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Research Article

Synergistic interaction between propolis extract, essential oils, and antibiotics against *Staphylococcus epidermidis* and methicillin resistant *Staphylococcus aureus*

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Abstract: The development of multi-drug-resistant bacteria pushed the scientific community to look for new alternatives to solve the problem. Propolis is a beehive substance and one of the richest natural products in bioactive compounds with antibacterial activity. This study was aimed to investigate the possible synergistic interaction between propolis and antibacterial drugs, such as essential oils (EOs) and antibiotics, in order to find increased activity with decreased concentrations. Two ethanol extracts of propolis were used for the test, which were collected from the north of Morocco. The chemical composition was determined by UHPLC-MS. The synergistic effect of propolis extracts with EOs and antibiotics was tested using the checkerboard technique. The chemical analysis showed the presence of more that 100 compounds in propolis extracts, belonging mainly to flavonoids. The combination of propolis with the other antibacterial drugs showed different types of interactions with FIC index values varied from 0.18 to 1, but no antagonist effect was noticed. With FICI<0.5, the synergistic effect was obtained with essential oils as well as with antibiotics. These results indicate that propolis can be a promising source of molecules with medical interest to treat bacterial infection and/or to increase the action of antibiotics.

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1. INTRODUCTION

Multi-drug-resistant bacteria became one the major problems of public health (Ventola, 2015). This fact is increasing, and the situation continues to be more complicated. The overuse and misuse of antibiotics, such as the use of broad-spectrum antibiotics without disease diagnostic, are the main reasons of this antibiotic resistance. To overcome the problem, researchers have been trying to find alternatives to compensate the less active antibiotics and/or to increase their

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efficacy. The combination antibiotic therapy has been known to reduce the evolution of drug resistance (Bantar *et al*., 2004). Indeed, nature is considered an inexhaustible source of bioactive compounds with significant antibacterial action. However, the search for specific molecules from the nature is challenging because of the complexity of the natural products.

Propolis is one of the richest natural products in bioactive compounds. It is a resinous substance collected by honeybees from plants exudates (Ghisalberti, 1979). It is constituted by a mixture of plant secondary metabolites and bee secretions. A highly complex hive products, propolis has been used in traditional medicine for a long time to treat several health problems. The traditional use of propolis has been proven by scientific studies (Kuropatnicki et al., 2013). Thus, it was reported as potent antimicrobial, antiviral, antiparasitic, anti-diabetic, anti-inflammatory, anti-leishmanial, immunomodulatory, and anticancer agent (Krol *et al*., 1993; Orsatti *et al*., 2010; Rivero-Cruz *et al*., 2020; Kwon *et al*., 2020). Recently, the interest in propolis highly increased around the world, especially with the development of sophisticated techniques of separation and identification. In fact, the instable chemical composition of propolis, which varies according to the geographical origin, is what makes it a target for several researchers. This variability led recently to the discovery of several new compounds and made propolis inexhaustible source of new bioactive compounds (Huang *et al*., 2014; Šturm and Ulrih, 2019). These compounds belong to several chemical classes such as phenolics, flavonoids, terpenoids, alkaloids, etc.

Propolis have been highly studied for its antibacterial activity, but few studies have been reported about its combined effect with other antibacterial drugs (Krol *et al*., 1993). Thus, the synergistic interaction between antibacterial drugs is very interesting in the medical field. In fact, the more the effective dose is low the more the product is desired. In this regard, the aim of this study was to investigate the chemical composition and to evaluate the possible interaction between propolis and other antibacterial drugs such as essential oils and antibiotics.

2. MATERIAL and METHODS

2.1. Propolis Collection and Preparation

Propolis samples were collected from the north of Morocco at two geographically different sites; namely, Beni Arouss and M'diq. The samples were harvested from traditional hives. After collection, propolis was congealed, crushed, and extracted using ethanol as solvent. Ethanol was eliminated using rotary evaporator, which allows to obtain sec extract called ethanol extract of propolis (EEP). The extracts were conserved at low temperature in the dark.

2.2. Chemical Analysis: UHPLC/MS

Chromatographic separation was accomplished with a Dionex Ultimate 3000RS UHPLC instrument, equipped with Thermo Accucore C18 (100 mm x 2.1 mm i. d., 2.6 µm) analytical column for separation of compounds. Water (A) and methanol (B) containing 0.1% formic acid were employed as mobile phases, respectively. The total run time was 70 minutes, the elution profile and all exact analytical conditions have been published (Zengin *et al*., 2018).

2.3. Bacterial Strains

Three bacterial strains were used for the test, namely methicillin resistant *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228. These bacteria were stocked in glycerol containing medium under -80°C. Before, use they were transferred to an enrichment medium (Brain Heart broth) in order to optimize their growth. All the tests were carried out using bacterial culture in exponential phase.

2.4. Determination of Minimal Inhibitory Concentration (MIC)

In order to determine the MICs of propolis extracts, essential oils, and antibiotics, the microdilution method was adopted. Briefly, a series of decreased concentrations of each tested agent was prepared in a sterile 92-microplate, in which the tested bacterial strain, in its exponential phase, was added (the final concentration of each bacterium was 10^6 CFU/mL). The microplates were incubated at 37°C for 18h and then 10 µL of resazurin was added to each well. Afterward, the microplates were reincubated at the same temperature during 2h. Finally, the MICs were determined based on the resazurin coloration change. The purple coloration of resazurin changes to pink by the products of bacterial metabolism. In this regard, MIC is the lowest concentration of the antibacterial agents, in which no change of resazurin color is noticed (absence of growth) (Yousif et al., 2020).

