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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

MRSA'YA KARŞI BİTKİSEL ÇAY ÖRNEKLERİNİN SİPROFLOKSASİN İLE SİNERJİK ETKİSİ VE ANTİOKSİDAN AKTİVİTELERİ

SYNERGISTIC EFFECT OF HERBAL TEA SAMPLES WITH CIPROFLOXACIN AGAINST MRSA AND THEIR ANTIOXIDANT ACTIVITIES

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ÖZET

AMAÇ: *Sambucus nigra L*. (mürver), *Salvia sclarea L*. (adaçayı), *Rosmarinus officinalis L*. (biberiye) ve *Coriandrum sativum L*. (kişniş) bitkileri gıdalarda tatlandırıcı ajan olarak kullanılmakta ve dünyada çeşitli hastalıkların tedavisinde halk hekimliğinde iyi bilinmektedir. Bu bitkiler ayrıca, enfeksiyonlarda özellikle öksürük, ateş ve soğuk algınlığı semptomlarını hafifletmek amacıyla yaygın olarak kullanılırlar. Bu çalışma, Türkiye'de *S. nigra*, *S. sclarea, R. officinalis* ve *C. sativum* 'un ticari örneğinden elde edilen üç farklı ekstrenin toplam fenol içeriğini ve antioksidan aktivite potansiyelini taramayı ve karşılaştırmayı amaçlamaktadır. Ayrıca, *Staphylococcus aureus* ATCC 29213 (metisiline duyarlı- MSSA) ve *Staphylococcus aureus* ATCC 43300'e (metisiline dirençli- MRSA) karşı Minimum İnhibitör Konsantrasyonu (MİK) değerlerini ve bir antibiyotik olan siprofloksasin ile sinerjistik aktivite gösterip göstermediği araştırılmıştır.

GEREÇ VE YÖNTEM: *S. nigra* ve *C. sativum*'un meyveleri, *S. sclarea*'nın toprak üstü kısımları ve *R. officinalis*'in yaprakları Türkiye'de bitki çayı pazarlayan bir ticari şirketten satın alınmıştır. Bu örneklerden elde edilen ekstrelerin toplam fenolik içeriği ve antioksidan potansiyeli araştırılmıştır. Ayrıca bitki örneklerinin metisiline dirençli *Staphylococcus aureus* 'a (MRSA) karşı siprofloksasin ile sinerjik etkisi belirlenmiştir.

BULGULAR: *R. officinalis* (biberiye) ve *S.sclarea* (misk adaçayı) ekstreleri yüksek fenolik içerik ve antioksidan aktivite gösterirken, *C. sativum* (kişniş) ve *S.nigra* (mürver) ekstreleri daha düşük fenolik madde içerdiği ve daha zayıf antioksidan aktivite gösterdiği tespit edilmiştir. Ayrıca, *S. sclarea*'nın etanol ekstresi ile birlikte siprofloksasinin, *S. aureus* ATCC 43300'e karşı additif antibakteriyel aktivite gösterdiği bulunmuştur.

SONUÇ: *R. officinalis* (biberiye) ve *S.sclarea* (misk adaçayı) ticari örneklerinden hazırlanan bitki çaylarının yüksek fenolik içeriğiyle antioksidan aktiviteyi destekleyebileceği ve siprofloksasinin misk adaçayının etanol ekstresi ile birlikte metisiline dirençli *S. aureus* ATCC 43300'e karşı additif antibakteriyel aktivite gösterdiği bulunmuştur. Söz konusu etkiye ait mekanizmanın ayrıntılı olarak anlaşılması için daha ileri çalışmalara ihtiyaç vardır.

ANAHTAR KELİMELER: Antioksidanlar, İlaç sinerjisi, Bitki preparatları.

