Black Sea Journal of Agriculture

doi: 10.47115/bsagriculture.1091459

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 5 - Issue 3: 248-254 / July 2022

THE ANTAGONISTIC AND SYNERGISTIC COMPARISON OF THE ANTIMICROBIAL CHARACTERISTICS OF EXTRACTS OF SOME HERBS

Ayşe Gül DOĞAN[1]*, Cihan DARCAN[2]

[1]*Bilecik Şeyh Edebali University, Institute of Science, Department of Molecular Biology and Genetics, 11230, Bilecik, Türkiye*
[2]*Bilecik Şeyh Edebali University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, 11230, Bilecik, Türkiye*

Abstract: In the present study, the antagonistic and synergistic effects of *Achillea millefolium* L., *Anthemis cretica* L., *Cichorium intybus* L., *Euphorbia seguieriana* Necker and *Hypericum perforatum* L plant extracts collected from Samsun were investigated. Gram negative bacteria; *Escherichia coli*, *Acinetobacter baumannii*, *Salmonella typhimurium*, Gram positive bacteria; *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* were used as research materials. In the research, methanol and diethyl were used as solvents. The antibacterial activities of the extracts were determined by microbroth dilution method. According to the results of the research; all plant extracts obtained using both methanol and diethyl ether solvent were determined to be more effective against gram positive bacteria. While the whole plant extract showed the most effect on Bacillus cereus bacteria, *Hypericum perforatum* L. methanol extract was the most effective plant against gram positive bacteria. *Achillea millefolium* L.: *Cichorium intybus* L., *Achillea millefolium* L.: *Hypericum perforatum* L. and *Cichorium intybus* L.: *Hypericum perforatum* L. methanol mixture extracts and *Achillea millefolium* L.: *Hypericum perfarotum* L. diethyl ether extract showed a synergistic effect, while other plant mixture extracts showed semi-synergistic or ineffective properties.

Keywords: Combination, Plant extracts, Synergistic effects, Antimicrobial activity, Herbal medicine

***Corresponding author:** Bilecik Şeyh Edebali University, İnstitute of Science, Department of Molecular Biology and Genetics, 11230, Bilecik, Türkiye
E mail: ayydogan@gmail.com (A. G. DOĞAN)
Ayşe Gül DOĞAN https://orcid.org/0000-0001-7642-1520
Cihan DARCAN https://orcid.org/0000-0003-0205-3774

Received: March 22, 2022
Accepted: May 11, 2022
Published: July 01, 2022

Cite as: Doğan AG, Darcan C. 2022. The antagonistic and synergistic comparison of the antimicrobial characteristics of extracts of some herbs. BSJ Agri, 5(3): 248-254.

1. Introduction

The treatment with plant extracts, which has survived from the earliest known civilizations to the present, has been the first method that comes to mind in the prevention and cure of many diseases. Treatments with herbal extracts are one of the oldest health care known to mankind (Gupta and Gupta, 2019) and have contributed greatly to people's health needs throughout their existence (Mehmood et al., 2012). It is estimated that there are between 250 and 500 thousand plant species on the planet, and only 1% to 10% are used by humans as food and medicine (Maciel et al., 2002).

Developing living conditions brought with it many diseases. The fact that the diseases experienced in the past become incurable again, and the inadequacy of the treatment of some serious diseases today, has increased the tendency to natural origin drugs. Plants are the most basic products used directly or indirectly in the treatment of such diseases (Çolak et al., 2020). Bacteria have become a serious problem due to their increasing frequency of infection as well as advanced antibiotic resistance (Nilson et al., 2014). Many studies have found that the plant species included in the study have effects on bacteria (Betoni et al., 2006; Stefanovic et al., 2012; Enerva et al., 2015; Leblebici et al., 2016; Gul et al., 2017; Riccobono et al., 2017; Hundur et al., 2018; Darcan et al., 2021; Yanar et al., 2021). Plant-based antibiotics and their synergistic effects could be a useful and practical solution to prevent antibiotic resistance. Studies of synergistic effects of plant extracts are therefore necessary to identify new combinations with highly desirable efficacy (Bahmani et al., 2019). Despite the obtained valuable information about *Achillea, Anthemis, Cichorium, Euphorbia* and *Hypericum* species their synergistic effects have not been sufficiently studied yet (Ma et al., 2009). The ability of plant extracts mixtures to act synergistically could be a new approach to solve the problem of bacterial resistance (Stefanovic and Comic, 2012).