2.5. Checkerboard Technique

To determine the interaction between propolis extracts and EOs (*Origanum compactum* and *Origanum elongatum*) and antibiotics (ampicillin, tetracycline, oxytetracycline, chloramphenicol, vancomycin, and neomycin TH) the checkerboard technique was used. This method was carried out in liquid medium using 92-microplate. A panel of EEP concentrations were combined with each antibacterial agent (essential oil and antibiotics). In the microplate the MIC of each agent was determined (EEPs, EOs, antibiotics) as well as the MIC of their combination. From the microplate the fractional inhibitory concentration index (FICI) was calculated applying this formula: Σ FICI = FIC (A) + FIC (B). With: FIC (A) = (MIC of A in combination) / (MIC of A alone), and FIC (B) = (MIC of B in combination) / (MIC of B alone).

The type of interaction was determined based on FICI values: FICI ≤0.5 means that the interaction is synergistic, 0.5< FICI ≤0.75 indicates the presence of a partial synergy, 0.76≤FICI<1 means an additive interaction, 1≤FICI≤4 FICI signifies that there is no interaction (not differential), and FICI>4 indicates an antagonism interaction (Denes & Hidri, 2009).

3. RESULTS and DISCUSSION

3.1. Chemical composition of EEP

The chemical profile of propolis extracts was determined based on their retention time and mass spectra (Figure 1). The results of the chemical analysis of the two extracts are represented in Table 1. More than 100 compounds were identified in the two propolis extracts. These molecules belong to numerous chemical groups such as flavonoids, phenolic acids, organic acids, alkaloids. etc. In fact, flavonoids represent the major part in term of compounds number, which represent more than 75%. There were slight differences between the two regions. This variability can be due to the difference in the vegetable source, bee races, date of collection, and other parameters (Bankova *et al*., 2014).

Alkaloids had not been identified in propolis until the last decade. In this present work, an alkaloid called trigonelline was identified in propolis extracts. This molecule has been known by its interesting biological activities (Zhou *et al*., 2012; Mohamadi *et al.*, 2018) . In addition, the chemical analysis showed also the presence of flavonoid glycosides, rare compounds in propolis with high pharmacological interest.

The chemical components containing in propolis extracts are the secondary metabolites of plants (Salatino *et al*., 2011). Thus, the chemical profile of propolis is highly diversified and depends on the plant species at the site of collection. In this study, two propolis samples were collected from two geographically different sites namely, Beni Arouss and M'diq. These sites exist in the north of Morocco. The north of Morocco is known by its diversified medicinal plants (Bouyahya, 2017), and the popularity of beekeeping. Indeed, propolis of this region could be rich in bioactive compounds, especially those from medicinal plants. In fact, this hypothesis

was proven in this study, since the chemical analysis showed the presence of several components known by their interesting biological activities. Among these important molecules, there are, as example, caffeic acid, ferulic acid, apigenin, kaempferide, quercetin, sakuranetin which are known to possess multiple biological properties such as antibacterial and antioxidant activities (Guz *et al*., 2001; Wu *et al*., 2008; Hirai *et al*., 2010).

3.2. MICs of EEPs, EOs and antibiotics

The minimal inhibitory concentration was determined in liquid medium using the microbroth method. The results are expressed in Table 2. As shown, the MICs of Beni Arouss and M'diq EEPs against *S. aureus* MRSA were 0.62 and 0.32 mg/mL, respectively. The MICs of essential oils of *O. compactum* and *O. elongatum* were 1 and 0.12%, respectively. While, the MICs of antibiotics were low and varied from 0.0025 to 0.02 mg/mL. Concerning *S. epidermidis*, the MICs of the two propolis extracts were 0.62 mg/mL for Beni Arouss and 1.25 mg/mL for M'diq extracts, and those of EOs were 0.5% for both species, and the antibiotic MIC values varied from 0.01 to 0.12 mg/mL. Finally, the MIC values against *S. aureus* 25923 were: 0.31 and 0.15 mg/mL, for Beni Arouss and M'diq extracts, 0.5 and 0.12% for OCEO and OEEO, respectively, and from 0.0025 to 0.04 for antibiotics.

The minimal inhibitory concentration of an antibacterial agent is the lowest concentration that prevent the bacterial growth in its optimal conditions. Therefore, MIC indicates the efficacy of the antibacterial drug. The antibacterial activity of propolis against *Staphylococcus spp.* has been reported previously (Lu *et al*., 2005). This activity was shown to vary from a region to another, and from a season to another depending on the chemical profile of propolis *(*Hegazi *et al.*, 2000; Lu *et al*., 2005). In the present study, EEPs showed a strong antibacterial activity against *S. aureus* and *S. epidermidis*, noticed by low MIC values. This high efficacy of Moroccan propolis extracts could be explained by their high content in flavonoid and phenolic compounds. These latter were reported as the responsible for the antibacterial action (Sforcin *et al.*, 2000). Indeed, the antibacterial activity is complicated and could not be attributed to a single molecule since several synergism effects can take place between minor molecules (Krol *et al*., 1993).

3.3. Interaction Between Propolis, Essential Oils, and Antibiotics

In order to evaluate the combined effect of EEPs, EOs, and antibiotics the checkboard method was used. The results are represented in Table 3. As shown, different types of interactions were recorded such as synergistic, partial synergy, additive, while there was no antagonistic interaction, and in some cases no interaction was noticed.

