constituents may be slower than to single entities. This might be considered as another advantage for using extracts rather than pure compounds as antimicrobial agents.[14]

Currently, MRSA is a worldwide problem with limited success with antimicrobial treatment. Accordingly, the reported antimicrobial activity of the *S. triloba* extract, against the sensitive reference strain of *S. aureus,* prompted us to evaluate its activity against MRSA. Our results demonstrated a bacteriocidal activity of the *S. triloba* ethanol extract and its volatile oil against the tested culture of MRSA. The reported results are of significant value and further studies are in progress to evaluate their antibiofilm and antiadhesion activities in combination with nonantibiotic agents.

The prevalent mode of growth in most ecological niches is biofilm. Some plant extracts and purified natural compounds were found to possess antibiofilm and antiadhesion activities.[12,14,28,29] Our findings indicated dose-dependent antibiofilm and antiadhesion activities of the *S. triloba* ethanol extract and its volatile oil. Previous studies on the antibiofilm activities of other *Salvia* species such as *S. pratensis* and *S. virgata* did not show any effect under the tested experimental conditions.[14] In the present study, the *S. triloba* extract did not achieve biofilm eradication under our experimental conditions. Nevertheless, this was demonstrated by its volatile oil. Moreover, both agents exhibited antiadhesive activities which count as a prophylactic measure toward biofilm establishment.

In comparison to chlorhexidine, the antibiofilm results of the *S. triloba* extract and its volatile oil demonstrated their effectiveness as antibiofilm agents at the tested concentrations where they have achieved considerable antibiofilm activities in comparison to that achieved by chlorhexidine. At predetermined MBC concentrations, chlorhexidine was capable to eradicate the planktonic cultures of *S. aureus*. At these concentrations, the best antibiofilm activity achieved by chlorhexidine was a 2 log cycle reduction. On the other hand, the antibiofilm activities reported in this study reached in some cases to 1–2 log cycle reduction in the population representing a significant effect relative to that of chlorhexidine.

CONCLUSIONS

In many situations, it is very difficult to eradicate and even to treat microbial biofilms. Hence, the inhibition of microbial adhesion to surfaces and the subsequent biofilm development would be of great value. The present study demonstrated that the *S. triloba* extract and its volatile oil should be considered of great value in preventing and treating biofilms due to their antibiofilm, antiadhesion, and anti-MRSA activities. Furthermore, these findings revealed that plants used in traditional medicine are still one of the significantly reliable sources in discovering cheap, safe, and easy accessible medicines. Based on the demonstrated activity of the *S. triloba* extract against *S. aureus* [Figure 1], it can be suggested that this plant extract might be used as an antiseptic in the prophylaxis and treatment of *S. aureus*-associated skin infections. In conclusion, although *Salvia* species have been previously demonstrated to possess antimicrobial activities against planktonic bacterial cultures, this is the first study to demonstrate the antibiofilm and antiadhesion activities for one of the *Salvia* species, namely, *S. triloba*. Accordingly, these findings justify the consideration of the *S. triloba* extract and oil as potential candidates in successful treatment of skin infections caused by *S. aureus* biofilms.

ACKNOWLEDGMENT

The authors are grateful to Mr Ismail Abaza for his technical help. This work was financially supported by the Deanship of Academic Research, University of Jordan.

REFERENCES

1. Lev E, Amar Z. Ethnopharmacological survey of traditional drugs sold in the Kingdom of Jordan. J Ethnopharmacol 2002;82: 131-45.
2. Afifi FU, Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J Ethnopharmacol 2000;72:101-10.
3. Oran S. Genetic resources of medicinal plants in Jordan. In: Jaradat A, editor. Plant genetic resources of Jordan. Proceedings of a national seminar. 2-4 August 1994, Amman: West Asia and North Africa Regional Office, IPGRI; 1994. p. 13-51.
4. Abu-Irmaileh BE, Afifi FU. Herbal medicine in Jordan with special emphasis on commonly used herbs. J Ethnopharmacol 2003;89:193-7.
5. El-Sayed NH, Khalifa TI, Ibrahim MT, Mabry TJ. Constituents from *Salvia triloba*. Fitoterapia 2001;72:850-3.
6. Oran SA, Al-Eisawi DM. Checklist of medicinal plants in Jordan. Dirasat 1998;25:84-111.
7. Bisio A, Romussi G, Russo E, Cafaggi S, Schito AM, Repetto B, *et al*. Antimicrobial activity of the ornamental species *Salvia corrugate* a potential new crop for extractive purposes. J Agric Food Chem 2008;56:10468-72.
8. Delamare AP, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem 2007;100:603-8.
9. Kamatou GP, Viljoen A, van Vuuren SF, van Zyl RL. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagea* and *Leontis leonurus*. S Afr J Bot 2006;72:634-6.
10. Kelen M, Tepe B. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. Bioresour Technol 2008;99:4096-104.
11. Kotan R, Kordali S, Cakir A, Kesdek M, Kaya Y, Kilic H. Antimicrobial and insecticidal activities of essential oil isolated