ABSTRACT

OBJECTIVE: *Sambucus nigra L*. (elderberry), *Salvia sclarea L*. (clary sage), *Rosmarinus officinalis L*. (rosemary), and *Coriandrum sativum L*. (coriander) are all consumed as flavoring agents for food and are well-known in traditional medicine for the treating various diseases worldwide. These herbs are also commonly used for microbial infections, especially to relieve cough, fever, and cold symptoms. This study aims to screen and compare the content of total phenols and antioxidant activity potential of three different extracts from each commercial sample of S. nigra, *S. sclarea, R. officinalis* and *C. sativum* from Türkiye. We also investigated the (Minimum Inhibitory Concentration (MIC) values against *Staphylococcus aureus* ATCC 29213 (methicillin-susceptible, MSSA) and *Staphylococcus aureus* ATCC 43300 (methicillin-resistant, MRSA) and the synergistic activity with an antibiotic, ciprofloxacin, by checkerboard assay.

MATERIAL AND METHODS: The fruits of *S. nigra* and *C. sativum*, the aerial parts of *S. sclarea,* and the leaves of *R. officinalis* were purchased from a trading company that marketed them as herbal tea in Türkiye. This study investigated the total phenolic content and antioxidant potential of extracts from commercial samples. We also determined the synergistic effect of herbal tea samples with ciprofloxacin against methicillin-resistant *Staphylococcus aureus* (MRSA).

RESULTS: *R. officinalis* (rosemary) and *Salvia sclarea* (clary sage) extracts showed high phenolic content and antioxidant activity, whereas it was determined that *C.sativum* (coriander) and *S. nigra* (elderberry) extracts exhibited lower antioxidant activity and low phenolic compounds. Moreover, ciprofloxacin in combination with the ethanolic extract of *S. sclarea* showed additive antibacterial activity against *S. aureus* ATCC 43300.

CONCLUSIONS: We conclude that herbal tea prepared from commercial *R. officinalis* (rosemary) and *S.sclarea* (clary sage) samples can support the antioxidant activity with high phenolic content and that ciprofloxacin combined with the ethanolic extract of clary sage showed additive antibacterial activity against methicillin-resistant *S. aureus* ATCC 43300. Further studies are needed to understand the mechanism of additive action in detail.

KEYWORDS: Antioxidants, Drug synergism, Plant preparations.

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INTRODUCTION

Staphylococcus aureus is a pathogen responsible for a wide spectrum of infections. Methicillin-resistant *S. aureus* (MRSA), an opportunistic bacterium that is resistant to various medicines, has been identified as one of the causes of hospital and outpatient infections. Aside from that, MRSA-related illnesses have been more common over the past years (1, 2).

Antimicrobial resistance is reported as significant public health issue both in our country and in the world. The World Health Organization (WHO) 2014 report states that antimicrobial resistance seriously threatens the prevention of various infectious diseases caused by bacteria, parasites, viruses, and fungi, and the success of treatment of these diseases. Although it is reported that antibiotic resistance can occur naturally, it has been stated that the overuse and misuse of antibiotics in humans accelerate the process. It has been remarked that antimicrobial resistance causes a decrease in the effectiveness of antibacterial, antiparasitic, antiviral, and antifungal drugs and makes treatment difficult, costly, and even impossible. For this reason, it is known that there is a need for new compounds that will increase the efficacy of antibiotics or that can be used alone or in combination with treatment protocols (3, 4).

Sambucus nigra L. (elderberry), *Salvia sclarea L.* (clary sage), *Rosmarinus officinalis L.* (rosemary) and *Coriandrum sativum L.* (coriander) are all consumed as flavoring agents for food and very well known plants as traditional remedies of various ailments worldwide. They have also a common use for microbial infectious especially relieving cough, fever, and cold symptoms related to RTI (Respiratory Tract Infection) (5 - 13). Previous phytochemical studies stated that these plants are rich sources of different polyphenols which could be linked to their biological activities depending on antioxidant properties (11, 14 - 16).