In current study, it was aimed to compare the antimicrobial properties of the extracts of *Achillea millefolium* L. (white yarrow), *Anthemis cretica* L. (mountain daisy), *Cichorium intybus* L. (wild chicory), *Euphorbia seguieriana* Necker (euphorbia), *Hypericum perforatum* L. (St. John's wort) as antagonistically and synergistically.

2. Material and Methods

Achillea millefolium L. (white yarrow), *Anthemis cretica* L. (mountain daisy), *Cichorium intybus* L. (wild chicory), *Euphorbia seguieriana* Necker (spurgery), and *Hypericum perforatum* L. (St. John's wort) were used as research materials (Figure 1). They were collected from Samsun-Alaçam (41 26' 47.88 N°, 35 28' 50.42 E°, elevation: 1657 m) on 8 August 2020. Plants were diagnosed at the flowering time by Prof. Sebahattin Albayrak who is an expert on the rangeland and forage plant management in the Ondokuz Mayıs University, Samsun.

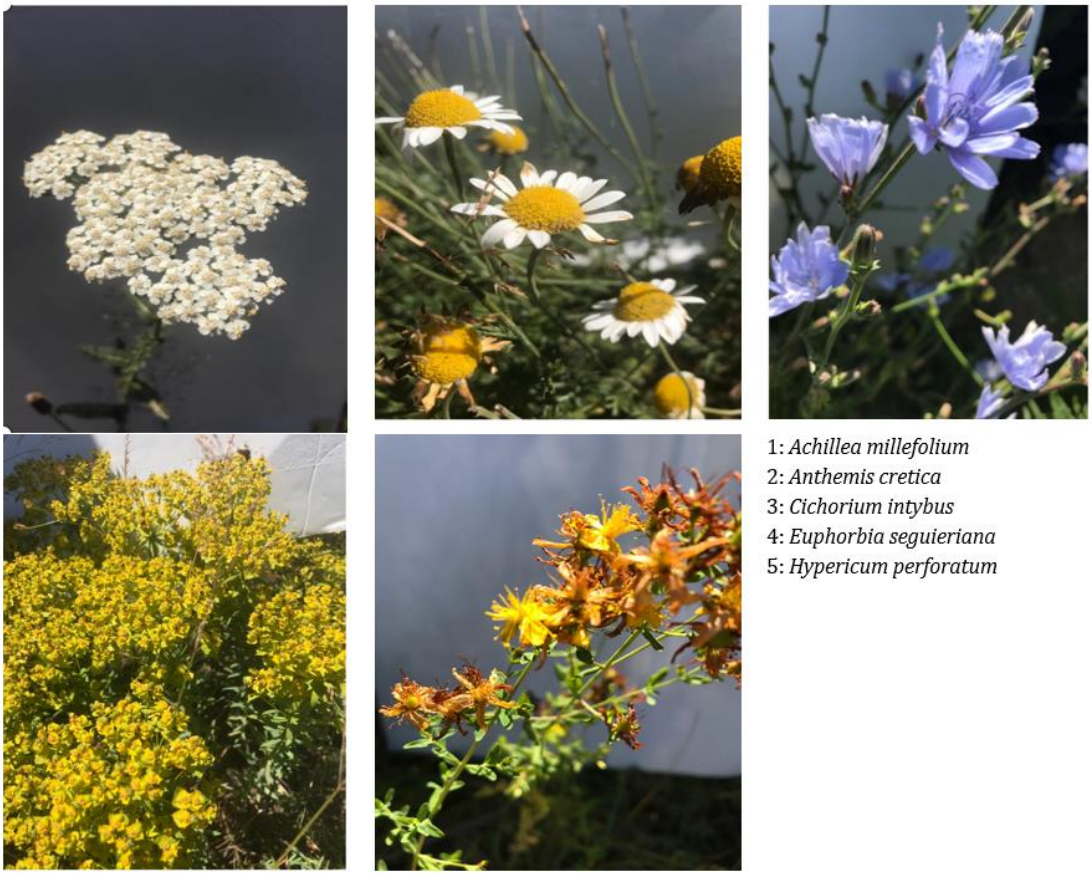

Figure 1. Original plant images used in the present research.

The test strains were obtained from the Faculty of Art and Science, Bilecik Şeyh Edebali University. In the research, gram negative bacteria; *Escherichia coli* W3110, *Acinetobacter baumannii* ATCC19606, *Salmonella typhimurium* ATCC 14028, Gram positive bacteria; *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 7644 were used.