from Turkish *salvia hydrangea* DC: Ex Benth. Biochem Syst Ecol 2008;36:360-8.

12. Kuzma L, Rozalski M, Walencka E, Rozalska B, Wysokinska H. Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclarea* L.: Salvipisone as a potential anti-biofilm agent active against antibiotic resistant *Staphylococci.* Phytomedicine 2007;14:31-5.
13. Mayekiso B, Magwa ML, Coopoosamy RM. The chemical composition and antibacterial activity of the leaf extract of *Salvia repens* Burch: Ex Benth. J Med Plant Res 2008;2:159-62.
14. Quave CL, Plano LR, Pantuso T, Bennett BC. Effects of extracts from Italian medicinal Plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol 2008;118:418-28.
15. WHO World Health Organization. Legal Status of Traditional Medicine and Complementary Alternative Medicine, a Worldwide Review. WHO, Geneva: WHO Publications; 2001.
16. Cunha BA. Antimicrobial therapy of multidrug-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*. Med Clin North Am 2006;90:1165-82.
17. Rice LB. Antimicrobial resistance in gram-positive bacteria. Am J Infect Control 2006;34:S11-9.
18. Feinbrun-Dothan N. Flora Palestina**.** Vol. 3. Jerusalem: Publication of the Israel Academy of Sciences and Humanities; 1978. p. 133-45.
19. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer Verlag; 1996. p. 144-5.
20. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA: NCCLS; 2003.
21. Al-Bakri AG, Othman G, Bustanji Y. The assessment of the antibacterial and antifungal activities of aspirin, EDTA and aspirin-EDTA combination and their effectiveness as antibiofilm agens. J Appl Microbiol 2009;107:280-6.
22. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiol Immunol 2005;20:221-5.
23. Gursoy UK, Gursoy M, Gursoy OV, Cakmakci L, Könönen E, Uitto VJ. Anti-biofilm properties of *Satureja hortensis* L. essential oil against periodontal pathogens. Anaerobe 2009;15:164-7.
24. Al-Bakri AG, Bustanji Y, Yousef A. Common consumption of antibacterial drugs within the Jordanian population: Sources, patterns and appropriateness. Int J Antimicrob Agents 2005;26:389-95.
25. Taha MO, Al-Bakri AG, Zaloum W. Discovery of potent inhibitors of *Pseudomonal quorum* sensing via pharmacophore modeling and in-silico screening. Bioorg Med Chem Lett 2006;16:5902-6.
26. Taha MO, Atallah N, Al-Bakri AG, Paradis-Bleau C, Zalloum H, Younis KS, *et al*. Discovery of new murF inhibitors via pharmacophore modeling and QSAR analysis followed by in-silico screening. Bioorg Med Chem 2008;16:1218-35.
27. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents 1999;11:217-21.
28. Dalleau S, Cateau E, Berges T, Berjeaud JM, Imbert C. *In vitro* activity of terpenes against *Candida* biofilm. Int J Antimicrob Agents 2008;31:572-6.
29. Schillaci D, Arizza V, Dayton T, Camarda L, Stefano VD. *In vitro* anti-biofilm activity of *Boswellia* spp. oleo gum resin essential oils. Lett Appl Microbiol 2008;47:433-8.
30. Aqil F, Ahmad I, Owais M. Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. J Biotechnol 2006;1:1093-102.
31. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol 2006;106:290-302.
32. Kamatou GP, Viljoen A, van Vuuren SF, van Zyl RL. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagea* and *Leontis leonurus*. S Afr J Bot 2006;72:634-6.

Source of Support: Deanship of Academic Research, University of Jordan, **Conflict of Interest:** None declared