The synergistic interaction against MRSA was noticed when the propolis of Beni Arouss was mixed with *O. compactum* EO, ampicillin, and vancomycin, with FIC indexes of 0.49, 0.49, and 0.44, respectively. While the M'diq extract showed synergistic interaction only with ampicillin against this strain with FIC index equal to 0.35. Against *S. epidermidis*, the extract of Beni Arouss interacted synergistically with *O. compactum* EO and neomycin, with FICI of 0.49 and 0.19, respectively. While the extract of M'diq acted synergistically against this bacterium when it was mixed with *O. compactum*, *O. elongatum*, and oxytetracycline, with FICI of 0.18, 0.49, and 0.31, respectively. There was no synergistic effect between the Beni Arouss extract and the tested products against S. aureus ATCC 25923, and only a partial synergy was recorded with chloramphenicol, neomycin, oxytetracycline, and *O. compactum*. On the other hand, the M'diq extract acted synergistically against this bacterial strain when it was mixed with ampicillin (FICI=0.29).

Propolis and essential oils are chemically complex substances, which contain a variety of bioactive molecules. In fact, a synergistic effect may exist between the components of the same sample. Giving as example propolis extract tested in this study, the chemical analysis showed

the presence of more than 100 compounds belonging to several chemical groups. Many of these molecules are known by their antibacterial activity such as galangin, kaempferide, caffeic acid, and others. In addition, the chemical characterization of the essential oils of *O. compactum* and *O. elongatum*, also showed a high complexity. In this regard, it is difficult to attribute specifically the synergistic effect to specific compounds. However, the recent insights of the mechanisms of action of propolis extracts and essential oils on bacteria could explain the synergistic effect of these natural products. In fact, by their amphipathic criteria, essential oils are known to affect the bacterial cell. (Ultee *et al*., 2002) reported that p-cymene, one of the main compounds of the studied EOs, caused swelling of cell membrane of *S. aureus*. Thus, the incorporation of p-cymene in lipid bilayer of *S. aureus* membrane could facilitate the transport of propolis compounds through the cytoplasmic membrane, and therefore increase the efficacy of this latter. In addition, other molecules exist in the studied EOs, namely carvacrol and thymol have been known to increase the permeability and depolarize bacterial cell (Lambert *et al*., 2001; Xu *et al*., 2008; Bouhdid *et al*., 2009). In this regard, the interaction of these compounds with other propolis molecules could explain the synergistic effect of propolis and essential oils.

Concerning the interaction between propolis and antibiotics, similar results were reported by (Fernandes Júnior et al., 2005) who showed that propolis interacts synergistically with chloramphenicol, vancomycin, tetracycline. In fact, antibiotics have been known to inhibit protein synthesis. The same authors did not notice any antagonistic effects between propolis and antibiotics, which is in concordance with the present work. The interaction differs as function of the two propolis extracts. This could be explained by the difference in the chemical composition as shown in the first part. The increase of the antibacterial activity of antibiotics could be explained by the fact that some propolis compounds like caffeic acid (CAPE) and quercetin, affect the membrane permeability by causing a disequilibrium at the level of bacterial membrane, which facilitate the entry of antibiotics into the bacterial cell. This could explain the high synergistic effect between EEPs and antibiotics.

Table 1. Chemical composition of ethanol extracts of propolis.

No.	Name	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Beni Arouss	M'diq
1	Trigonelline	$C_7H_7NO_2$	1.22	138.05550		110.0604	96.0453	94.0657	92.0501	65.0393	+	+
2	Esculetin (6,7-Dihydroxycoumarin)	$C_9H_6O_4$	14.71	179.03444		151.0391	135.0445	133.0287	123.0443	117.0335	+	+
3[1]	Chlorogenic acid (3-O-Caffeoylquinic acid)	$C_{16}H_{18}O_9$	14.89	355.10291		193.0499	163.0391	145.0285	135.0443	89.0390	+	+
4	Caffeic acid	$C_9H_8O_4$	15.08		179.03444	135.0438	107.0487				+	+
5	Dihydroxy-methoxycoumarin	$C_{10}H_8O_5$	17.13	209.04500		194.0212	181.0499	166.0261	153.0544	149.0235	+	+
6	Fraxetin (7,8-Dihydroxy-6-methoxycoumarin)	$C_{10}H_8O_5$	17.59	209.04500		194.0212	181.0500	163.0391	149.0235	135.0444	+	+
7	Eriodictyol-O-hexoside isomer 1	C21H22O11	18.38		449.10839	287.0563	151.0024	135.0439	125.0231	107.0125	-	+
8[1]	4-Coumaric acid	C9H8O3	18.44		163.03952	119.0487	93.0330				+	+
9	Caffeoylshikimic acid	C16H16O8	18.46		335.07670	179.0340	161.0233	135.0439	111.0434	93.0329	-	+
10[1]	Scopoletin (7-Hydroxy-6-methoxycoumarin)	C10H8O4	19.09	193.05009		178.0263	165.0547	149.0598	137.0599	133.0287	+	+
11	Eriodictyol-O-hexoside isomer 2	$C_{21}H_{22}O_{11}$	19.24		449.10839	287.0562	151.0023	135.0438	125.0230	107.0123	-	+
12	Luteolin-di-O-glucuronide	$C_{27}H_{26}O_{18}$	19.68		637.10409	461.0719	285.0405	151.0019			-	+
13[1]	Taxifolin (Dihydroquercetin)	$C_{15}H_{12}O_7$	19.83		303.05048	285.0406	217.0498	175.0389	153.0185	125.0229	+	+
14[1]	Ferulic acid	$C_{10}H_{10}O_4$	19.85		193.05009	178.0263	149.0595	137.0229	134.0360		-	+
15	Eriodictyol-O-hexoside isomer 3	$C_{21}H_{22}O_{11}$	20.74		449.10839	287.0565	151.0024	135.0438	125.0226	107.0121	-	+
16	Isoferulic acid	$C_{10}H_{10}O_4$	20.98		193.05009	178.0260	149.0595	137.0231	134.0360		-	+
17	Tetrahydroxyflavanone-O-rhamnosylhexoside	$C_{27}H_{32}O_{15}$	21.06		595.16630	459.1136	287.0562	175.0025	151.0024	135.0438	+	+
18	Myricetin-3'-O-glucoside (Cannabiscitrin)	$C_{21}H_{20}O_{13}$	21.33		479.08257	317.0301	316.0224	287.0195	271.0250	178.9975	+	+