In light of all these findings, this study was designed to search for potential candidates that have synergistic effects with ciprofloxacin against infections triggered by methicillin-resistant *Staphylococcus aureus* (MRSA). This study aims to screen and compare the content of total phenols and antioxidant activity potential of three different extracts from each commercial sample of *S. nigra, S. sclarea, R. officinalis* and *C. sativum* from Türkiye. We also investigated the minimum inhibitory concentration (MIC) values against *S. aureus* ATCC 29213 (methicillin-susceptible, MSSA) and *S. aureus* ATCC 43300 (methicillin-resistant, MRSA) and the synergistic activity with an antibiotic, ciprofloxacin, by checkerboard assay.

MATERIALS AND METHODS

Plant Material

The fruits of *S. nigra* and *C. sativum*, the aerial parts of *S. sclarea*, and the leaves of *R. officinalis* were supplied by a trading company that purchased them as herbal tea in Türkiye.

Preparation of Extracts

3 different extraction methods were used and a total of 12 extracts were prepared. The names of the samples and their codes are listed **(Table 1)**.

Table 1: The names and codes of extracts

	Extraction procedure		
Plant name	Infusion	Ultrasound extraction with water	Ultrasound extraction with EtOH 70%
Sambucus nigra, fruit	1a	1b	1c
Salvia sclarea, aerial part	2a	2b	2c
Rosmarinus officinalis, leaves	3a	3b	3c
Coriandrum sativum, fructus	4a	4b	4c

a. Extraction based on the label information: The infusions were prepared from 5 g of each powdered sample by adding 100 mL of boiling water and waiting for 10 minutes, and then filtered. The aqueous extracts were lyophilized.

b. Extraction in an ultrasonic bath with water: 5 g of each powdered sample went through extraction with 100 mL of water in an ultrasonic bath with 35 kHz frequency (Bandelin Sonorex) for 1 hour and then filtered. The aqueous extracts were lyophilized

c. Extraction in an ultrasonic bath with ethanol: 5 g of each powdered sample was extracted with 100 mL of 70% ethanol in an ultrasonic bath with 35 kHz frequency (Bandelin Sonorex) for 1 hour and then filtered. The extracts obtained with ethanol were evaporated to dryness.

Total Phenolic Content

With a few minor adjustments, the Folin-Ciocalteu technique was employed to figure out

the total amount of phenolics present in the extracts. Briefly, plant extract samples (1 mg/mL) were prepared and reacted with 10% Folin-Ciocalteau's reagent. The tubes were then filled with 7.5% Na_2CO_3, and they were subjected to incubation at 45°C for 15 minutes. The absorbance value of the samples was obtained at 765 nm. The experiments were done in triplicate. The same procedure was performed with gallic acid and the outcomes were stated as mg of gallic acid equivalent (GAE) per g of crude extract for each sample (17).

Total Flavonoid Content

The aluminum chloride method was performed to determine the total quantity of flavonoids present in the extracts with slight modifications. Briefly, plant extract samples (1 mg/mL) were prepared and 20% $NaNO_2$ was added to each tube. Following 5 minutes of incubation, 10% AlCl3 was added. 1 M NaOH was added 6 min later and the mixture was diluted with distilled water to a total volume of 1 mL. At 510 nm, the absorbance was recorded. The tests were carried out in triplicate. The same procedure was performed with quercetin and the outcomes were stated as mg of quercetin equivalent (QE) per g of crude extract for each sample (18).

Total Antioxidant Capacity

The total antioxidant capacity of the extracts were measured by using phosphomolybdate assay. 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were reacted to get working solution. Different concentrations of plant extract samples (0.1-1mg/mL) were added to this working solution in a ratio of 1:10. Then incubation was performed at 95°C for 90 min. The tubes were cooled following the end of the incubation. The absorbance value of the samples was recorded at a wavelength of 765 nm. The tests were carried out in triplicate. Total antioxidant capacities were determined as mg ascorbic acid (AAE) per gram crude extract for each sample (19).

