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Original Article

Combination of medicinal plants with antibiotics against *Klebsiella pneumoniae* and *Acinetobacter baumannii*

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Abstract

Background and aims: One essential plant-based strategy to deal with infections is antimicrobial synergism, which can make antimicrobials more efficient. The aim of this study was to investigate the interaction between these extracts and two widely used antibiotics, meropenem and gentamicin, on two multidrug-resistant (MDR) bacteria, *K. pneumoniae* and *A. baumannii*, in vitro.

Methods: Different concentrations of *Rosa damascena* Mill., *Malva sylvestris* L., and *Zataria multiflora* Boiss. hydroalcoholic extracts (2-fold serial dilution from 131072 to 256 µg/mL) were administered against two MDR bacteria, and their combination with gentamicin and meropenem (serial dilution from 32 to 0.015 µg/mL) was investigated by the resazurin-based microdilution and the checkerboard method. The phytochemical properties of the extracts were also examined, and the total phenolic content (TPC), total flavonoid content (TFC), anthocyanin content, and antioxidant capacity of the extracts were determined.

Results: *Z. multiflora* and *R. damascena* showed high antibacterial activity, and their minimal inhibitory concentrations on *Acinetobacter baumannii* were 1024 and 2048 µg/mL, respectively. *Z. multiflora* also had high TFC, TPC, and antioxidant activity and demonstrated additive interaction with meropenem and gentamicin with fractional inhibitory concentrations of 1 and 0.75, respectively.

Conclusion: We suggest the potency of *Z. multiflora*-antibiotic combinations in treating MDR *A. baumannii* after future clinical studies.

Keywords: Drug combination, Phytochemicals, Anti-bacterial agents, *Klebsiella pneumoniae*, *Acinetobacter baumannii*

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Introduction

Health communities are facing a new era of infectious disease treatment. According to the World Health Organization, the emergence of antibiotic-resistant bacteria is threatening medical achievements regarding infection treatment (1). For example, in the United States, two million people are affected by drug-resistant infections each year, 23 000 of whom die; these numbers are higher in Europe and especially in low- and middle-income countries. Despite all these challenges against infection treatment, certain strategies in modern medicine, such as transplantation, cancer chemotherapy, and intensive care for preterm newborns, have increased reliance on antibiotics (2,3). Among antibiotic-resistant bacteria, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. from the ESKAPE group are more important due to their drug resistance, transmission, and pathogenesis (4).

Klebsiella pneumoniae carries a beta-lactamase gene in its chromosome that makes penicillins, including amoxicillin and ampicillin, ineffective, and its resistance to co-trimoxazole, third-generation cephalosporins, carbapenems, and fluoroquinolones is spreading globally. Although there is no clinically effective treatment for many people with *K. pneumoniae* infection (5), some clinicians use combination therapy by administering polymyxins, followed by tigecycline, aminoglycosides, and carbapenems for severe *K. pneumoniae* infection (6). *Acinetobacter baumannii* is another bacterium from the ESKAPE group that is considered a great threat to inpatients and may cause certain complications such as pneumonia, surgical site infection, meningitis, and bacteremia. The ability of *A. baumanii* to survive in hard conditions makes its transmission easy through medical instruments and other equipment to which patients' exposure (7). Many mechanisms, such as the expression of β-lactamases (cephalosporinases and carbapenemases), efflux pumps, changes in cell wall channels, and mutations, cause *A. baumanii* to acquire resistance to many antibiotics, such as chloramphenicol, quinolones, tigecyclines, tetracyclines, and disinfectants. This

bacterium can also express aminoglycoside-modifying enzymes that overshadow its susceptibility to this group of antibiotics (8).