The above-ground parts of the plants were dried at room temperature, in a shade and moisture-free environment. The leaf-flower parts of the dried plants were grinded separately with the mill in the laboratory and turned into powder. 5 g of each plant was weighed and treated in two different solvents (80% methanol, 20% water (80ml:20 ml) and (100 ml diethyl ether) and extracted in a Soxhlet device for 7 hours (Del monte et al. 2015).

The antimicrobial activities of the extracts were determined using the microbroth dilution method. U-bottom 96 microtiter plates were used for the experiment. Nutrient broth (NB) medium was used as a growth medium for the growth of bacterial strains. 5.2 g nutrient broth (Biolife) was weighed and dissolved in 400 ml distilled water and sterilized in an autoclave (Core) for 15 minutes at 121 °C. 6 g nutrient broth (Biolife) was poured into petri dishes by applying aseptic techniques when it reached the temperature of 55 °C. According to the method reported by (Aydın and Sevindik, 2018) different medium were added for each bacterial group, the first well in which the decrease in turbidity color in the wells was observed was accepted as the Minimum Inhibitory Concentration (MIC). In MIC experiments, one drop was taken from the wells without growth and allowed to grow on Nutrient Agar media. Therefore, it was determined whether the inhibition was caused by a static or cidal effect. Thus, cidal concentration values were determined (Darcan et al., 2021).

Plants were extracted in a soxhlet device for 7 hours. After using only one extract on each bacterial species, 50-50 were mixed together to evaluate the synergist effectiveness in each bacterial species (Al-Terehi et al. 2015). In vitro interactions between antimicrobial agents were determined by calculating the fractional inhibitory concentration (FIC) index using the following formula: FIC=FICA+FICB; FIC A=Combination effect/MIC A: The effect of MIC A alone; FIC B=Combination effect/MIC B: The effect of MIC B alone; FIC≤0.5, total synergism; 0.5<FIC≤0.75, partial synergism; 0.75<FIC≤2, no effect; FIC>2, antagonism (Sharma et al. 2020).

3. Results

The results of antimicrobial activity *Achillea millefolium*, *Anthemis cretica*, *Cichorium intybus, Euphorbia seguieriana* and *Hypericum perforatum* are shown in Table 1-5, synergistic activities of those plants are given in Table 6 and 7.

In research of present study, the effect of methanol solvent on all bacterial groups was more effective than diethyl ether solvent. The control group of diethyl ether was more effective on *E.coli* bacteria than all the plants in the study. In methanol, *A.baumanni* and *L. monocytogenes* in Euphorbia and *A. baumanni* in Hypericum control group were found to be more effective (Table 1 to 5).

All plant extracts were more effective on gram positive bacteria than gram negative bacteria. Among the plants, only *Anthemis* extract showed a very high effect on the gram-negative bacteria *A. baumannii* (5.375 mg ml^{-1}). Extracts of all plants in the study showed the greatest effect on *B. cereus* bacteria (1.321 to 5.562 mg ml^{-1}). The most effective plant extract was *H. perforatum* (1.321 mg ml^{-1}). *Euphorbia* extract showed the lowest effect on both bacterial groups (except for *B. cereus*, 5.562 mg ml^{-1}) (Table 1 to 5).

Table 1. Antibacterial activities of *Achillea millefolium* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	11.85	23.70	25.00	50.00	14.15	14.15	12.50	25.00
A.baumannii	11.85	23.70	12.50	25.00	14.15	28.30	25.00	50.00
S.typhimurium	11.85	23.70	25.00	50.00	14.15	14.15	25.00	25.00
Gram- positive								
S. aureus	5.925	11.85	25.00	25.00	10.61	10.61	25.00	25.00
B. cereus	2.962	5.924	25.00	50.00	10.61	10.61	25.00	25.00
L. monocytognes	5.925	11.85	12.50	25.00	10.61	10.61	12.50	25.00

Table 2. Antibacterial activities of *Anthemis cretica* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	21.50	21.50	25.00	50.00	21.37	14.25	12.50	25.00
A.baumannii	5.375	10.75	12.50	25.00	14.25	14.25	25.00	50.00
S.typhimurium	10.75	21.50	25.00	50.00	14.25	14.25	25.00	25.00
Gram-positive								
S. aureus	5.375	10.75	25.00	25.00	10.68	10.68	25.00	25.00
B. cereus	2.680	5.36	25.00	50.00	10.68	10.68	25.00	25.00
L. monocytognes	10.75	10.75	12.50	25.00	10.68	10.68	12.50	25.00