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of the plant extracts were investigated by measuring their ferric-reducing antioxidant potential. The method is operated on the conversion of [Fe(III)-TPTZ] complex, which is formed as a result of the reaction of iron (III) with Tripyridyltriazine (TPTZ), to [Fe(II)-TPTZ]. 10 mM TPZT, 20 mM FeCl3 $6H_2O$, and 300 mM sodium acetate buffer (pH= 3.6) was reacted to prepare the FRAP reagent. This reagent was applied to the prepared plant extracts which was then left to incubate at 37°C for 30 min. The absorbance value of the samples was measured at 593 nm. The experiments were performed in triplicate. The findings were presented as Trolox (Vitamin E) equivalent per gram crude extract for each sample (20).

(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) Free Radical Scavenging Assay

The antioxidant capacity of the extracts was assessed by testing their ability to scavenge ABTS free radicals. 2.45 mM potassium persulfate was added to a 7 mM ABTS aqueous solution to get ABTS working solution. Overnight, the reaction mixture was allowed to rest at room temperature in the dark. As a result of diluting ABTS radical cation with ethanol, the final absorbance at 734 nm(pH=7.4) was 0.700±0.02. Serial dilutions of samples (0.01-1 mg/mL) were prepared and mixed with ABTS working solution in a ratio of 1:10. The mixture was incubated at ambient temperature for 6 minutes. The absorbance value of the samples was measured at 734 nm. Three duplicates of each experiment were run. The outcomes were represented as Trolox (Vitamin E) equivalent per gram crude extract for each sample (21).

Antibacterial Activity Test

S. aureus ATCC (American Type Culture Collection) 29213 (methicillin-susceptible, MSSA) and *S. aureus* ATCC 43300 (methicillin-resistant, MRSA) were employed as test bacteria in the antibacterial activity test. The broth microdilution method was utilized to calculate the minimum inhibitory concentration (MIC) values. A series of two-fold dilutions were produced using Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA), with concentrations between 512 g/mL to 4 g/mL. The inoculums were prepared from subcultures for 24 hr. The final setting for the bacterial count was 5 $x10^5$ CFU/mL. The microplates were subjected to incubation at 35 °C for 18 to 24 hours. The final well where observable microbial growth was completely sup-

pressed was recorded to estimate the minimum inhibitory concentration (MIC, g/mL). Alcohol was used to dissolve the test substances. utilized as the negative control. Ciprofloxacin (Sigma, USA) and gentamicin (Sigma, USA) were employed as reference drugs (22, 23).

Broth Microdilution Checkerboard Method

The broth microdilution method, as previously reported, was used to test checkerboard synergy. The extract which has the lowest MIC value was chosen for the checkerboard test between samples. Ciprofloxacin (Sigma, USA) concentrations ranging from 0.03 to 16 g/ mL were examined. Ciprofloxacin was mixed with ethanolic extract of *S.sclarea* at concentrations between 16 and 512 µg/mL against *S. aureus* ATCC 43300 (MRSA) (24). The microtiter plate was subjected to incubation at 37°C for 24 h. FICIwas calculated using the first non-turbid well in each column and row of a 96-well U-bottom microplate (25). The formula that comes next was used to get the ΣFIC (Fractional Inhibition Concentration) for the resulting mixture of agent A (ciprofloxacin) and agent B (ethanolic extract of *Salvia sclarea*). ΣFICI(Fractional Inhibition Concentration Index) = FICA (MIC Value of Substance A In the Combination) + FICB (MIC Value of Substance B In the Combination) , where FICA = MICA (in combination with B) /MICA (alone), and FICB = MICB (in combination with A) /MICB (alone) (26). The following was the interpretation of the combination test results using the FIC index: FICI ≤ 0.5 is considered synergistic;>0.5 to <4 additive /indifference, and ≥ 4 antagonistic (27).

Ethical Committee

This research does not require ethical approval.