To effectively fight these pathogens, new antimicrobial agents could be found in natural sources, including plant extracts that contain many therapeutic agents. One problem is that many of these extracts are not strong enough in low dosages to treat infections; however, their effect could be enhanced by combining them with antibiotics to produce their synergistic effects with antibiotics. Certain plant-derived agents may cause certain interactions with antibiotics that could increase their effects in inhibiting bacterial growth or lysing bacteria (9,10). Phytopharmacological studies on *Rosa damascena* Mill. have demonstrated its substantial potential to treat diseases. The plant is rich in polyphenols such as gallic acid, 3,5-D-glycoside, kaempferol, quercetin, and cyanidin and can, therefore, exhibit noticeable antibacterial properties in treating infections (11). Researchers have identified numerous active ingredients in *R. damascena* with high antimicrobial and antioxidant properties, which indicates its potential to be used in treatment regimens (12). The anti-inflammatory and antibiofilm effects of *Malva sylvestris* L. and its ability to reduce the side effects of gentamicin in animal studies have led to the argument that this plant could be an appropriate complementary choice for antimicrobial drugs (13,14). The antibacterial effect of *Zataria multiflora* Boiss., in addition to its appropriate effects in healing injuries and its anti-inflammatory activity, has led researchers to identify its active ingredients. This plant contains certain antimicrobial agents that cause synergistic interactions with some antibiotics. All these properties made this plant an excellent antibacterial source, so that the Food and Drug Administration has approved its essential oil for use in antibacterial packaging in the food industry (15-17).

This study aimed to evaluate the interaction between these extracts and two widely used antibiotics, meropenem and gentamicin, on two multidrug-resistant (MDR) bacteria, *K. pneumoniae* and *A. baumannii*, in vitro.

Materials and Methods

Collection of plant material

The flowers of *R. damascena* and *M. sylvestris* and the aerial parts (stems and leaves) of *Z. multiflora* were purchased from local groceries in Shahrekord, Iran, and after identification by a botanist, voucher specimens (No. 187, 107, and 443, respectively) were deposited at the Herbarium of the Medical Plants Research of Shahrekord University of Medical Sciences. The maceration method was used to extract plants; briefly, pulverized plants were macerated in 70% ethanol (1:5 w/v) for 72 hours, and then the Whatman paper filter was utilized to separate plant residues from the extract. Vacuum distillation was performed by a rotary evaporator, and finally, the dried extract was obtained after incubation at 37 ˚C. The extracts were stored at -20 ˚C until use.

Phytochemical Studies

Antioxidant activity

The 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the antioxidant activity of the extracts (18). Different concentrations of the extracts were prepared by dissolving them in methanol (99%), and then the DPPH solution was added to the extracts at different concentrations. The optical absorbance of wells was read at 517 nm after 15-minute incubation at room temperature. The following formula was applied to determine the half-maximal inhibitory concentration of the extracts:

DPPH inhibition (%) = (absorbance of control – absorbance of sample/absorbance of control) × 100

Anthocyanin content

The pH-differential method was utilized to measure anthocyanin content (19). To this end, stock solutions of the extracts were prepared in distilled water, and 1–5 mL of these solutions were added to two volumetric flasks. Afterward, two buffers were added to each flask to a final volume of 25 mL. The optical absorbance of the buffers was read under optical densities of 520 nm and 700 nm, and the anthocyanin contents of the extracts were determined by the following formula:

$$Absorbance(A) = (absorbance\,520 - absorbance\,700)\,PH$$
$$= 1 - (absorbance\,520 - absorbance\,700)\,PH = 4.5$$

$$Anthocyanin\,content = \frac{A * MW * Df * V * 1000}{4 * l * m}$$

Total phenolic content

The Folin-Ciocalteu method was used to measure the TPC of extracts (20). Briefly, the stock solutions of extracts were prepared by dissolving 0.01 g dried extract in 10 mL methanol (60%). Then, 100 µL of the stock solutions were added to 500 µL of the Folin-Ciocalteu reagent (10%). After 3–8 minutes of incubation at room temperature, 400 µL of the aqueous sodium bicarbonate (7.5%) were added to the solution. Finally, the optical absorbance of the resulting solution was spectrophotometrically read at 765 nm after it was incubated at room temperature for 30 minutes.

Total flavonoids content

The TFC of the extract was measured by the aluminum chloride colorimetric method (21). Briefly, 500 µL of the stock solution, after it was prepared for the determination of TPC, was added to 500 µL of aluminum chloride (2%) and 3 mL of potassium acetate (5%). The optical absorbance of the resulting solution was measured at an optical density of 415 nm after it was incubated in the dark for 40 minutes.

Bacteriological studies

Lyophilized strains of *K. pneumoniae* (ATCC® 700603™) and *A. baumannii* (ATCC® BAA-747™) were purchased

from the Iranian Research Organization for Science and Technology and activated according to their respective instructions. Resazurin sodium (R 7017), gentamicin, and meropenem were purchased from Sigma (batch No. #22ka12876), Exir Pharmaceutical (batch No. 0681218), and Dana Pharmaceutical (batch No. 9709VMR5184) Companies, respectively.