Table 3. Antibacterial activities of *Cichorium intybus* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	12.35	12.35	25.00	50.00	13.60	13.60	12.50	25.00
A.baumannii	12.35	12.35	12.50	25.00	13.60	27.30	25.00	50.00
S.typhimurium	12.35	12.35	25.00	50.00	13.60	13.60	25.00	25.00
Gram- positive								
S. aureus	6.175	6.175	25.00	25.00	6.82	6.82	25.00	25.00
B. cereus	5.375	10.75	25.00	50.00	6.82	6.82	25.00	25.00
L. monocytognes	6.175	12.35	12.50	25.00	6.82	6.82	12.50	25.00

Table 4. Antibacterial activities of *Euphorbia seguieriana* Necker Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	44.50	44.5	25.00	50.00	18.50	18.50	12.50	25.00
A.baumannii	22.50	22.5	12.50	25.00	18.50	18.50	25.00	50.00
S.typhimurium	44.50	44.5	25.00	50.00	18.50	18.50	25.00	25.00
Gram positive								
S. aureus	11.12	11.12	25.00	25.00	13.90	13.90	25.00	25.00
B. cereus	5.562	5.562	25.00	50.00	13.90	13.90	25.00	25.00
L. monocytognes	22.25	22.25	12.50	25.00	13.90	13.90	12.50	25.00

Table 5. Antibacterial activities of *Hypericum perforatum* L.Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	21.50	21.50	25.00	50.00	21.55	21.55	12.50	25.00
A.baumannii	21.50	21.50	12.50	25.00	10.75	10.75	25.00	50.00
S.typhimurium	10.57	10.57	25.00	50.00	21.55	21.55	25.00	25.00
Gram positive								
S. aureus	2.643	2.643	25.00	25.00	10.70	10.70	25.00	25.00
B. cereus	1.321	1.321	25.00	50.00	10.70	10.70	25.00	25.00
L. monocytognes	2.643	2.643	12.50	25.00	10.70	10.70	12.50	25.00

Table 6. Binary mixture antibacterial activity FIC values of methanol extracts

	CP	CY	CS	CK	PY	PS	PK	YS	YK	SK
Gram- negative										
E.coli	0.67	0.67	0.90	1.02	0.61	0.84	0.96	0.84	0.96	1.19
A.baumannii	0.83	0.66	1.09	0.97	0.85	1.27	1.16	1.11	0.99	1.41
S.typhimurium	0.65	0.64	0.88	1.06	0.64	0.87	1.05	0.86	1.04	1.27
Gram positive										
S. aureus	0.65	0.49	1.10	0.49	0.65	1.26	0.64	1.10	0.49	1.10
B. cereus	0.61	0.49	0.77	0.48	0.60	0.88	0.59	0.77	0.48	0.76
L. monocytognes	0.57	0.49	0.90	0.48	0.57	0.97	0.56	0.90	0.47	0.89

C= *Achillea millefolium*, P= *Anthemis cretica*, Y= *Cichorium intybus*, S= *Euphorbia seguieriana*, K= *Hypericum perforatum.*

Table 7. Binary mixture antibacterial activity FIC values of diethyl ether extracts

	CP	CY	CS	CK	PY	PS	PK	YS	YK	SK
Gram- negative										
E.coli	1.12	1.12	1.13	1.14	1.57	1.58	1.58	1.58	1.59	1.60
A.baumannii	1.08	1.16	1.16	1.16	1.55	1.55	1.55	1.63	1.63	1.63
S.typhimurium	1.10	1.14	1.14	1.11	1.56	1.56	1.53	1.59	1.59	1.56
Gram positive										
S. aureus	0.73	0.84	1.16	0.24	0.90	1.21	0.64	1.31	0.74	1.06
B. cereus	0.72	0.81	1.18	0.25	0.80	1.17	0.60	1.26	0.69	1.06
L. monocytognes	0.75	0.88	1.15	0.24	0.89	1.17	0.63	1.29	0.75	1.03

C= *Achillea millefolium*, P= *Anthemis cretica*, Y= *Cichorium intybus*, S= *Euphorbia seguieriana*, K= *Hypericum perforatum.*

When the cidal (MCC) and static (MIC) values are compared, it can be found that while the active substances have a static effect on Gram-negative bacteria, a cidal effect on the Gram-positive bacteria at MIC value (Table 1 to 5).

In the study, it was determined whether the inhibition effect of the extracts of 5 plants obtained with methanol and diethyl ether was cidal or static, according to the MIC value seen on the bacteria. For this purpose, reproduction status was tested by sowing on the solid medium from wells that did not show growth.