Statistical Analysis

IBM SPSS 25 was performed to analyze the data statistically. Three separate runs of each experimental test were operated. The findings were displayed as mean±standard deviation (SD). The post-hoc Tukey test was followed by a one-way analysis of variance. A p-value less than 0.05 was used to indicate statistical significance.

RESULTS

Yields of The Extracts

In this study, the synergistic activity of the extracts from the fruits of S. nigra and C. sativum, the aerial parts of *S. sclarea*, and the leaves of *R. officinalis* obtained by three different methods were evaluated and compared, and the content of phenolic compounds and antioxidant activities of the extracts were determined. In the first method, the extracts were prepared as an infusion based on the label information. In the second method, the extraction was performed with water for one hour in an ultrasonic bath, and in the last method, the extraction was also performed for one hour in an ultrasonic bath with an extraction solvent of 70% ethanol (EtOH). The yields of extracts according to the codes are as follows: (1) *S. nigra*, fruit 1a-27.44%; 1b-35.24%;1c-42.73%. (2) *S. sclarea*, herba; 2a-5.95%; 2b-28.90%; 2c-32.88%. (3) *R. officinalis,* leaves 3a-6.45%; 3b-12.97%; 3c-21.74%. (4) *C. sativum*, fruit 4a-11.76%; 4b-14.28%; 4c-22.36%. According to the results, the highest yields are obtained by ultrasonic extraction with EtOH 70%.

Total Phenolic Content

The total phenolic content of the extracts were derived from the calibration curve of gallic acid (y=0.005x+0.0965, R^2=0.9953) and reported in GAE per gram dry extract **(Table 2)**.

Table 2: Total phenolic and flavonoid contents of plant extracts

Plant extract	Total phenolic content mg GAE / g crude extract		Total flavonoid content mg QE / g crude extract	
	Mean	SD	Mean	SD
1a	55,4400	1,5974	45,0000	3,2222
1b	60,8800	0,3904	44,0741	1,7368
1c	57,8000	2,8703	51,4444	1,2522
2a	73,6300	0,6930	139,0000	5,5076
2b	61,8600	2,8402	83,0000	3,9487
2c	86,6733	1,0633	138,0370	0,7883
3a	90,9667	0,7798	145,6667	3,6124
3b	82,3667	1,4342	128,9630	2,1839
3c	87,9600	1,0182	149,7778	0,4714
4a	22,8733	0,3630	53,0741	3,4647
4b	21,4533	0,0808	40,1111	2,9856
4c	16,4400	0,9606	56,4074	1,0082

mg GAE / g crude extract: mg of gallic acid equivalent per g of crude extract; **mg QE / g crude extract:** mg of quercetin equivalent per g of crude extract

Rosmarinus officinalis contains the highest amount of phenolics among all plants. The infusion of 3a extract with 90.9667± 0.7798 mg GAE/g crude extract yielded the highest concentration of phenolics which was significantly greater than the other extracts (p=0.0001). 3a was followed by 3c, 2c, 3b, and 2a, in that order.

Total Flavonoid Content

The total flavonoid content of the extracts were derived from the calibration curve of quercetin (y=0.0009x+0.0271, R^2 = 0.9955) and expressed

in QE per gram dry extract (Table 2). The lowest level of flavonoids was found in "b" extracts which were prepared by extraction in an ultrasonic bath with water. When methods "a" and "c" were used, a significantly higher amount of flavonoids was found compared to method "b" in *Salvia sclarea*, *Rosmarinus officinalis*, and Coriandrum sativum extracts (p=0.001). However, there was no significant difference between the flavonoid contents of the extracts obtained by method "a" and "c" in S*ambucus nigra, Salvia sclarea*, *Rosmarinus officinalis* and *Coriandrum sativum* (p=0.230, 1.000 0.927, 0.965, respectively). In general, *Rosmarinus offcinalis* contains higher amount of flavonoids than the others. The greatest amount of flavonoids was observed in 3c with a level of 149.7778±0.4714 mg QE/g crude extract. Total flavonoid concent of 3c was significantly higher than other extracts except 3a (p=0.008 for 2a, p=0.003 for 2c, and p=0.0001 for the other samples). No significant difference was observed in 3a and 3c based on flavonoid amounts (p=0.927). The second highest flavonoid content was determined in 3a followed by 2a, 2c, and 3b, respectively.

