagonism

In this study, according to both disc diffusion and broth microdilution method, *E. coli*, *S. aureus* and *C. albicans* were found to be rather sensitive to *C. sinensis* EO (Table 2). In the disc diffusion method, CsEO strongly inhibited *E. coli*, *S. aureus* and *C. albicans*. The inhibition zones were noted as 3.4 mm for *E. coli*, 3.3 mm for *S. aureus*, and 8.3 mm for *C. albicans*. It was 6.8 and 9.9 mm for *E. coli* and *S. aureus* in AMP, 27 mm for *C. albicans* in FLC as a positive control, respectively (Figure 2).

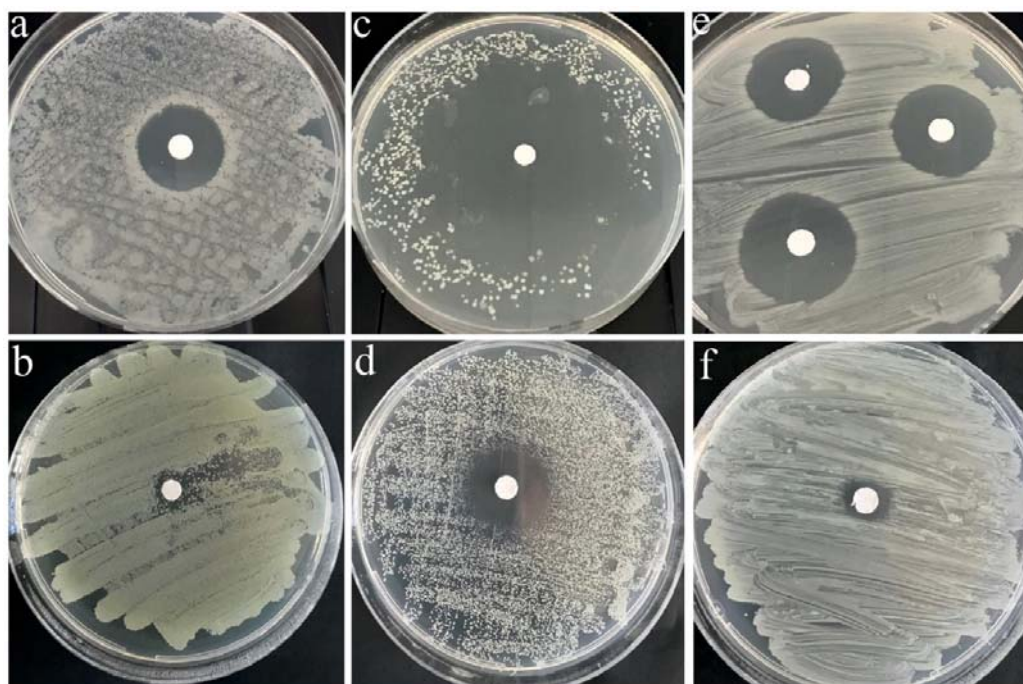


Fig. 2 – The images of inhibition zones of 15 μ L CsEO (pure) and antibiotics against *E. coli*, *S. aureus* and *C. albicans*. a: *E. coli* for AMP, b: *E. coli* for CsEO, c: *C. albicans* for FLC, d) *C. albicans* for CsEO, e: *S. aureus* for AMP, f: *S. aureus* for CsEO.

Table 2

MICs ($\mu\text{g/mL}$) and FICIs of combinations of CsEO (3.5 mg/mL) with antibiotics (128 $\mu\text{g/mL}$ AMP or 5128 $\mu\text{g/mL}$ FLC) and MICs values of CsEO and antibiotics alone and IZs (mm) of CsEO (pure), AMP (128 $\mu\text{g/mL}$) on *E. coli*, *S. aureus* and FLC (5128 $\mu\text{g/mL}$) on *C. albicans*