MIC and MCC values of *A. millefolium* were determined to be different in all bacteria. The MIC value was 11.85 mg/ml in Gram-negative bacteria, the MCC value was 23.7 (Table 1). The MIC value of S. aureus and *L. monocytogenes* (Gram-positive bacteria) were 5.92. In addition, the MIC value of *B. cereus* was determined as 2.96, the MCC value was 5.92. It was clearly seen that the effect of the methanol extract of this plant on MIC concentrations was a static effect. While the methanol extract of *Anthemis cretica* plant had a cidal effect on *E. coli* and *L. monocytogenes*, the diethyl ether extract had a cidal effect on all bacteria except *E. coli* (Table 2). Cichorium intybus methanol extract had a cidal effect except B. ceraus and L. monocytogenes. Diethyl ether extract obtained from this plant showed a static effect only on *A. baumanni* bacteria, while MIC value was found to be cidal concentration in other bacteria (Table 3). Both methanol and diethyl ether extracts of the 2 plants (*Euphorbia* and *Hypericum*) had cidal effects on gram negative and gram positive bacteria. Therefore, the MIC values of these 2 plants were the cidal concentration (Table 4 and 5).

Binary mixtures of plants were determined as effects of synergistic or antagonistic on bacteria. According to fractional inhibitory concentration (FIC) index; FIC≤0.5, total synergism; 0.5<FIC≤0.75, partial synergism; 0.75<FIC≤2, no effect; FIC>2, antagonism (Sharma et al. 2020).

Partial synergism was observed in the combination of *Achillea* and *Anthemis* (FIC 0.57 to 0.75), except for gram-negative bacteria of Diethyl ether and *A.baumannii* of methanol. In the *Achillea+Cichorium* combination, synergism was found in gram positive bacteria and partial synergism in gram negative bacteria of methanol, ineffectiveness in diethyl ether. *Achillea* and *Euphorbia* mixture was ineffective with both methanol and ether solvents. *Achillea+Hypericum* combinations showed a synergistic effect of both methanol and ether solvents on gram-positive bacteria (FIC: 0.24 to 0.49), but not effect on gram-negative bacteria (FIC: 0.97 to 1.16). Except for the methanol solvent *A. baumannii*, the *Anthemis+Cichorium* mixture showed partial synergism, while the diethyl solvent had no effect on all bacteria. The *Anthemis +Euphorbia* combination did not show any effect on both gram positive and gram negative bacteria (FIC: 0.84 to 1.58). The *Anthemis* and *Hypericum* mixture was semi-synergist against gram positive bacteria (FIC: 056 to 0.64) and ineffective against gram negative bacteria in both solvents. It was determined that the *Cichorium+Euphorbia* combination did not affect any bacterial group (FIC: 0.77 to 1.63). The methanol extract of *Cichorium+Hypericum* combination showed a synergist effect, diethyl ether extracts semi-synergistic effect on gram positive bacteria; it had no effect on other groups. The *Euphorbia+Hypericum* mixture was not effective on any bacteria group (FIC: 0.76 to 1.63) (Table 6 and 7).

4. Discussion

Achillea millefolium, Anthemis cretica, Cichorium intybus, Euphorbia seguieriana and *Hypericum perforatum* methanol and diethyl ether extracts were generally found to be more effective on gram-positive bacteria. On the other hand, the effect of methanol on all bacterial groups was more effective than diethyl ether (Table 1 to 5).

The effect of methanol extracts of *Achillea millefolium* on gram-negative bacteria used in this study was the same (MIC 11.85), the effect on gram-positive bacteria was in the range of 2.96-5.92 mg ml^{-1}. In diethyl ether solvent, the MIC value of *A. millefolium* extract was 14.15 on gram-negative bacteria, and it was 10.612 on gram-positive. Kharma and Hassawi (2006) reported that *Achillea spp*. extract had the greatest effect on *S. aureus* bacteria. Salvagnini et al. (2006) found that *Achillea millefolium* extract was effective only against *Bacillus subtilis* from gram-positive bacteria. Kharma and Hassawi (2006) and Salvagnini et al. (2006)'s findings are consistent with our research results.

B. cereus (MIC: 2.68 mg ml^{-1}) was the most sensitive bacteria compared to other bacteria according to the antibacterial effect of *Anthemis cretica* extracts. The finding shown that *Anthemis* extracts had a greater effect on gram-positive bacteria (Formisano et al., 2012; Riccobono et al., 2017) was consistent with our research results.

