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Investigating the Antimicrobial Effect of *Loranthus europeaus* Leaf Hydroalcoholic Extract Against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: *Staphylococcus aureus* is one of the important factors that causes hospital infections. In recent years, the emergence of antibiotic resistance has become widespread. Methicillin-resistant *S. aureus* (MRSA) infection is caused by a type of staphylococcus bacteria that is resistant to many antibiotics. The use of medicinal plants for the treatment of various diseases has increased recently. Hence, we decided to evaluate the antimicrobial effect of *Loranthus europaeus* leaf hydroalcoholic extract against methicillin-resistant *S. aureus*.

Methods: The leaves of *L. europeaus* were collected from Khorramabad in Lorestan province. After drying the leaves, a hydroalcoholic extract of this plant was prepared. Finally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the hydroalcoholic extract of *L. europeaus* were measured by the CLSI microdilution method.

Results: Based on the results, the MIC and MBC values for the hydroalcoholic extract of the leaves of *L. europaeus* were 6.13 µg/mL and 98 µg/mL for MRSA, respectively.

Conclusion: Our results showed that MRSA clinical strain was sensitive to the hydroalcoholic extract of *L. europaeus*. Therefore, this extract can be proposed as a good solution for routine antibiotics.

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Intorduction

Different types of microbial infections affect many people around the world every year. These types of infections bring huge costs to the health system worldwide annually. Some of these mialicrobinfections, such as staphylococcus infection, cause antibiotic resistance. Microbial resistance has created serious concerns for

scientists (Mancuso et al., 20121). It is estimated that antibiotic resistance will cause the death of 10 million people annually by 2050. Also, estimates show that antibiotic resistance will cost the health system as much as 100 trillion dollars (Chinemerem Nwobodo et al. 2022). Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is caused by a type of *Staphylococcus* bacteria that is resistant to many antibiotics used in the treatment of staphylococcal infections (Lee et al., 2018). In most cases, methicillin-resistant *S. aureus* infection occurs in people who are in hospitals or treatment centers such as dialysis centers. Patients who get these infections in medical centers have often undergone surgery or had artificial devices, tubes, or joints inserted in their bodies. The common methicillin-resistant *S. aureus* infection in hospitals is called *S. aureus* nosocomial infection or HA-MRSA (Chen et al., 2022). Another type of MRSA infection occurs in the wider community. This type of MRSA infection is often accompanied by painful skin rashes. This type of methicillin-resistant *S. aureus* infection, abbreviated as CA-MRSA, is spread through skin contact. People who live in crowded communities are at risk of contracting this infection with methicillin-resistant *S. aureus* (Shiadeh et al., 2022). The use of medicinal plants in the treatment of microbial diseases has along history. Medicinal plants with a wide range of chemical compounds show good antimicrobial activity (Mickymaray et al., 2019). *L. europeaus* is a semi-parasitic plant that, due to its type of life, gets its nutritional needs completely from the host plant during the establishment and growth stage, and then it is able to make the organic materials it needs during the leaf production and photosynthesis stage. But in terms of water and minerals, it still depends on the host plant (Gleb et al., 2019). However, recently it has been found that this plant has useful therapeutic properties, especially antimicrobial properties for the treatment of a wide range of diseases (Ambrosio et al., 2019). Hence, the aim of this study was to evaluate the antimicrobial effect of *L .europeaus* leaf hydroalcoholic extract against methicillin-resistant *S.aureus*.

Materials and Methods:

Plant Preparation

The leaves of *L. europeaus* were collected and dried from May to June 2022 from the forest area of Khorramabad, Lorestan province, western Iran.

Hydroalcoholic Extract Preparation

The leaves of the dried *L. europeaus* were powdered. 50 grams of the resulting powder was placed in a soxhle using 400 mL of 30% ethanol, 40% methanol and 30% water at a temperature of 70°C for 3 hours to prepare the extract. The resulting green hydroalcoholic solution (400 mL) was placed in a rotary machine with a speed of 30 rpm and a temperature of 50°C for one hour to evaporate ethanol, water and methanol, then the resulting extract of *L. europeaus* was dried for 3 days. It was placed in the oven with a temperature of 40 °C (Bahmani et al., 2019).

Antibacterial Evaluation

In this study, the antibacterial effect of *L. europeaus* hydroalcoholic extract was determined by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods using the micro dilution method using the Clinical & Laboratory Standards Institutemethod (CLSI, 2009). For this purpose, first, 50 microliters of Mueller-Hinton medium was added to all the wells. So, 50 microliters, equivalent to McFarland half suspension 5 x 105 (CFU/mL) of bacteria was added to wells number 2 to 10. Then they were incubated at 37 °C. Then 50 microliters of hydroalcoholic extract was added to well number 1. Then dilution was done from the second to the tenth row. The plates were incubated for 48 to 72 hours and the MIC was measured. Also, the protocol proposed by CLSI (2009) was also used to determine MBC. Standard antibiotics methicillin and cholestin were used as controls (Bahmani et al., 2019).

Results

As shown in Table 1, the MIC and MBC values for the hydroalcoholic extract of the leaves of Loranthuseuropeaus were 6.13 µg/mL and 98 µg/mL for MRSA, respectively. Furthermore, the MIC and MBC values for methicillin as positive control were 6.13 µg/mL and 98 µg/mL for MRSA, respectively.