19	Scoparone (6,7-Dimethoxycoumarin)	$C_{11}H_{10}O_4$	21.69	207.06574		192.0420	191.0343	179.0707	163.0393	151.0756	+	+
20	Verbascoside or isomer	$C_{29}H_{36}O_{15}$	22.32		623.19760	461.1658	315.1087	179.0339	161.0232	133.0282	+	+
21	Dihydrokaempferol (3,4',5,7-Tetrahydroxyflavanone)	$C_{15}H_{12}O_6$	22.42		287.05556	269.0457	259.0610	243.0660	177.0545	125.0229	+	+
22	Padmatin (7-Methoxy-3,3',4',5-tetrahydroxyflavanone) or isomer	$C_{16}H_{14}O_7$	22.60	319.08178		301.0710	286.0482	273.0758	153.0183	137.0599	+	+
23	Luteolin-7-O-glucuronide	$C_{21}H_{18}O_{12}$	22.74		461.07201	285.0405	217.0496	199.0389	175.0390	133.0280		+
24	Luteolin-7-O-glucoside (Cynaroside)	$C_{21}H_{20}O_{11}$	22.81		447.09274	327.0513	285.0406	284.0329	256.0386	151.0025	-	+
25	Luteolin-O-rhamnosylhexoside	$C_{27}H_{30}O_{15}$	22.85		593.15065	327.0515	285.0404	284.0327	133.0284	107.0124	+	+
26	Isorhamnetin-O-rhamnosylhexoside	$C_{28}H_{32}O_{16}$	23.02		623.16121	315.0513	314.0436	300.0276	299.0201	271.0249	+	+
27	Methoxy-tetrahydroxy(iso)flavone-O-glucuronide	$C_{22}H_{20}O_{13}$	23.02		491.08257	315.0512	300.0277	272.0322	113.0227		-	+
28	Hyperoside (Quercetin-3-O-galactoside)	$C_{21}H_{20}O_{12}$	23.18		463.08765	301.0354	300.0277	271.0248	255.0296	178.9978	+	+
29	Trimethoxycoumarin	$C_{12}H_{12}O_5$	23.39	237.07630		222.0524	207.0290	193.0499	191.0341	176.0469	+	+
30[1]	Isoquercitrin (Quercetin-3-O-glucoside)	$C_{21}H_{20}O_{12}$	23.41		463.08765	301.0355	300.0276	271.0247	255.0296	178.9976	+	+
31	Eriodictyol-O-hexoside isomer 4	$C_{21}H_{22}O_{11}$	23.64		449.10839	287.0562	151.0024	135.0439	125.0228	107.0123	-	+
32	Vanillin acetate	$C_{10}H_{10}O_4$	23.71	195.06574		153.0548	125.0601	111.0444	93.0342	65.0393	+	_
33	Padmatin (7-Methoxy-3,3',4',5-tetrahydroxyflavanone) or isomer	$C_{16}H_{14}O_7$	23.76	319.08178		301.0708	286.0470	273.0758	153.0184	137.0599	+	+
34	Reinutrin (Quercetin-3-O-xyloside)	$C_{20}H_{18}O_{11}$	23.74		433.07709	301.0359	300.0278	271.0249	255.0299	178.9981	+	-
35	Avicularin (Quercetin-3-O-arabinofuranoside)	$C_{20}H_{18}O_{11}$	24.01		433.07709	301.0356	300.0277	271.0250	255.0295	178.9974	+	+

36	Apigenin-O-rhamnosylhexoside	$C_{27}H_{30}O_{14}$	24.36		577.15574	269.0455	268.0376	117.0327			-	+
37	Methoxy-trihydroxy(iso)flavone-O-rhamnosylhexoside	$C_{28}H_{32}O_{15}$	24.54	609.18195		463.1240	301.0709	286.0475	129.0550	85.0290	-	+
38[1]	Myricetin (3,3',4',5,5',7-Hexahydroxyflavone)	$C_{15}H_{10}O_8$	24.68		317.02974	271.0238	178.9975	165.0179	151.0024	137.0231	+	+
39	Guaijaverin (Quercetin-3-O-arabinoside)	$C_{20}H_{18}O_{11}$	24.74		433.07709	301.0354	300.0277	271.0249	255.0304	178.9976	+	+
40	Dimethoxy-trihydroxy(iso)flavone-O-glucuronide	$C_{23}H_{22}O_{13}$	24.75		505.09822	329.0666	314.0435	299.0199	271.0250	113.0230	+	+
41	Chrysoeriol-7-O-glucuronide	$C_{22}H_{20}O_{12}$	24.76		475.08766	299.0562	284.0328	256.0385			-	+
42[1]	Quercitrin (Quercetin-3-O-rhamnoside)	$C_{21}H_{20}O_{11}$	24.95		447.09274	301.0355	300.0277	271.0249	255.0298	178.9976	+	+
43	Isorhamnetin-O-hexoside isomer 1	$C_{22}H_{22}O_{12}$	25.22		477.10330	315.0515	314.0435	285.0406	271.0248	243.0295	+	–
44[1]	Eriodictyol (3′,4′,5,7-Tetrahydroxyflavanone)	$C_{15}H_{12}O_6$	25.39		287.05556	269.0469	151.0024	135.0439	125.0230	107.0124	+	+
45	Isorhamnetin-O-hexoside isomer 2	$C_{22}H_{22}O_{12}$	25.40		477.10330	315.0513	314.0434	285.0406	271.0248	243.0295	+	–
46	Methoxy-tetrahydroxy(iso)flavone-O-hexoside	$C_{22}H_{22}O_{12}$	25.69		477.10330	315.0510	314.0435	299.0198	271.0246	243.0298	+	–
47	N-trans-Feruloyltyramine	$C_{18}H_{19}NO_4$	25.52	314.13924		194.0811	177.0548	149.0599	135.0443	121.0651	-	+
48	Cedeodarin (6-Methyl-3,3',4',5,7-pentahydroxyflavanone) or isomer	$C_{16}H_{14}O_7$	26.72	319.08178		301.0707	273.0760	245.0811	167.0341	163.0391	+	+
49	N1,N5,N10-Tricoumaroylspermidine cis/trans isomer 1	$C_{34}H_{37}N_3O_6$	26.74		582.26042	462.2028	342.1457	316.1657	145.0283	119.0487	+	+
44	Acetyltaxifolin	$C_{17}H_{14}O_8$	27.07		345.06105	327.0508	303.0510	285.0406	151.0024	125.0229	-	+