In Methanol solvent, *Cichorium intybus* extracts showed similar antibacterial effects with *E. Coli, A.baumannii* and *S.typhimurium* (MIC: 12.35 mg ml^{-1}). MIC values of gram-positive bacteria were found in the range of 5.375-6.175. In diethyl ether solvent, the MIC value was 13.65 gram-negative bacteria, and it was 6.825 in gram-positive. Koner et al. (2011) the effect of chicory root extract had more bacteriostatic effect on Gram-positive bacteria than Gram-negative bacteria; Nandagopal and Kumari (2007) concluded that chicory root extracts showed more inhibitory effect on gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus)* bacteria than gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria. The researchers' findings were in agreement with our results.

It had been observed that *Euphorbia seguieriana* extracts (MIC: >44.5 mg ml^{-1}) had a low antibacterial effect on E.coli and S.typhimurium. Rocha et al. (2021) found *Euphorbia macroclada* had no effect on some bacteria, on the other hand, Enerva et al. (2015) reported that *Euphorbia hirta* extract had high effects on *P. aeruginosa, Staphyloccus aureus, Candida albicans* and *Trichopyton mentagrophytes* bacteria. It could be thought that the variability between the findings of different studies may be due to the differences in the material used or the method applied.

Antibacterial activity of *Hypericum perforatum* extracts on *S. aureus* (MIC: 2.643 mg ml^{-1}), *B. aureus* (MIC: 1.321 mg ml^{-1}) and *L. monocytogenes* (MIC: 2.643 mg ml^{-1}) are more effective compared to gram negative bacteria in methanol solvent. On the other hand, the MIC values of the diethyl ether solvent on bacteria were found to be less efficient. *H. perforatum* was reported to be good antibacterial agents in many sources (Meral and Karabay, 2002; Okmen and Balpınar, 2017; Önem and Çevik Baş, 2018; Özkan et al., 2018). The finding of our research the antibacterial activity of *Hypericum perforatum* extract on gram-negative bacteria was weaker than gram-positive bacteria was consistent with other research results (Düzgüner and Erbil, 2020; Okmen and Balpınar, 2017).

In Methanol solvent, *Achillea millefolium* and *Cichorium intybus, Achillea millefolium* and *Hypericum perforatum, Cichorium intybus* and *Hypericum perforatum* extracts showed synergistic effects on gram- positive bacteria (*S. aureus, B. cereus and L. monocytoges*) (FIC values ranged from 0.47-0.49). The synergistic effect of *Achillea millefolium L: Hypericum perforatum* mixture was found to be high in the extract obtained by using diethyl ether solvent (FIC 0.24, 025 and 024, respectively). Two or more agents in the combination interact in different manners leading to one of the four possible effects-synergistic, partial synergistic, no effect, and antagonism (Kasrati et al., 2014). Synergistic interactions are the most important because they enhance the antimicrobial and antioxidant activity by utilizing the efficiencies of the combined agents in the best possible manner and thereby result in several fold reduction in the required doses of the combined agents (Sharma et al. 2020).

Bahmani et al. (2019) reported that *Origanum vulgare* and *Hypericum perforatum* had a synergistic effect of 0.5 and that this plant combination could be used as a new antibacterial strategy against *S. aureus*. It was stated that their synergistic studies not only show promise in the fight against drug-resistant pathogens and in the future treatment of infectious diseases, but they could also change the purpose of traditional antibiotics, which were often ineffective when used alone. Gram-negative bacteria were generally more resistant to the antagonistic effects of essential oils than Gram-positive ones, due to the lipopolysaccharide and porin proteins found in the outer membrane (György 2010; Darcan 2012). It was stated that their synergistic studies not only show promise in the fight against drug-resistant pathogens and in the future treatment of infectious diseases, but they could also change the purpose of traditional antibiotics, which are often ineffective when used alone (Fatemi et al., 2020). Synergy was a situation that occurs when two or more herbal ingredients mutually increase the effect of each other more than the simple sum of these ingredients (Ma et al., 2009). Studies examining the interactions of plant extracts in combination increase their antibacterial activity compared to studies examined as single extracts. It should be noted that in addition to the synergistic effects obtained with Gram-positive bacteria, antagonistic effects may also occur in Gram-negative bacteria (Obuekwe, 2020).

5. Conclusion

All plant extracts obtained using both methanol and diethyl ether solvents were determined to be more effective against gram positive bacteria. It was determined that methanol solvent was more effective on bacteria than diethyl ether. *Hypericum perforatum* had been an effective herb against gram-positive bacteria. *Achillea millefolium: Cichorium intybus*, *Achillea millefolium: Hypericum perforatum* and *Cichorium intybus.: Hypericum perforatum* methanol mixture extracts and *Achillea millefolium: Hypericum perforatum* diethyl ether extract showed a synergistic effect, other plant mixture extracts showed semi-synergistic or ineffective properties. It will be useful to conduct new research on the antimicrobial single and mixture extracts of the plants used in the study at the point of combating bacteria.