Determination of the minimum inhibitory and bactericidal concentrations

A resazurin microdilution assay was conducted to determine the minimum inhibitory concentration of antibiotics and plant extracts. Briefly, the stock solutions of the agents were prepared in 20% dimethyl sulfoxide (the final concentration of DMSO in the first well of the microplate was 10%). Then, the serial dilution of stock solutions was performed in the wells of the microplate containing 50 µL of Mueller-Hinton broth; next, 10 µL of the resazurin solution, 30 µL broth, and 10 µL of the bacterial suspension were added to all wells, except for the second well of each row that was determined as the negative control. Positive control in the assay was considered to be bacterial suspension, resazurin solution, and culture medium with 10% DMSO, and negative control was considered to be resazurin solution, culture medium, and 10% DMSO. The plates were incubated at 37 ˚C for 18–24 hours. The minimum inhibitory concentration (MIC) was the concentration of agents in the last well at which there was no color change from blue to pink. For the determination of the minimum bactericidal concentration (MBC) of the agents, 10 µL of wells with no color change were cultured on agar plates, and the concentration of those whose original inoculum decreased by 99.9% was regarded as MBC (22).

Checkerboard method

After determining the MIC of the plant extract and antibiotics, the checkerboard method was used to evaluate their interaction (23). To this end, antibiotics and extracts were diluted throughout the rows and columns of the microplate, and then 10 µL of the resazurin solution, 30 µL broth, and 10 µL of the bacterial suspension were added to all wells; the 9th and 10th columns were utilized as negative control and positive control, respectively. Finally, the fractional inhibitory concentration index (FICi) of antibiotics and extracts was obtained by the following formula:

$$MICc/MICe + MICc/MICa = FICe + FICa = FICi$$

MICc: MIC of drugs in combination
MICe: MIC of extracts
MICa: MIC of antibiotics
FIC: Fractional inhibitory concentration
The following ranges were considered for this test:
Synergy (FICi < 0.5), additive (0.5 < FICi < 1), subtractive (1 < FICi < 4), and antagonist (FICi > 4) (24).

Statistical analysis

Statistical analyses (except for antibacterial tests) were performed by GraphPad Prism software (version 8) using a one-way ANOVA. Linear regression was also used to calculate the concentration required to reduce the DPPH radicals by 50%.

Results

The antibacterial activity of the extracts on the studied bacteria is presented in Table 1.

*R. damascena*had the highest bacteriostatic effect on *A. baumannii* and *K. pneumoniae*, followed by *Z. multiflora* and *M. sylvestris*, while *Z. multiflora* showed a greater bactericidal effect on *A. baumannii* than *R. damascena* with an MBC of 4096 µL/mL. The results also revealed that the standard strain of *A. baumannii* was more sensitive to the studied extracts than *K. pneumoniae*. The *M. sylvestris* extract had the lowest antibacterial effect in the present study, as its MBC on *K. pneumoniae* was not found. Figure 1 illustrates the interaction between gentamicin and the *Z. multiflora* extract on *A. baumannii*. In this test, the combination of these two agents decreased the MICs of gentamicin and *Z. multiflora* extract by 1.4 and 1.2 of their independent MICs, respectively, and the final FICi for this interaction was 0.75.

The results regarding other interactions are provided in Table 2.

Based on the results, the combination of the *Z. multiflora* crude extract with meropenem on *A. baumannii* also showed an additive interaction between them with FICi of 1; other combinations in this study demonstrated subtractive interactions with FIC indexes 2–3.

The results of phytochemical investigations (Table 3) revealed that *R. damascena* exhibited the highest antioxidant activity in neutralizing free radicals, and its activity was about four times higher than that of butylated hydroxytoluene (a standard antioxidant) as their half-maximal inhibitory concentrations were 7.755 ± 2.01 µg/mL and 33.5 ± 1.9 µg/mL, respectively. *Z. multiflora* also represented higher antioxidant activity than *M. sylvestris*. In addition, *Z. multiflora* had the highest TPC and TFC, followed by *R. damascena* and *M. sylvestris*, while *M. sylvestris* had the highest anthocyanin content, followed by *R. damascena* and *Z. multiflora* (Table 3).