50	Quercetin-O-coumaroylhexoside	$C_{30}H_{26}O_{14}$	27.30		609.12444	463.0886	301.0354	300.0276	271.0249	255.0295	+	–
51[1]	Quercetin (3,3',4',5,7-Pentahydroxyflavone)	$C_{15}H_{10}O_7$	27.49		301.03483	273.0404	178.9975	151.0024	121.0280	107.0124	+	+
52	N1,N5,N10-Tricoumaroylspermidine cis/trans isomer 2	$C_{34}H_{37}N_3O_6$	27.74		582.26042	462.2033	342.1458	316.1663	145.0283	119.0487	+	+
53[1]	Luteolin (3',4',5,7-Tetrahydroxyflavone)	$C_{15}H_{10}O_6$	28.36		285.03991	217.0501	175.0388	151.0024	133.0281	107.0124	+	+
54	N1,N5,N10-Tricoumaroylspermidine cis/trans isomer 3	$C_{34}H_{37}N_3O_6$	28.60		582.26042	462.2031	342.1450	316.1657	145.0284	119.0487	+	+
55	Quercetin-3-O-methyl ether	$C_{16}H_{12}O_7$	28.74		315.05048	300.0276	271.0249	255.0296	243.0296	227.0346	+	+
56	Kaempferol-O-coumaroylhexoside	$C_{30}H_{26}O_{13}$	28.78		593.12952	447.0938	285.0405	284.0327	255.0295	119.0489	–	+
57	Kaempferol-O-coumaroylhexoside isomer 1	$C_{30}H_{26}O_{13}$	28.79		593.12952	447.0936	285.0405	284.0327	255.0296	119.0485	+	-
58	O-Acetylpadmatin or isomer	$C_{18}H_{16}O_8$	28.91		359.07670	341.1380	317.0663	299.0560	289.0724	284.0327	-	+
59	Dimethoxy-tetrahydroxy(iso)flavone	$C_{17}H_{14}O_8$	29.00		345.06105	330.0380	315.0147	287.0199	271.0247	259.0246	+	+
60	Kaempferol-O-coumaroylhexoside isomer 2	$C_{30}H_{26}O_{13}$	29.19		593.12952	447.0934	285.0405	284.0328	255.0296	119.0489	+	-
61	N1,N5,N10-Tricoumaroylspermidine cis/trans isomer 4	$C_{34}H_{37}N_3O_6$	29.45		582.26042	462.2036	342.1460	316.1659	145.0282	119.0488	+	+
62[1]	Kaempferol (3,4',5,7-Tetrahydroxyflavone)	$C_{15}H_{10}O_6$	29.84		285.03991	257.0453	229.0495	169.0648	151.0022	107.0123	+	+
63[1]	Apigenin (4',5,7-Trihydroxyflavone)	$C_{15}H_{10}O_5$	30.20		269.04500	227.0340	225.0550	151.0024	149.0232	117.0330	+	+
64[1]	Isorhamnetin (3'-Methoxy-3,4',5,7-tetrahydroxyflavone)	$C16H_{12}O_7$	30.33		315.05048	300.0276	283.0254	271.0246	164.0102	151.0023	+	+
65	Chrysoeriol (3'-Methoxy-4',5,7-trihydroxyflavone)	$C_{16}H_{12}O_6$	30.48		299.05556	284.0327	256.0373	227.0351	151.0020	107.0128	+	+