Total Antioxidant Capacity

The total antioxidant capacity of the extracts were calculated from the calibration curve of ascorbic acid (y=0.0099x+0.0268, R^2= 0.9961) and expressed in AAE per gram dry crude extract **(Table 3)**.

Table 3: Total antioxidant capacity of plant extracts

Plant extract	mg AAE / g crude extract		FRAP value		TEAC value (µM TE/g crude extract)	
	Mean	SD	Mean	SD	Mean	SD
1a	250,6809	39,9648	17,3113	0,4261	38,0079	1,884051
1b	224,8822	5,0871	19,1207	0,4359	45,1625	2,246536
1c	226,3636	3,7415	18,4080	0,1225	40,3023	2,616737
2a	251,7172	7,0729	50,5980	1,5502	65,0965	0,418678
2b	217,3079	79,5735	30,3300	0,6212	42,5227	0,732686
2c	262,5589	5,8971	49,0760	0,7828	58,2874	0,628017
3a	312,7273	2,8641	56,8353	0,2065	85,3266	2,539538
3b	287,0707	2,3667	48,7960	0,6416	63,6903	1,570042
3c	290,7071	3,4742	47,4947	0,7123	76,7905	1,046695
4a	126,1953	0,7581	12,0753	0,5054	"-"	-
4b	127,3737	2,2153	10,6813	0,4486	"-"	-
4c	110,8081	4,7140	8,9030	0,3352	"-"	-

mg AAE / g crude extract: ascorbic acid equivalents (AAE) in milligrams per gram of crude extract; **FRAP:** Ferric ion reducing antioxidant power assay; **TEAC:** Trolox equivalent antioxidant capacity; **µM TE/g extract:** µM Trolox equivalent per gr crude extract; **"-":** no activity

The total antioxidant capacity of all extracts was at significant levels compared to solvent control (p=0.0001). "c" and "a" extracts were found to have higher total antioxidant capacity than "b" extracts. *Rosmarinus officinalis* was the most potent antioxidant among all samples. 3a had the greatest total antioxidant capacity (312.7273±2.8641 mg AAE/g crude extract), which was significantly higher than the others (p=0.0001). The second was 3c followed by 3b, 2c, and 2a, respectively.

Ferric Ion Reducing Antioxidant Power Assay

The ferric ion-reducing antioxidant power of the extracts were calculated from the calibration curve of trolox (y=0.05x+0.2073, R^2= 0.9982). The results were expressed (Table 3). The antioxidant power of all extracts were significantly higher than the control (p=0.0001). *Rosmarinus officinalis* and *Salvia sclarea* was found more effective than the other plants in this assay. However, applied extraction techniques had no specific discernible impact on the antioxidant capacity of the extracts. 3a had the greatest ferric ion reducing power which was significantly higher than the other extracts (p=0.0001). The second active one was 2a followed by 2c, 3b, and 3c, respectively.

ABTS Free Radical Scavenging Assay

ABTS free radical scavenging potential of the extracts were calculated from the calibration curve of Trolox and the results were expressed as µM Trolox equivalent per gr dry extract. The results were shown in (Table 3). Except plant 4, the other extracts possessed statistically significant activity than solvent control (p=0.0001). *Rosmarinus officinalis* was the most potent one. 3a had the greatest ABTS free radical scavenging potential which was significantly higher than the other extracts (p=0.017 for 3c and p=0.0001 for the other extracts). The second active one was 3c followed by 2a and 3b, respectively. The ability of the extracts to scavenge this free radical was not significantly impacted by the extraction technique.