	<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>	
IZ CsEO→	3.4		3.3		8.3	
IZ control→	6.8		9.9		27	
	MIC	FICI	MIC	FICI	MIC	FICI
Ad	152.06 ^b ±3.1	–	27.07 ^c ±16.4	–	26.74±11.9	–
CsEOd	479.26±30.4	–	20.0 ^{ac} ±8.4	–	54.41±12.9	–
Ad+CsEO ₁₀₀	346.11±26.1	2.9 IndE	298.08±26.7	25.91 AntE	50.5±32.3	2.8 IndE
Ad+CsEO ₅₀	126.82 ^b ±11.1	1.09 IndE	134.03±21.3	11.65 AntE	34.24±19.2	1.9 IndE
Ad+CsEO ₂₅	369.79±38.3	3.2 IndE	111.50±14.3	9.69 AntE	27.23±12.08	1.5 IndE
Ad+CsEO _{12.5}	829.61±25.4	7.1 AntE	312.13±22.4	27.14 AntE	34.86±12.1	1.9 IndE
Ad+CsEO _{6.25}	850.55±63.2	7.3 AntE	217.41±15.1	18.91 AntE	50.94±32.3	2.8 IndE
Ad+CsEO _{3.12}	778.78±44.0	6.7 AntE	228.15±18.4	2.45 IndE	53.62±16.9	2.9 IndE
Ad+CsEO _{1.56}	1018.53±29.7	8.8 AntE	699.36±46.5	60.80 AntE	46.49±17.8	2.5 IndE

Statistically, a, b and c demonstrate the difference from the (Ad+CsEO_{6.25}) fraction in *S. aureus*, the (Ad+CsEO_{1.56}) fraction in *E. coli* and the (Ad+CsEO_{1.56}) fraction in *S. aureus*, respectively. n = 3, d:dilution.

According to broth microdilution method, MICs on microorganisms of CsEO were found to be 479.26 $\mu\text{g/mL}$ for *E. coli*, 20.0 $\mu\text{g/mL}$ for *S. aureus* and 54.41 $\mu\text{g/mL}$ for *C. albicans* while the MICs of antibiotics results were 152.06 $\mu\text{g/mL}$, 27.07 $\mu\text{g/mL}$ and 26.74 $\mu\text{g/mL}$ for *E. coli*, *S. aureus* and *C. albicans*, respectively (Table 2).

When the combined antimicrobial activities of CsEO and antibiotics were examined, the values of the MICs in all combinations were found to be statistically different ($p \leq 0.05$) from the MICs determined from CsEO alone against pathogen microorganisms. The study of combination on *E. coli* showed that the highest efficacy was found with MIC of 152.06 $\mu\text{g/mL}$ in the antibiotic alone, while the lowest efficacy was 1018.53 $\mu\text{g/mL}$ in the fraction of Ad+CsEO_{1.56}. On *S. aureus*, the highest efficacy was found with MIC of 20.0 $\mu\text{g/mL}$ in CsEO alone, while the lowest efficacy was 699.36 $\mu\text{g/mL}$ in the fraction of Ad+CsEO_{1.56}. Finally, on *C. albicans*, the highest efficacy was MIC of 26.74 $\mu\text{g/mL}$ in CsEO alone, while the lowest efficacy MIC was 54.41 $\mu\text{g/mL}$ in CsEO alone (Table 2).

The interaction data in the form of the FICI, exposing bacteria to CsEO-Ad combinations

overnight, AntE and IndE effects occurred as 57.14% and 42.86% for *E. coli*, 14.29% and 85.71% *S. aureus*, respectively. Remarkable IntE action was observed against *C. albicans* (FICI: 100%) by the combination of CsEO*FLC. Interestingly, no synergy was noted between CsEO and antibiotic combinations against microorganisms (FICIsyn:0) as shown in Figure 3. This might be attributed to the fact that the antibiotics are more powerful than CsEO against microorganisms.

Recent studies revealed the antimicrobial activity of the essential oils of *Citrus* species and their combination with antibiotics.³⁰⁻³⁵

Many terpene compounds such as limonene, thymol, linalool, eugenol, vanillin, citral have been accepted as preservatives and flavorings in the food industry by the European Commission. Besides, they are marked as GRAS (generally recognized as safe) in the United States Food and Drug Administration (FDA).³⁶ However, some limitations on their use were introduced because of their interaction with food components which reduces their antimicrobial effectiveness.³⁷ In our study, we showed that *C. sinensis* essential oil reduced the antibacterial effect by competing with ampicillin, especially against *E. coli*.