66	Isokaempferide (3-Methoxy-4',5,7-trihydroxyflavone)	$C_{16}H_{12}O_6$	30.87	301.07122		286.0474	285.0399	258.0524	212.0466	121.0283	+	+
67	Dimethoxy-trihydroxy(iso)flavone isomer 1	$C_{17}H_{14}O_7$	31.06		329.06613	314.0433	299.0197	285.0406	271.0248	243.0294	+	+
68	Hydroxy-methoxy(iso)flavone	$C_{16}H_{12}O_4$	31.11	269.08138		254.0574	226.0626	167.0337			+	+
69	Trihydroxy-trimethoxy(iso)flavone isomer 1	$C_{18}H_{16}O_8$	31.68		359.07670	344.0537	329.0302	314.0071	301.0355	286.0120	+	+
70	Rhamnetin (7-Methoxy-3,3',4',5-tetrahydroxyflavone)	$C_{16}H_{12}O_7$	32.31		315.05048	300.0277	193.0133	165.0181	121.0280	97.0280	+	+
71	Pinocembrin (5,7-Dihydroxyflavanone)	$C_{15}H_{12}O_4$	32.69		255.06573	227.0706	213.0551	151.0024	107.0123	83.0122	+	+
72	Dimethoxy-trihydroxy(iso)flavone isomer 2	$C_{17}H_{14}O_7$	32.71		329.06613	314.0434	313.0355	299.0197	285.0405	271.0248	+	+
73	Isosakuranetin (5,7-Dihydroxy-4'-methoxyflavanone)	$C_{16}H_{14}O_5$	32.72		285.07630	270.0535	243.0660	164.0103	151.0024	136.0153	+	–
74	Acetyltrihydroxy(iso)flavanone	$C_{17}H_{14}O_6$	33.07		313.07122	271.0611	253.0503	225.0553	151.0024		–	+
75	Trihydroxy-trimethoxy(iso)flavone isomer 2	$C_{18}H_{16}O_8$	33.09		359.07670	344.0537	329.0303	314.0066	301.0355	286.0124	+	+
76	Dihydroxy-trimethoxy(iso)flavone isomer 1	$C_{18}H_{16}O_7$	33.11	345.09743		330.0734	329.0655	315.0501	299.0552	287.0552	+	+
77	Dimethoxy-trihydroxy(iso)flavone isomer 3	$C_{17}H_{14}O_7$	33.26		329.06613	314.0433	299.0197	285.0415	271.0248	243.0300	+	+
78	Dihydroxy-methoxy(iso)flavone isomer 1	$C_{16}H_{12}O_5$	33.37	285.07630		270.0524	269.0445	257.0813	242.0575	229.0859	–	+
79[1]	Chrysin (5,7-Dihydroxyflavone)	$C_{15}H_{10}O_4$	33.77	255.06573		209.0593	153.0183	129.0339	103.0546	67.0185	+	+
80	Caffeic acid phenethyl ester	$C_{17}H_{16}O_4$	34.07		283.09703	179.0339	178.0254	161.0231	135.0438	133.0281	+	+
81	Acacetin (5,7-Dihydroxy-4'-methoxyflavone)	$C_{16}H_{12}O_5$	34.39	285.07630		270.0523	242.0573	153.0181	133.0652		+	+

82	Trihydroxy(iso)flavone	$C_{15}H_{10}O_5$	34.65	271.06065		253.0504	215.0704	197.0597	165.0187	153.0185	_	+
83	Dihydroxy-methoxy(iso)flavone isomer 2	$C_{16}H_{12}O_5$	35.00	285.07630		270.0525	269.0445	242.0573	167.0340		+	+
84	Dihydroxy-trimethoxy(iso)flavone isomer 2	$C_{18}H_{16}O_7$	35.13	345.09743		330.0734	329.0669	315.0498	301.0705	287.0549	+	+
85	Dihydroxy-dimethoxy(iso)flavone	$C_{17}H_{14}O_6$	35.43		313.07122	298.0483	283.0249	269.0450	255.0297	227.0338	+	+
86	Dihydroxy-tetramethoxy(iso)flavone	$C_{19}H_{18}O_8$	35.45		373.09235	358.0694	343.0458	328.0219	315.0516	313.0355	+	+
87	Dihydroxy-trimethoxy(iso)flavone isomer 3	$C_{18}H_{16}O_7$	35.50	345.09743		330.0735	329.0657	315.0499	301.0712	287.0549	+	+
88	Isoimperatorin	$C_{16}H_{14}O_4$	36.20	271.09704		203.0341	175.0390	159.0442	147.0442	131.0495	+	+
89	Hydroxy-tetramethoxy(iso)flavone	$C_{19}H_{18}O_7$	37.02	359.11308		344.0893	343.0815	329.0659	315.0863	301.0709	+	+
90	Pinostrobin (5-Hydroxy-7-methoxyflavanone)	$C_{16}H_{14}O_4$	37.10	271.09704		229.0859	173.0598	167.0341	131.0495	103.0548	–	+
91	Unidentified compound 1	$C_{20}H_{30}O_3$	37.54		317.21167	299.1992	273.1853	247.1693	189.0912	173.0596	+	+
92	Tectochrysin (5-Hydroxy-7-methoxyflavone)	$C_{16}H_{12}O_4$	38.02	269.08138		254.0574	226.0626	167.0340			+	+
93	Unidentified compound 2	$C_{20}H_{34}O_3$	38.53		321.24297	303.2330					+	_
94	Hydroxy-trimethoxy(iso)flavone	$C_{18}H_{16}O_6$	39.26		329.10252	314.0786	313.0709	299.0552	285.0763	271.0600	+	-
95	Unidentified carboxylic acid	$C_{20}H_{32}O_3$	39.80		319.22732	275.2383	259.2067				+	-
96	Apigenin-4',7-dimethyl ether (4',7-Dimethoxy-5-hydroxyflavone)	$C_{17}H_{14}O_5$	38.67	299.09195		284.0679	256.0730	167.0341	133.0650		+	+
97	Unidentified compound 2	$C_{20}H_{32}O_3$	38.84		319.22732						_	+
98	Hexadecanedioic acid	$C_{16}H_{30}O4$	40.72		285.20659	267.1964	241.2167	223.2062			+	+
99	Unidentified caffeic acid derivative	$C_{29}H_{36}O_6$	41.65		479.24336	317.2112	299.2015	179.0339	135.0438		+	+

100	Unidentified compound 3	$C_{22}H_{36}O_4$	42.12		363.25353	321.2447	303.2329	59.0122			+	+
101	Unidentified compound 4	$C_{20}H_{36}O_3$	42.64		323.25862	305.2492	279.2694	263.2379	247.2067		+	-
102	Linoleamide	$C_{18}H_{33}NO$	44.40	280.26404		263.2371	245.2264	109.1016	95.0861	81.0705	+	+
103	Palmitic amide (Hexadecanamide)	$C_{16}H_{33}NO$	45.38	256.26404		144.1388	130.1224	116.1072	102.0918	88.0763	+	+
104	Oleamide	$C_{18}H_{35}NO$	45.68	282.27969		265.2526	247.2422	135.1171	83.0861	69.0706	+	+
105	Ginkgoic acid or isomer	$C_{22}H_{34}O_3$	47.64		345.24298	301.2536	175.1117	133.0645	119.0486	106.0410	+	+

[1] *Confirmed by standard*
- Absent
+ present

Table 2. MIC values of propolis extracts, EOs and antibiotics.