A positive yet statistically insignificant correlation was also observed between the antimicrobial properties of the extracts and their phenolic and flavonoid contents and antioxidant capacity. The only significant correlation was found between the antioxidant activity of *Z. multiflora* and its phenolic content (R = 0.998, *P* = 0.035).

Discussion

Plants contain many antimicrobial agents that, depending on their structures, have been classified as polyphenols, terpenoids, flavonoids, and glycosteroids. Although most of these compounds are not as effective as those produced by other organisms such as fungi, plants

Table 1. Antimicrobial activity of the extracts against the standard strain of *Klebsiella pneumoniae* and *Acinetobacter baumannii*

	***Rosa damascena* Mill.**		***Zataria multiflora* Boiss.**		***Malva sylvestris* L.**	
	MIC*	**MBC***	**MIC**	**MBC**	**MIC**	**MBC**
Klebsiella pneumoniae	4096	16384	32768	32768	65536	-
Acinetobacter baumannii	1024	8192	2048	4096	32768	131072

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.
*MIC and MBC are expressed in µg/mL.

Table 2. FIC indexes evaluated in the checkerboard assay and interaction results

Variables	**FICg***	**FICe****	**FICi**	**Remarks**	**FICm*****	**FICe**	**FICi**	**Remarks**
Rosa damascena Mill.								
Klebsiella pneumoniae	1	2	3	Subtractive	1	1	2	Subtractive
Acinetobacter baumannii	1	1	2	Subtractive	1	0.5	1.5	Subtractive
Zataria multiflora Boiss.								
Klebsiella pneumoniae	2	1	3	Subtractive	1	1	2	Subtractive
Acinetobacter baumannii	0.25	0.5	0.75	Additive	0.5	0.5	1	Additive
Malva sylvestris L.								
Klebsiella pneumoniae	1	1	2	Subtractive	2	1	3	Subtractive
Acinetobacter baumannii	1	1	2	subtractive	1	1	2	Subtractive

Note. FIC: Fractional inhibitory concentration; FICi: Fractional inhibitory concentration index;
* FICg: Fractional inhibitory concentration of gentamicin; ** FICe: The fractional inhibitory concentration of extract; *** FICm: Fractional inhibitory concentration of meropenem.

Table 3. Phytochemical properties of extracts

Variables	***Rosa damascena* Mill.**	***Zataria multiflora* Boiss.**	***Malva sylvestris* L.**	***P* value**
Antioxidant activity (IC_{50})*	7.755 ± 2.009	85.23 ± 3.825	194.6 ± 0.697	< 0.0001
Total flavonoids content**	10.07 ± 0.032	13.44 ± 0.008	4.053 ± 0.002	< 0.0001
Total phenolic content**	57.44 ± 0.267	60 ± 1.715	28.89 ± 0.15	< 0.0001
Anthocyanin content**	44.53 ± 1.584	13.16 ± 1.082	181.1 ± 1.994	< 0.0001

Note. IC50: Half-maximal inhibitory concentration. The results are reported as means ± standard deviations. *Antioxidant activity (IC_{50}) is expressed in µg/mL. The mean IC_{50} for butylated hydroxytoluene was 33.5 ± 1.9 µg/mL. **Total phenolic content values are expressed in mg equivalent GAE/g extract. In addition, the total flavonoid content and anthocyanin content are expressed in mg equivalent rutin/g extract and mg/g extract, respectively.

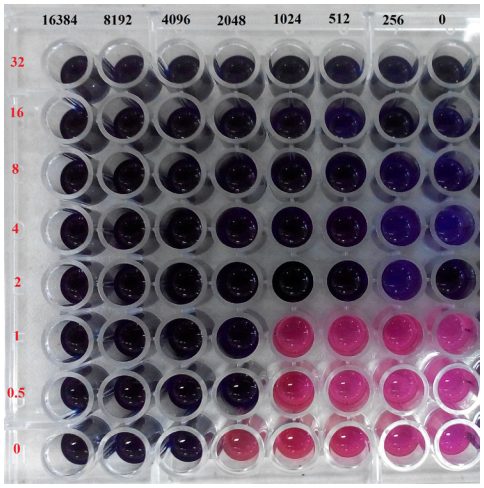

Figure 1. Checkerboard assay results on gentamicin and the *Zataria multiflora* extract interaction on *Acinetobacter baumannii*. *Note*. The concentrations are expressed in µg/mL