Author Contributions

A.G.D (50%) and C.D. (50%) design of study. A.G.D (50%) and C.D. (50%) data acquisition and analysis. A.G.D (50%) and C.D. (50%) writing up. A.G.D (50%) and C.D. (50%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

This study includes a part of the master thesis of Bilecik Şeyh Edebali University, Institute of Science, Department of Molecular Biology and Genetics.

References

Al-Terehi M, Abed-Neama Z, Al-Askeri M, Zaida HK, Al-Saadi AH, Behjet RH, Haleem Z. 2015. Efficiency of Plants Extracts Synergism as Antibacterial Activity on Pathogenic Bacteria. Adv Life Sci Tech, 36: 8-15.

Aydın S, Sevindik E. 2018. Achillea millefolium L. subsp. millefolium essential oil's antifungal effect. European J Biol Res, 8(3): 153-156.

Bahmani M, Taherikalani M, Khaksarian, M, Kopaei MR, Ashrafi B, Nazer M, Soroush S, Abbasi N, Rashidipour M. 2019. The synergistic effect of hydroalcoholic extracts of Origanum vulgare, Hypericum perforatum and their active components carvacrol and hypericin against Staphylococcus aureus. Future Sci OA, 5(3): FSO371.

Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes A. 2006. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem Inst Oswaldo Cruz, 101: 387.

Çolak S, Çolak S, Dağlı F, Çömlekcioğlu N, Kocabaş YZ, Aygan A. 2020. Achillea aleppica subsp. aleppica'nın farklı organlarından elde edilen ekstraktların antimikrobiyal aktivitesi ve bazı fitokimyasal özellikleri. Gıda, 45(5): 929-941.

Darcan C. 2012. Expression of OmpC and OmpF porin proteins and survival of Escherichia coli under photooxidative stress in Black Sea water. Aquatic Biol, 17(2): 97-105.

Darcan C, Kaygusuz Ö, Leblebici S, Gülümser E, Acar Z. 2021.

Antimicrobial activity of leaf extracts of Bituminaria sp. genotypes at different growth stages Indian J Exp Biol, 59: 302-309.

Del Monte D, De Martino L, Marandino A, Fratianni F, Nazzaro F, De Feo V. 2015. Phenolic content, antimicrobial and antioxidant activities of Hypericum perfoliatum L. Indust Crops Prod, 74: 342-347.

Düzgüner V, Erbil N. 2020. Ardahan yöresinde yetişen kılıç otu bitkisinin (Hypericum perforatum) antimikrobiyal ve antioksidan etkilerinin araştırılması. Türk Tarım Doğa Bil Derg, 7(1): 27-31.

Enerva LT, Atienza TV, Glifonea ZR, Villamor OB, Villa NA. 2015. Cytotoxicity and antimicrobial property of the leaf extract of euphorbia hirta. Open J Soc Sci, 3: 162-170.

Fatemi N, Sharifmoghadam MR, Bahreini M, Khameneh B, Shadifar H. 2020. Antibacterial and synergistic sffects of herbal extracts in combination with amikacin and imipenem against multidrug-resistant Isolates of Acinetobacter. Curr Microbiol, 77: 1959-1967.

Formisano C, Rigano D, Senatorea F, Raimondo FM, Maggio A, Bruno M. 2012. Essential oil composition and antibacterial activity of Anthemis mixta and A. tomentosa (Asteraceae). Natural Prod Commun, 7(10): 1379-1382.

Gul M, Cali IO, Cansaran A, Idil O, Kulu I, Celikoglu U. 2017. Evaluation of phytochemical content, antioxidant, antimicrobial activity and DNA cleavage effect of endemic Linaria corifolia Desf. (Plantaginaceae). Cogent Chem, 3(1): 1337293.

Gupta R, Gupta J. 2019. Investigation of antimicrobial activity of euphorbia hirta leaves. Int J Life Sci Pharma Res, 9(3): 32-37.

György É. 2010. Study of the antimicrobial activity and synergistic effect of some plant extracts and essential oils. Rev Română de Medicină de Lab, 18(1/4): 49-56.

Hundur DO, İdil Ö, Kandemir N, Gül M, Konar V. 2018. Phytochemical screening and in-vitro antioxidant, antimicrobial activity and DNA interaction of Leucojum aestivum. Fres Env Bul, 27(10): 6704-6710.