	EEPBA	EEPM	OCEO	OEEO	VA	OT	TE	C	AM	N
S. aureus ATCC 43000 MRSA	0.62	0.31	1%	0.12%	0.005	0.005	0.0025	0.01	0.02	ND
S. epidermis ATCC 12228	0.62	1.25	0.5%	0.5%	ND	0.04	0.01	0.12	ND	0.08
S. aureus ATCC 25923	0.31	0.15	0.5%	0.12%	0.0025	0.01	0.005	0.01	0.012	0.04

MIC values of essential oils are expressed in % (v/v)
MIC values of EEPs and antibiotics are expressed in mg/mL
EEPBA: Ethanol extract of propolis of Beni Arouss
EEPM: Ethanol extract of propolis of M'diq
OEEO: *Origanum elongatum* essential oil
OCEO*: Origanum compactum* essential oil

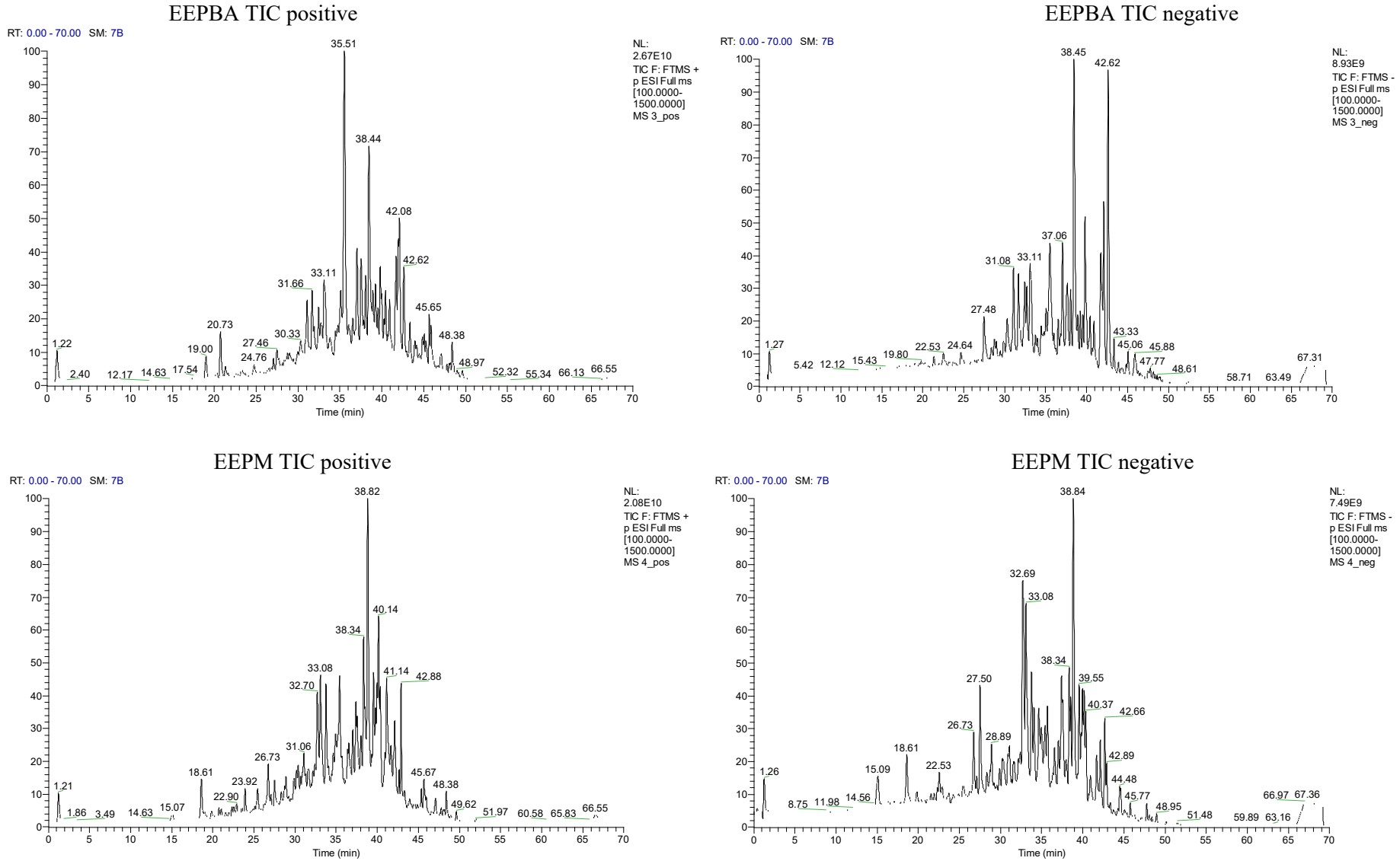
Table 3. Combined effect of EEPs, EOs, and antibiotics.