have been reported to act successfully in dealing with infections for certain reasons, including the interaction between these compounds (10). In recent years, antibiotic resistance to bacteria and their side effects have triggered pharmaceutical companies to seek out new antibiotics or change the structure of available antibiotics to overcome these problems. But certainly, these measures do not suffice, and more strategies, in addition to antibiotics alone, are needed, including immune-based, bacteriophage, probiotic, and combination therapies using plant derivatives with antimicrobial properties (17, 25). Antibacterial compounds in plants target their hosts through numerous mechanisms, such as the disruption of the cell membrane, inactivation of bacterial enzymes, substrate deprivation, bacterial content coagulation, and disruption of other vital activities of bacteria, making them appropriate solutions to certain bacterial strategies for their survival against antibiotics (26). Some bacteria use efflux pumps in their membrane to survive against antibiotics; for example, many of them have been identified in *Acinetobacter* spp. (27), and some studies have shown that many plant extracts and their derivatives, such as some flavonoids and phenols in *Thymus vulgaris* and *Rosmarinus officinalis*, could inhibit these pumps and help overcome resistance to tetracycline, fluoroquinolones, and erythromycin (28); the additive interaction between the *Z. multiflora* extract and gentamicin observed in the present study is likely to be produced by the same mechanism, and

some components in this extract could inhibit the efflux pumps of *A. baumannii* that assists in the accumulation of gentamicin in bacteria and reduction in its MIC. This argument, however, deserves further investigation; for instance, a study on the clinical isolates of *A. baumannii* reported the synergistic effect of *Lavandula stoechas* and *Z. multiflora* essential oils through inhibiting efflux pumps (29).

In the present study, an additive interaction was found between the *Z. multiflora* extract and meropenem. It is likely that some components in this extract, as with other plant derivatives, act as β-lactamase inhibitors, increase membrane permeability, or directly attack the same site in the cell wall in the same manner as of meropenem (17,30). For example, a study on the hydroethanolic extract of *Leonotis nepetifolia* demonstrated that this extract disturbed the membrane of *Enterococcus faecalis* and *Shigella flexneri* and facilitated the entrance of hydrophobic antibiotics into the bacteria (31), but the same interaction effect on *K. pneumoniae* was not observed in the current study. A study on the antibacterial effect of *Olea europaea*, *Tabebuia avellanedae*, and green propolis extracts against several respiratory bacterial pathogens revealed their potent antibacterial effects, as their MIC on *K. pneumoniae* was detected at 12500 µL/mL. The findings of this study also confirmed several additives and synergistic interactions between antibiotics and plant extracts, highlighting the potency of these natural components in overcoming antibiotic resistance (32). The *R. damascena* extract in the present study also showed higher antibacterial efficacy against this bacterium.

This may be attributed to its polysaccharide capsule that can act as a barrier to components with these properties, preventing them from reaching the bacterial cell membrane, as *K. pneumoniae* polysaccharide capsule is an important factor for its resistance to antimicrobial peptides and prevents complement activation. The hydrophilicity of the capsule and the negative charge between the capsule and core lipopolysaccharide, which supports their linking, can cause the capsule to act as an additional barrier against antibacterial agents in reaching their targets in bacteria (33-36). Further resistance of *K. pneumoniae* observed in the present study may be due to the same reason, and this layer might have prevented the antimicrobial agents in extracts from reaching the bacteria.

The results of the present study, consistent with those of other studies (17,37), revealed that the interaction between groups of antibiotics and plant derivatives varied depending on bacteria spp., drug resistance mechanism, and even the temperature of the environment; as a result, it is recommended that researchers focus on ingredients that have potential to overcome the resistance of many bacteria, including inhibition of efflux pumps.

A subtractive interaction in the current study was observed between some of the studied agents. Although the antagonistic mechanism of the interaction between natural compounds and other antimicrobials has not been studied enough, some researchers consider several mechanisms for this type of interaction, including reactions between the plant-derived agents and antimicrobials through the influence on solubility, the competition between bacteriostatic and bactericidal agents, and the like (37).

The phytochemical investigations demonstrated that *Z. multiflora* was rich in phenolic and flavonoid compounds that contribute to its antimicrobial properties; in other words, *Z. multiflora*, in addition to exhibiting a substantial antibacterial property, was also found to contain the highest phenolic compounds. A positive correlation was also found between the phenolic content of *Z. multiflora* and its antioxidant activity, which reveals the balance between the antioxidant capacity and phenolic components of this extract, making it an appropriate therapeutic agent for infections.