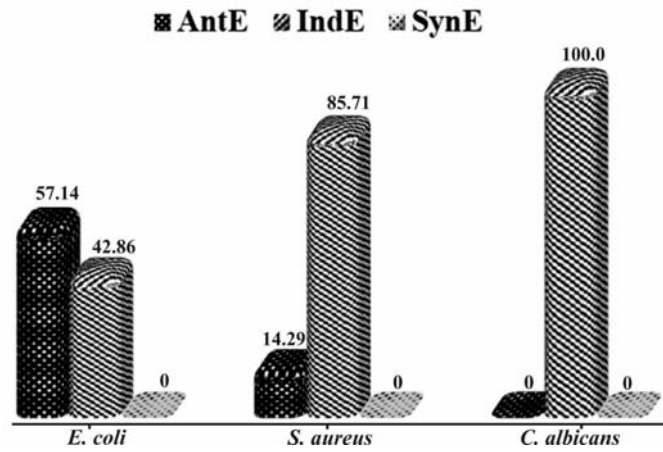


Fig. 3 – Percentage of FICI values of antibiotics and *C. sinensis* EO combinations against *E. coli*, *S. aureus* and *C. albicans*. Antagonistic: AntE, synergistic: SynE, Indifferent: IndE.

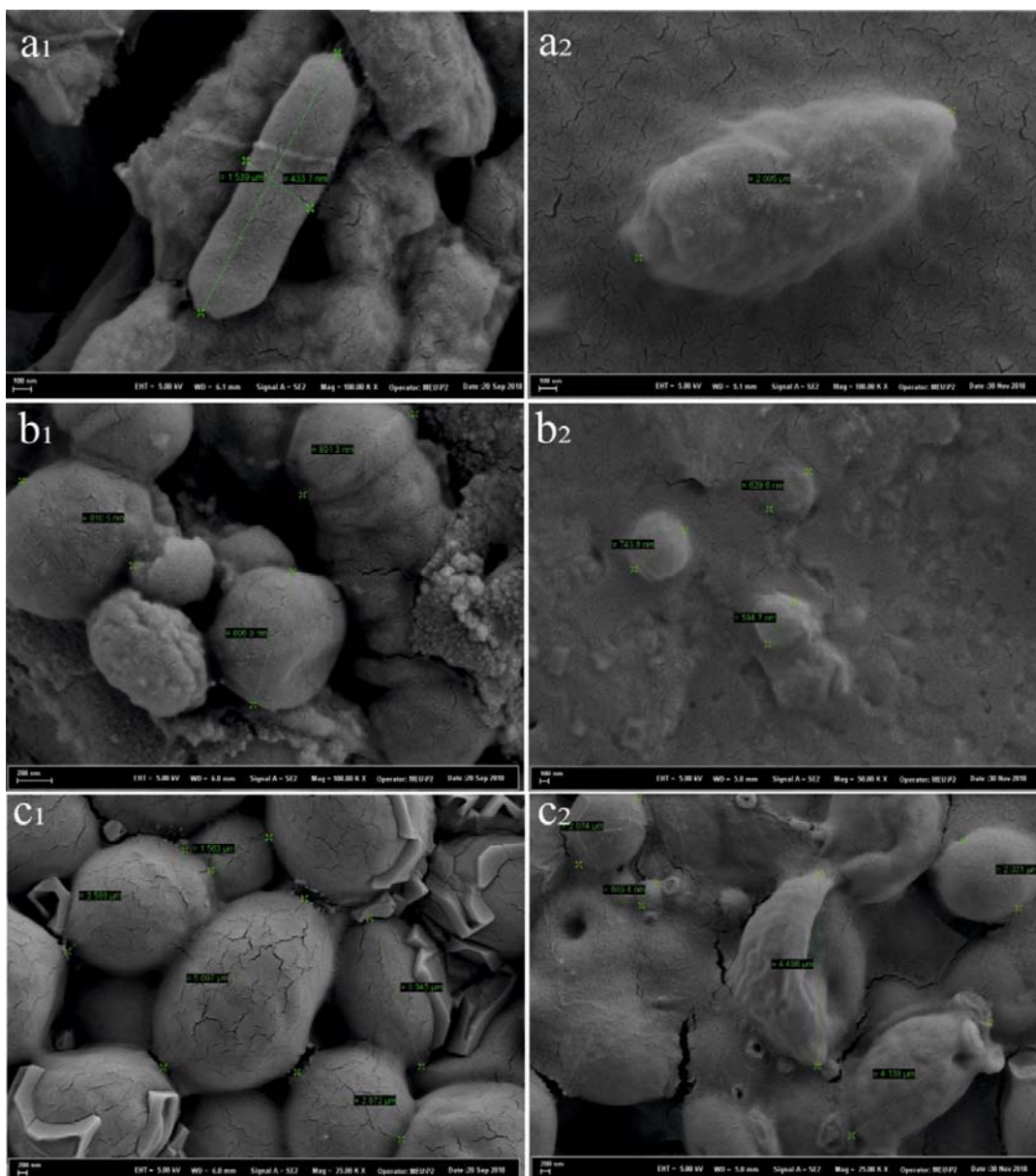


Fig. 4 – Representative SEM images of attached *E. coli* (a₁), *S. aureus* (b₁) and *C. albicans* (c₁) taken from non-treated and incubated with CsEO and *E. coli* (a₂), *S. aureus* (b₂) and *C. albicans* (c₂), respectively, after 24-h incubation.

3. SEM Images of Microorganisms Treated with CsEO

SEM micrographs of *E. coli*, *S. aureus* and *C. albicans* in the presence of CsEO were pointed out that morphological changes do occur in the microorganisms' surfaces. Similar observations have been made with *Enterococcus species* in the presence of citrus EOs.³⁸

Changes in the cell morphology were observed in high magnification SEM images (Figure 4). Figures 4 (a₁, b₁, and c₁) shows untreated *E. coli*, *S. aureus* and *C. albicans*, respectively while figures 4 (a₂, b₂, and c₂) shows incubated *E. coli*, *S. aureus* and *C. albicans* with CsEO, respectively. When treated with CsEO, morphological deterioration of the cell surface was observed. A spreading pattern change was seen on cell surfaces on *E. coli* and *S. aureus* matrix, whereas an intracellular collapse in *C. albicans*. In particular, the swelling and expansion in *E. coli* were quite remarkable. These changes may occur due to differences in cell wall structures of microorganisms.

CONCLUSIONS