Strain	Combination	MIC of EOs and antibiotics		MIC of EEPs		FICi	Interpretation
		Alone	in combination	Alone	in combination		
S. aureus ATCC 43000 MRSA	EEPBA + *OCEO*	1	0.25	0.62	0.15	0.49	**Synergy**
	EEPBA + *OEEO*	0.12	0.06	0.62	0.15	0.74	Partial synergy
	EEPBA + C	0.01	0.005	0.62	0.312	1.00	No interaction
	EEPBA + TE	0.0025	0.0012	0.62	0.15	0.72	Partial synergy
	EEPBA + OT	0.005	0.0025	0.62	0.04	0.56	Partial synergy
	EEPBA + AM	0.02	0.005	0.62	0.15	0.49	**Synergy**
	EEPBA + VA	0.005	0.001	0.62	0.15	0.44	**Synergy**
	EEPM + OC	1	0.5	0.31	0.15	0.98	Additive
	EEPM + *OE*	0.12	0.06	0.31	0.15	0.98	Additive
	EEPM + C	0.01	0.005	0.31	0.15	0.98	Additive
	EEPM + TE	0.0025	0.0012	0.31	0.07	0.71	Partial synergy
	EEPM + OT	0.005	0.0025	0.31	0.07	0.73	Partial synergy
	EEPM + AM	0.02	0.0025	0.31	0.07	0.35	**Synergy**
S. epidermis ATCC 12228	EEPBA + *OCEO*	0.5	0.125	0.62	0.15	0.49	**Synergy**
	EEPBA + *OEEO*	0.5	0.25	0.62	0.04	0.56	Partial synergy
	EEPBA + C	0.12	0.06	0.62	0.31	1.00	No interaction
	EEPBA + TE	0.01	0.005	0.62	0.31	1.00	No interaction
	EEPBA + OT	0.04	0.02	0.62	0.15	0.74	Partial synergy
	EEPBA + N	0.08	0.01	0.62	0.04	0.19	**Synergy**
	EEPM + *OCEO*	0.5	0.06	1.25	0.075	0.18	**Synergy**
	EEPM + *OE*	0.5	0.12	1.25	0.31	0.49	**Synergy**
	EEPM + TE	0.01	0.005	1.25	0.62	1.00	Additive
	EEPM + OT	0.04	0.01	1.25	0.07	0.31	**Synergy**
	EEPM + N	0.08	0.0025	1.25	0.625	0.53	Partial synergy

S. aureus ATCC 25923	EEPBA + OCEO	0.5	0.12	0.31	0.15	0.72	Partial synergy
	EEPBA + OEEO	0.125	0.06	0.31	0.15	0.96	Additive
	EEPBA + C	0.01	0.005	0.31	0.07	0.73	Partial synergy
	EEPBA + TE	0.005	0.0025	0.31	0.15	0.98	Additive
	EEPBA + OT	0.01	0.005	0.31	0.04	0.63	Partial synergy
	EEPBA + N	0.04	0.02	0.31	0.07	0.73	Partial synergy
	EEPBA + AM	0.012	0.006	0.31	0.15	0.98	Additive
	EEPBA + VA	0.0025	0.00125	0.31	0.15	0.98	Additive
	EEPM + OCEO	0.5	0.25	0.15	0.02	0.63	Partial synergy
	EEPM + OEEO	0.12	0.06	0.15	0.07	0.97	Additive
	EEPM + C	0.01	0.005	0.15	0.07	0.97	Additive
	EEPM + TE	0.005	0.0025	0.15	0.02	0.63	Partial synergy
	EEPM +OT	0.01	0.0024	0.15	0.07	0.71	Partial synergy
	EEPM + N	0.04	0.02	0.15	0.04	0.77	Additive
	EEPM + AM	0.012	0.0003	0.15	0.04	0.29	**Synergy**
	EEPM + VA	0.0025	0.00125	0.15	0.04	0.77	Additive

EEPBA: Ethanol extract of propolis of Beni Arouss; EEPM: Ethanol extract of propolis of M'diq; AM: Ampicillin; C: Chloramphenicol; VA: Vancomycin; N: Neomycin; TE; Tetracycline; OT; Oxytetracycline; OEEO: Origanum elongatum essential oil; OCEO: Origanum compactum essential oil. FICI ≤0.5 = synergistic interaction, 0.5<FICI ≤0.75 = Partial synergy, 0.76 ≤FICI<1 = additive interaction, FICI between 1 and 4 = no interaction (not differential), FICI > 4 = antagonism (Denes & Hidri, 2009)

Figure 1. Total ion chromatograms of EEPs. *EEPBA: Ethanol extract of propolis of Beni Arouss EEPM: Ethanol extract of propolis of M'diq*.



4. CONCLUSION

Combination antibiotic therapy is an effective way to reduce the emergence of bacterial resistance and to fight infections. Thus, natural products are known by their diversified bioactive components with antibacterial action. In this study, the combination of propolis extracts with essential oils and antibiotics was investigated. The results showed some synergistic interaction between these antibacterial products against methicillin resistant *S. aureus* and *S. epidermidis*. The chemical analysis showed the presence of several compounds known by their antibacterial activity in the tested propolis extracts. In this regard, the synergistic effect could be the result of the interaction of major or minor molecules contained in propolis with antibiotics and essential oils compounds. It can be concluded from this study that propolis extract is a promising source of bioactive antibacterial compounds that can be used in combination therapy against infectious diseases.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Omar Belmehdi: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Abdelhakim Bouyahya:** Investigation, Resources, and Writing-original draft. **József Jekő:** Methodology, Supervision. **Zoltán Cziáky:** Methodology, Supervision**. Gokhan Zengin:** Methodology, Supervision. **Gyula Sotkó:** Methodology, Supervision**. Aicha El baaboua:** Investigation, Resources, Visualization, Software, Formal Analysis. **Nadia Skali Senhaji:** Investigation, Resources, Visualization, Software, Formal Analysis**. Jamal Abrini:** nvestigation, Resources, Visualization, Software, Formal Analysis

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