Regarding phytochemical properties and antimicrobial outcomes, although there was a positive correlation between extracts' antioxidant activities, TPC and TFC, and their antimicrobial activities, none of the correlations were statistically significant. The absence of significant correlations could be eliminated by the fractionation of extracts, testing of the phytochemical properties of each fraction, and their comparison with their antimicrobial activity against more Gram-positive and Gram-negative bacteria.

Studies on the antimicrobial effects of different *R. damascena* extract fractions showed that the crude extract was more effective on some of the tested bacteria compared with the chloroform, ethyl acetate, and butanol fractions (38). This may be due to the interaction between several groups of the active ingredients of the plant in its crude extract, enhancing its antimicrobial properties. These investigations, in line with the present study, indicate the diversity of different interaction effects of compounds of plant extracts on different bacteria. Another study reported that the administration of the aqueous *R. damascena* extract in rabbits reduced gentamicin nephrotoxicity by the reversal of electrolyte imbalance and hematological disturbance (39). Moreover, one study confirmed the protective effect of *M. sylvestris* on gentamicin-induced toxicity (14). The results of our study imply that the combination of this plant and gentamicin should be administered for infectious diseases more cautiously because of their diverse interaction effects on different bacteria. Further in vivo studies are also needed because many factors may influence the interaction between two drugs, including their pharmacokinetics (40).

Conclusion

Our findings revealed the potency of *R. damascena* and *Z. multiflora* to be administered against MDR bacteria which are important challenges facing healthcare systems. Treatment with *Z. multiflora* in combination with gentamicin and meropenem could enhance their

antibacterial properties against *K. pneumoniae* and *A. baumannii*, although more in vivo studies are required to thoroughly investigate their interactions. The findings also demonstrated diverse interactions between the compounds in plant extracts and other antimicrobials, making it difficult to predict the outcome; nevertheless, it is recommended that the researchers purify extracts' potent compounds and administer them independently and in combination with other antimicrobials. Given the widely various results regarding the interaction of antimicrobials, we also suggest focusing on the interactions that disrupt common vital strategies for bacteria, such as the inhibition of efflux pumps.

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Competing Interests

The authors declare that there was no conflict of interests.

Ethical Approval

The protocol of the study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (No. IR.SKUMS.REC.1395.260).