Kasrati A, Jamali CA, Fadli M, Bekkouche K, Hassani L, Wohlmuth H, Leach D, Abbad A. 2014. Antioxidative activity and synergistic effect of Thymus saturejoides Coss. Essential oils with cefixime against selected food-borne bacteria. Ind Crops Prod, 61: 338-344.

Kharma A, Hassawi D. 2006. The antimicrobial activity and the genetic relationship of achillea species. Biotechnol, 5(4): 501-507.

Koner A, Ghosh S, Roy P. 2011. Isolation of antimicrobial compounds from chicory (Cichorium intybus L.) root. Int J Res Pure Appl Microbiol, 1(2): 13-18.

Leblebici S, Kaygusuz Ö, Korkmaz T, Darcan C. 2016. The antimicrobial activities of the leaves of some endemic stachys species spreading in west Anatolia Turkey. Mitteilungen Klosterneuburg, 66(6): 18-28.

Ma XH, Zheng CJ, Han LY, Xie B, Jia J, Cao ZW, Chen YZ. 2009. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. Drug Discov Today, 14(11-12): 579-588.

Maciel MAM, Pinto AC, Veiga Jr VF, Grynberg NF, Echevarria A. 2002. Medicinal plants: the need for multidisciplinary scientific studies. Quim Nova, 25(3): 429-438.

Mehmood N, Zubaır M, Rızwan K, Rasool N, Shahid M, Ahmad VU. 2012. Antioxidant, antimicrobial and phytochemical analysis of cichoriumintybus seeds extract and various organic fractions. Iranian J Pharm Res, 11(4): 1145.

Meral GE, Karabay NÜ. 2002. In vitro antibacterial activities of three Hypericum species from west Anatolia. Turkish E J Biotechnol, Special Issue: 6-10.

Nandagopal S, Kumari BR. 2007. Phytochemical and antibacterial studies of Chicory (Cichorium intybus L.)-A multipurpose medicinal plant. Adv Biol Res, 1(1-2): 17-21.

Nilson S, Gendron F, Bellegarde J, McKenna B, Louie D, Manson G, Alphonse H. 2014. Prelimınary scientific investigation of the effectiveness of the medicinal plants plantago major and Achillea millefolıum against the bacteria Pseudomonas aerugınosa and Staphylococcus aureus in partnership with indigenous elders. Global J Res Medic Plants Ind Medic, 3(11): 402.

Obuekwe IS, Okoyomo EP, Anka US. 2020. Effect of plant extract combinations on some bacterial pathogens. J App Sci Environ Manag, 24(4): 627-632.

Okmen G, Balpınar N. 2017. The biological activities of Hypericum perforatum L. African J Trad Complem Altern Medic, 14(1): 213-218.

Önem E, Çevikbaş H. 2018. Hypericum atomarium Boiss. farklı çözücü ekstraktlarının klinik stafilokok türleri üzerine antibakteriyel etkisi. Süleyman Demirel Üniv Fen Edeb Faki Fen Derg, 13(2): 164-172.

Özkan EE, Çelik BÖ, Mat A. 2019. Antimicrobial activities of five endemic Hypericum species from Anatolia compared with Hypericum perforatum. J Res Pharm, 23(1): 114-119.

Riccobono A, Maggio M, Bruno V, Spadaro FM, Raiomondo R. 2017. Chemical composition and antimicrobial activity of the essential oils of some species of Anthemis sect. Anthemis (Asteraceae) from Sicily, 31: 23.

Rocha ARF, Sousa HG, Júnior EPV, Lima FL, Costa A, Araújo AR, Lago EC. 2021. Extracts and fractions of Croton L. (Euphorbiaceae) species with antimicrobial activity and antioxidant potential. Food Sci Technol, 139: 110521.

Salvagnini LE, Migliato KF, Isaac VLB, Correa MA, Salgado HRN, Pietro CLR. 2006. Evaluation of efficacy of preservatives associated with achillea millefolium l. extract against bacillus subtilis. Brazilian J Microbiol, 37: 75-77.

Sharma K, Guleria S, Razdan VK, Babu V. 2020. Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. Indust Crops Prod, 154: 112569.

Stefanovic OD, Stanojevic DD, Comic LR. 2012. Synergistic antibacterial activity of Salvia officinalis and Cichorium intybus extracts and antibiotics. Acta Pol Pharm, 69(3): 457-463.

Yanar O, Aykol G, Topkara EF, Solmaz FG, Mercan S, Eski A. 2021. An example of the effect of diet quality on the immune system of an infected insect: Uresiphita gilvata (Fabricius, 1794), Fres Envir Bul, 30(5): 4749-4755.