Table 1. The MIC and MBC values for the hydroalcoholic extract of the leaves of *Loranthuseuropeaus*, methicillin and cholestin.

Bacterial strain	Hydroalcoholic extract		Antibiotics			
	MIC	MBC	Methicillin		Cholestin	
			MIC	MBC	MIC	MBC
MRSA	6.13	98	380	380	-	-

MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Discussion

Due to the increasing resistance of bacteria to a variety of antibiotics, efforts have been made to obtain and use the compounds found in plants and their use in the treatment of various diseases (Górniak et al., 2019). Plants have played a very important role in maintaining health and improving the quality of life for thousands of years. Medicinal plants have useful properties, such as antibacterial, antiparasitic, antifungal, antiulcer,antiinflammatoryand antioxidant properties (Baidya et a., 2020; Das et al., 2018; Muduli et al., 2022; Bose et al., 2019). The essential oils of medicinal plants with antimicrobial effects on a wide range of organisms, as well as their ability to be used as food in some cases, and their side effects being less than common antibiotics, can eventually replace antibiotics (Anand et al., 2019). Hence, the aim of this study was to evaluate the antimicrobial effect of *L. europeaus* leaf hydroalcoholic extract against methicillin-resistant *S. aureus*. The results of the current study highlighted that the MIC and MBC values for the hydroalcoholic extract of the leaves of *L. europeaus* were 6.13 µg/mL and 98 µg/mL for MRSA, respectively. Furthermore, the MIC and MBC values for methicillin as positive control were 6.13 µg/mL and 98 µg/mL for MRSA, respectively.There are several pieces of evidence about the presence of various types of

phytochemical constituents such as catechin, epicatechin, chlorogenic acid, hyperoside and pinoresinol in methanolic extract of various parts of *L.europeaus* including fruits, stalks and leaves. Furthermore, the strong antioxidant capacity of methanolic extract of various parts of *L. europeaus*t hrough the ferric reducing ability of plasma (FRAP), cupric reducing antioxidant capacity (CUPRAC), ABTS, DPPH and phosphomolybdenum assays. It has been found that the high antioxidant activity of this plant can be due to the presence of various types of phytochemical constituents especially catechin (Benabderrahim et al., 2019). Khalifa E. Sharquie and his colleagues demonstrated that Loranthuseuropaeuscould be proposed as a therapeutic and anti-parasitic agent for the treatment of cutaneous *Leishmaniasisis*. They also found that the therapeutic effect of this plant could be related to the presence of several phytochemical compounds such as alkaloids, flavonoids, phenolic acids, terpenoides, and others (Sharquie et al., 2016). The results of another similar study conducted by Rosa Luisa Ambrosio and her colleagues in agreement with the results of our investigation revealed that the extract protein compounds from *L.europaeus* could exert anti-bacterial and anti-fungal properties against several types of pathogenic fungi such as *Aspergillusniger, Alternaria spp., Penicillium spp., Botritiscinereus* and pathogenic bacteria including *Listeria monocytogenes, Staphylococcus aureus strains, Salmonella Typhimurium and Escherichia coli*. Furthermore, the authors concluded that the extract protein compounds from *L.europaeus*can possess remarkable bactericidal activity against above mentioned pathogenic bacterial strains. The results of their study documented that *S.aureus* was the most susceptible pathogenic bacterial strains with the MIC and MBC values between 0.2 and 0.5 mg/mL, respectively (8). Abbas yaseen Hasan and Thuraya Kadhum Ismael in a similar study with our investigation evaluated anti-bacterial effect of *L. europaeus* L. aqueous and alcohol extracts against clinical Methicillin-resistant *S. aureus*. The results of their study in line with the findings of our study showed that the aqueous and alcohol extracts of *L. europaeus* L. had strong anti-bacterial capacity. They also understood that the aqueous extract of *L. europaeus* L. exerted an anti-bacterial effect with the diameters of inhibition zone found as 14.57, 15.28, 16.00, 23.71 and 25.00 mm for concentrations of 12.5, 25, 50, 100 and 200 mg/mL, respectively. furthermore, the observed that the alcohol extract of *L. europaeus* L. possessed an anti-bacterial property with the diameters of inhibition zone found as 7.57, 8.42, 10.57,13.28, 17.28 mm for concentrations of 12.5, 25, 50, 100 and 200 mg/mL, respectively (Yaseen Hasa et al., 2018n)

Conclusions

Taking together, the results of our study indicated that the hydroalcoholic extract of the leaves of *L. europeaus* could display a potent antibacterial effect against MRSA. Further studies in the field of identification of phytochemical compounds are recommended for the recognition of other biological properties of this plant.

Declarations

Conflict of interest

There is no conflict of interest among the authors.

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Consent for publications

The authors approved the manuscript for publication.

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This study was carried out with personal funds from the authors.

Authors' contributions

RMKH conceived the research idea and SKM designed the work. SSH carried out the experiment, SKM and RMKH wrote the first draft of the manuscript, PM and AB carried out the literature search, AB carried out the statistical analysis, and RMKH supervised the study. All authors read and approved the final manuscript for publication.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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