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References

1. Nathan C, Cars O. Antibiotic resistance--problems, progress, and prospects. N Engl J Med. 2014;371(19):1761-3. doi: 10.1056/NEJMp1408040.
2. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-18. doi: 10.1179/2047773215y.0000000030.
3. Peters L, Olson L, Khu DTK, Linnros S, Le NK, Hanberger H, et al. Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: a cohort study among neonatal intensive care patients with hospital-acquired infections caused by gram-negative bacteria in Vietnam. PLoS One. 2019;14(5):e0215666. doi: 10.1371/journal.pone.0215666.
4. Chang RYK, Nang SC, Chan HK, Li J. Novel antimicrobial agents for combating antibiotic-resistant bacteria. Adv Drug Deliv Rev. 2022;187:114378. doi: 10.1016/j.addr.2022.114378.
5. Carvalho I, Alonso CA, Silva V, Pimenta P, Cunha R, Martins C, et al. Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated from healthy and sick dogs in Portugal. Microb Drug Resist. 2020;26(6):709-15. doi: 10.1089/mdr.2019.0205.
6. Jacobs DM, Safir MC, Huang D, Minhaj F, Parker A, Rao GG. Triple combination antibiotic therapy for carbapenemase-producing *Klebsiella pneumoniae*: a systematic review. Ann Clin Microbiol Antimicrob. 2017;16(1):76. doi: 10.1186/s12941-017-0249-2.
7. Tiwari V, Roy R, Tiwari M. Antimicrobial active herbal compounds against *Acinetobacter baumannii* and other pathogens. Front Microbiol. 2015;6:618. doi: 10.3389/fmicb.2015.00618.
8. Leus IV, Weeks JW, Bonifay V, Smith L, Richardson S, Zgurskaya HI. Substrate specificities and efflux efficiencies of RND efflux pumps of *Acinetobacter baumannii*. J Bacteriol. 2018;200(13):e00049-18. doi: 10.1128/jb.00049-18.
9. Bartmańska A, Wałecka-Zacharska E, Tronina T, Popłoński J, Sordon S, Brzezowska E, et al. Antimicrobial properties of spent hops extracts, flavonoids isolated therefrom, and their derivatives. Molecules. 2018;23(8):2059. doi: 10.3390/molecules23082059.
10. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, et al. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. Chem Biol Interact. 2019;308:294-303. doi: 10.1016/j.cbi.2019.05.050.
11. Nayebi N, Khalili N, Kamalinejad M, Emtiazy M. A systematic review of the efficacy and safety of *Rosa damascena* Mill. with an overview on its phytopharmacological properties. Complement Ther Med. 2017;34:129-40. doi: 10.1016/j.ctim.2017.08.014.
12. Sivaraj C, Abhirami R, Deepika M, Sowmiya V, Saraswathi K, Arumugam P. Antioxidant, antibacterial activities and GC-MS analysis of fresh rose petals aqueous extract of *Rosa damascena* Mill L. J Drug Deliv Ther. 2019;9(4S):68-77. doi: 10.22270/jddt.v9i4-s.3248.
13. Braga AS, Pires JG, Magalhães AC. Effect of a mouthrinse containing *Malva sylvestris* on the viability and activity of microcosm biofilm and on enamel demineralization compared to known antimicrobials mouthrinses. Biofouling. 2018;34(3):252-61. doi: 10.1080/08927014.2018.1428957.
14. Mohamadi Yarijani Z, Najafi H, Shackebaei D, Madani SH, Modarresi M, Jassemi SV. Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by *Malva sylvestris* extract in gentamicin induced renal toxicity. Biomed Pharmacother. 2019;112:108635. doi: 10.1016/j.biopha.2019.108635.
15. Mahmoodi M, Koohpeyma F, Saki F, Maleksabet A, Zare MA. The protective effect of *Zataria multiflora* Boiss. hydroalcoholic extract on TNF-α production, oxidative stress, and insulin level in streptozotocin-induced diabetic rats. Avicenna J Phytomed. 2019;9(1):72-83.
16. Dashipour A, Razavilar V, Hosseini H, Shojaee-Aliabadi S, German JB, Ghanati K, et al. Antioxidant and antimicrobial carboxymethyl cellulose films containing *Zataria multiflora* essential oil. Int J Biol Macromol. 2015;72:606-13. doi: 10.1016/j.ijbiomac.2014.09.006.
17. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: are synergistic combinations of

plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev. 2017;11(22):57-72. doi: 10.4103/phrev.phrev_21_17.
18. Torki A, Khalaji-Pirbalouty V, Lorigooini Z, Rafieian-Kopaei M, Sadeghimanesh A, Rabiei Z. *Anchusa italica* extract: phytochemical and neuroprotective evaluation on global cerebral ischemia and reperfusion. Braz J Pharm Sci. 2018;54(1):e17251. doi: 10.1590/s2175-97902018000117251.
19. Taghavi T, Patel H, Rafie R. Comparing pH differential and methanol-based methods for anthocyanin assessments of strawberries. Food Sci Nutr. 2022;10(7):2123-31. doi: 10.1002/fsn3.2065.
20. Carmona-Hernandez JC, Taborda-Ocampo G, González-Correa CH. Folin-Ciocalteu reaction alternatives for higher polyphenol quantitation in Colombian passion fruits. Int J Food Sci. 2021;2021:8871301. doi: 10.1155/2021/8871301.
21. Mohamed WA, Ismail NZ, Muhamad M, Omar EA, Abdul Samad N, Ooi JP, et al. Q-TOF LC-MS compounds evaluation of propolis extract derived from Malaysian stingless bees, *Tetrigona apicalis*, and their bioactivities in breast cancer cell, MCF7. Saudi J Biol Sci. 2022;29(10):103403. doi: 10.1016/j.sjbs.2022.103403.
22. Huq MA, Akter S. Biosynthesis, characterization and antibacterial application of novel silver nanoparticles against drug resistant pathogenic *Klebsiella pneumoniae* and *Salmonella enteritidis*. Molecules. 2021;26(19):5996. doi: 10.3390/molecules26195996.
23. Berditsch M, Jäger T, Strempel N, Schwartz T, Overhage J, Ulrich AS. Synergistic effect of membrane-active peptides polymyxin B and gramicidin S on multidrug-resistant strains and biofilms of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2015;59(9):5288-96. doi: 10.1128/aac.00682-15.
24. Iten F, Saller R, Abel G, Reichling J. Additive antimicrobial effects of the active components of the essential oil of *Thymus vulgaris*–chemotype carvacrol. Planta Med. 2009;75(9):PJ100. doi: 10.1055/s-0029-1234905.
25. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15(8):639-52. doi: 10.1016/j.phymed.2008.06.008.
26. Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. N Engl J Med. 2013;368(4):299-302. doi: 10.1056/NEJMp1215093.
27. Silva NC, Fernandes Júnior AJ. Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis. 2010;16(3):402-13. doi: 10.1590/s1678-91992010000300006.
28. Brzoska AJ, Hassan KA, de Leon EJ, Paulsen IT, Lewis PJ. Single-step selection of drug resistant *Acinetobacter baylyi* ADP1 mutants reveals a functional redundancy in the recruitment of multidrug efflux systems. PLoS One. 2013;8(2):e56090. doi: 10.1371/journal.pone.0056090.
29. Agahi P, Mahdavi-Ourtakand M, Noorbakhsh F. Synergistic effect of *Zataria multiflora* and *Lavandula stoechas* essential oils on phenotype detection of efflux pump against multidrug-resistant *Acinetobacter baumannii* isolates. Rev Res Med Microbiol. 2021;32(1):39-44. doi: 10.1097/mrm.0000000000000229.
30. Mikulášová M, Chovanová R, Vaverková Š. Synergism between antibiotics and plant extracts or essential oils with efflux pump inhibitory activity in coping with multidrug-resistant staphylococci. Phytochem Rev. 2016;15(4):651-62. doi: 10.1007/s11101-016-9458-0.
31. Abreu AC, McBain AJ, Simões M. Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep. 2012;29(9):1007-21. doi: 10.1039/c2np20035j.
32. Ramata-Stunda A, Petriņa Z, Valkovska V, Borodušķis M, Gibnere L, Gurkovska E, et al. Synergistic effect of polyphenol-rich complex of plant and green propolis extracts with antibiotics against respiratory infections causing bacteria. Antibiotics (Basel). 2022;11(2):160. doi: 10.3390/antibiotics11020160.
33. Oliveira DM, Melo FG, Balogun SO, Flach A, de Souza EC, de Souza GP, et al. Antibacterial mode of action of the hydroethanolic extract of *Leonotis nepetifolia* (L.) R. Br. involves bacterial membrane perturbations. J Ethnopharmacol. 2015;172:356-63. doi: 10.1016/j.jep.2015.06.027.
34. Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, Bengoechea JA. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun. 2004;72(12):7107-14. doi: 10.1128/iai.72.12.7107-7114.2004.
35. Merino S, Camprubí S, Albertí S, Benedí VJ, Tomás JM. Mechanisms of *Klebsiella pneumoniae* resistance to complement-mediated killing. Infect Immun. 1992;60(6):2529-35. doi: 10.1128/iai.60.6.2529-2535.1992.
36. Tomás JM, Benedí VJ, Ciurana B, Jofre J. Role of capsule and O antigen in resistance of *Klebsiella pneumoniae* to serum bactericidal activity. Infect Immun. 1986;54(1):85-9. doi: 10.1128/iai.54.1.85-89.1986.
37. Bassolé IH, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989-4006. doi: 10.3390/molecules17043989.
38. Shohayeb M, Abdel-Hameed ES, Bazaid SA, Maghrabi I. Antibacterial and antifungal activity of *Rosa damascena* Mill. essential oil, different extracts of rose petals. Glob J Pharmacol. 2014;8(1):1-7. doi: 10.5829/idosi.gjp.2014.8.1.81275.
39. Mumtaz F, Khaliq T, Zia-ur-Rahman IJ, Iftikhar A, Aslam B, Ali A, et al. Effects of *Rosa damascena* Mill flowers, *Cichorium intybus* Linn roots and their mixtures on serum electrolytes and hematological parameters against gentamicin induced toxicity in albino rabbits. Indo Am J Pharm Res. 2014;4(1):236-42.
40. Nagy F, Tóth Z, Nyikos F, Forgács L, Jakab Á, Borman AM, et al. In vitro and in vivo interaction of caspofungin with isavuconazole against *Candida auris* planktonic cells and biofilms. Med Mycol. 2021;59(10):1015-23. doi: 10.1093/mmy/myab032.