Many of the compounds (α -pinene, β -myrcene, sabinene, linalool, 4-terpineol, α -terpineol, carvacrol, *d*-limonene, geraniol, nerol and *p*-vinylguaiaicol) detected in CsEO as a result of GC-MS analysis were determined to show antimicrobial activity or contribute to the total antimicrobial activity of the essential oil compared to literature data. In particular, it can be argued that *d*-limonene plays a major role in antimicrobial activity. This was supported by the results of the antimicrobial activity results obtained in this study. The antimicrobial effect of *C. sinensis* essential oil was updated by both disc diffusion and microdilution method. The most important emphasis here is the interaction with antibiotics. This paper provided an example of indifferent and antagonist effect interactions of *C. sinensis* EO and antibiotic combinations against *E. coli*, *S. aureus* and *C. albicans* microorganisms. Since the target of both antibiotics and essential oils is primarily cell wall destruction, it is predictable that there will be competition between them. The most important result from the study suggests that *C. sinensis* decreased antibiotic activity on bacteria by competing with ampicillin antibiotic. Compounds exist in the essential oil of *C. sinensis* often show

antagonistic effects on microorganisms, meaning that the antibiotic will partially slow down its effectiveness. This may also slow the resistance to antibiotics. Thus, essential oil drug interactions can shed light on us to prevent and/or understand the resistance that occurs in this time frame.

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