Antibacterial Activity

MIC values (µg/mL) of the extracts are given **(Table 4)**.

Table 4: . Minimum inhibitory concentration values (µg/mL) of the extracts

Plant extract	Gram-positive bacteria	
	S. aureus ATCC 29213(MSSA)	*S.aureus* ATCC 43300 (MRSA)
1a	-	-
1b	-	-
1c	-	-
2a	-	-
2b	-	-
2c	256	128
3a	-	-
3b	-	-
3c	256	256
4a	-	-
4b	-	-
4c	-	512
%70 ethanol	-	-
Ciprofloxacin	<0.25	0.5
Gentamicin	0.5	<0.25

"-": No activity

Broth Microdilution Checkerboard Method

Checkerboard Assay was used to evaluate the synergistic effect of ciprofloxacin with the ethanolic extract of *S.sclarea* which showed the lowest MIC value against *S. aureus* ATCC 43300. Ciprofloxacin in combination with the ethanolic extract of *S.sclarea* exhibited additive antibacterial activity against *S. aureus* ATCC 43300. FICA was 0,24 while FICB was 0,5. The ΣFIC value was 0,74 for *S. aureus* ATCC 43300 thus indicating an additive interaction between ciprofloxacin and the ethanolic extract of clary sage.

DISCUSSION

It is well known that the extraction procedures strongly influence the yield and chemical composition of the extracts. Therefore, it is not surprising that in this study, the yield of all extracts was increased by longer extraction time and sonication in the ultrasonic bath. In addition, aqueous ethanol as an extraction solvent has a positive effect on the yield. According to our results, extract 3a from *R. officinalis* had the lowest extraction yield but the highest total phenolic content and antioxidant potential. Our results are in agreement of previous reports. In fact, the extract of rosemary is accepted as a natural antioxidant attributed to phenolic acids (such as rosmarinic acid), flavonoids and diterpenoids mainly, carnosol and carnosic acid (11, 28, 29).

Both rosemary and clary sage are popular in the food, cosmetic, and medicinal industries for their antioxidant constituents such as phenolic acids, flavonoids, and diterpenoids. Rosmarinic acid, carnasol, and carnosic acid are also found in the genus Salvia. Cuvelier et al. (30) reported the relationship between antioxidant activity potential and the presence of rosmarinic acid, carnosic acid, and carnosol as major constituents. In this study, rosemary and clary sage extracts were found to have high phenolic content and antioxidant activity, while coriander and elderberry had lower antioxidant activity with low phenolic content.

Based on the results of the checkerboard assay, the ethanolic extract of clary sage could be used safely in combination with ciprofloxacin in the treatment of MRSA infections. Polyphenols are compounds with different structural properties and different functional groups. They are naturally present in plants and have a wide variety of applications in the medical field in regards to having antioxidant, and anti-inflammatory actions, such as; allergic diseases, diabetes, cardiovascular and neurodegenerative diseases. Besides, polyphenol-rich plant extracts were reported as having the potential to inhibit bacterial and fungal growth. Earlier reports mentioned that plant extracts with high levels of polyphenols have the ability to block the development of pathogenic bacteria and fungi. They could have potential in clinical settings. Ultrasonication could be a useful tool to increase the yield of the extract, but on the other hand, continuous cavitation could cause the degradation of some polyphenolic compounds depending on the extraction parameters (temperature, solvent, time, frequency, drog-solvent ratio, etc.). In this study, the highest yields are obtained by ultrasonic extraction with EtOH 70% (31-33).

We conclude that herbal tea prepared from commercial rosemary and clary sage samples can support the antioxidant activity with high phenolic content and that ciprofloxacin combined with the ethanolic extract of clary sage showed additive antibacterial activity against methicillin-resistant *S. aureus* ATCC 43300. Further studies are needed to understand the mechanism of additive action in detail.

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