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Exploration of Phytochemical and Antibacterial Potentiality of *Anagallis arvensis* L. Extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JSR, SMHA, AM, Majid Sharifi-Rad, Marzieh Sharifi-Rad, MH and Mehdi Sharifi-Rad designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JSR, SMHA and AM managed the analyses of the study. Author Mehdi Sharifi-Rad reviewed critically the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Context: *Anagallis arvensis* L. (Scarlet pimpernel) was used to treatment of several ailments in several countries.
Objective: The aim of this study was to evaluate the *in vitro* antimicrobial activity of leaf methanolic extract of *A. arvensis* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: In this study *A. arvensis* leaf was shade dried, powdered and extract was made by using methanol. The antimicrobial activity of methanolic extract was investigated against clinical isolates of MRSA by both the disc diffusion method and the microbroth dilution method.
Results: The result of disc diffusion method showed that the plant extract recorded different degrees of antibacterial activity on MRSA as evidenced by the inhibited zones. The MICs of the plant extract and vancomycin were >100 and 14.5±0.1 µg/mL, respectively. This results showed that the plant extract although have slightly effect on MRAS but it was not sufficient strong.
Discussion and Conclusion: *A. arvensis* leaf extract has anti-MRSA properties, corroborating the traditional therapeutic uses of this plant, and can be used in the therapy of infectious diseases as well as an antimicrobial supplement in food industries.

Keywords: Scarlet pimpernel; Inhibition zone; disc diffusion method; microbroth dilution method; MIC.

1. INTRODUCTION

The excessive and repeated use of the same drugs used in modern medicine cause to the evolution of antibiotic-resistant microbes, including *Staphylococcus aureus* whose emergence of antibiotic-resistant strains reduces the number of antibiotics available to treat clinical infections caused by this bacterium [1,2]. Many medicinal plants are considered as important natural remedies for the treatment of health conditions and diseases. Recently, natural products were used as the source and inspiration for a large fraction of the common pharmacopoeia. These have played an important role in development and drug discovery especially for agents against infectious diseases [3-7]. Plants are very significant natural source due to production of complex molecular substances. The plant produce structures such as secondary metabolites (phenolic compounds, flavonoids, alkaloids, coumarins, tannins, glycosides, terpens and isoflavonoids) and their derivatives have antimicrobial properties [8-12]. Despite plants are extremely exploited in traditional healing systems, only in some cases their curative potential in human has been substantiated [13]. The need of herb-based medicines, cosmetics health products, food supplements, and pharmacevauticals is successively expanding worldwide, since in some cases, natural products *i*) are non or low toxic, *ii*) have low side effects and *iii*) are available at affordable costs [14].

Anagallis arvensis L. (Scarlet pimpernel; also known as red chickweed, red pimpernel, poorman's barometer, shepherd's weather glass, poor man's weather-glass or shepherd's clock) is a low-growing annual plant. It was widely distributed throughout the world, being found in all temperate regions in both hemispheres [15-17]. It was used for treatment of several ailments such as gout, epileptic attacks, dropsical affections, leprosy, hydrophobia, mania, cerebral affections and other complains of the nervous system. The plant was also used as diaphoretic, diuretic, expectorant for the treatment of rheumatism, dropsy and hepatic and renal complaints [18,19].

There is no information concerning antimicrobial activity of *A. arvensis* against methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, the aim of this study was to investigate *in vitro* antimicrobial activity of leaf methanolic extract of this plant against clinical isolates of MRSA.

2. MATERIALS AND METHODS

2.1 Collection and Plant Preparation of Extract

The *Anagallis arvensis* L. were collected during flowering period, May 2013, from the area surrounding Hamun Lake, Zabol (Coordinates: 31° 1′ 43″ N, 61° 30′ 4″ E), in Sistan and Baluchestan Province of Iran. The plant was taxonomically identified by a botanist at the herbarium of Pharmacognosy, Department of the Faculty of Pharmacy affiliated to Zabol University of Medical Sciences of Iran. Twenty grams of leaf part were powdered and then dissolved in 200 mL methanol 85% using a shaker water bath for

24 h at 25℃. After filtering through Whatman No. 1 filter paper, the filtrate was concentrated with a rotary evaporator (Laborota 4000, Heidolph, Germany) at 45℃ for 35 min to remove solvent from the extract. The solid-like material that precipitated (i.e., the extract) was stored at 4℃ until further experiments. Solid extracts were dissolved in 20 mL of distilled water. These working solutions were used for all the tests in this study.

2.2 Preliminary Phytochemical Analysis

Phytochemical screening for major constituents each part include saponins, glycoside, flavonoids, tannins, alkaloids, resin and phenols was undertaken by using standard qualitative methods [20-23].

2.3 Bacterial Isolates and Antibiotic Susceptibility Assay

In this study, Four hundred and seventy-seven clinical specimens such as burn, wound, urine and throat swab were collected from patients attended in emergency hospital and internal laboratory of hospital and central laboratory in Zabol, Sistan and Baluchestan Province of Iran for various bacteriological examinations. Identification of *S. aureus* was approved by standard techniques accord on colonial and microscopic morphology, coagulase test and biochemical activities [24]. Detection of methicillin-resistant *S. aureus* (MRSA) was done by the use of cefoxitin disc (30 µg) diffusion test. The strains of *S. aureus* with a zone diameter of < 19 mm based on clinical and laboratory standard Institute (CLSI) was taken as showing of methicillin resistance [25]. All strains of *S. aureus* were confirmed as methicillin resistant by oxacillin agar dilution using Muller Hinton agar (beef extract, acid hydrolysate of casein 17.5 g/L, starch 1.5 g/L, agar 17.0 g/L, final 7.3±0.1 at 25℃) supplemented with 2% NaCl. The concentrations tested ranged from 1 µg/mL to 16 µg/mL of oxacillin. The strains having minimum inhibitory concentration (MIC) ≥ 4 µg/mL were taken as resistant.

2.4 Disc Diffusion Method and Determination of Minimum Inhibitory Concentration (MIC)

The MRSA bacteria were cultured for 14-24 hour at 37ºC and the densities were adjusted to 0.5 McFarland standards at A_{530} nm. The antibacterial tests were carried out by the disc diffusion method [26]. Of the 100 µL microbial suspensions was spread on Mueller hinton agar (Merck, Germany) plates (100 mm × 15 mm). Discs were impregnated with 100 µL of different concentrations of extract (50,100, 150, 200, 250, 300, 400 and 500 µg/mL) and placed on the inoculated agar. All the inoculated plates were incubated for 24 h at 37ºC. The vancomycin disc (30-µg disc) and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. Antimicrobial activity was appraised by measuring the zone of inhibition. Minimum inhibitory concentration (MIC) was determined using serial dilutions of the extract (0-500 µg/mL) using microdilution assay approved by Clinical and Laboratory Standards Institute [27]. The bacteria were suspended in Luria Bertani broth and the densities were regulated to 0.5 McFarland standards at A_{530} nm.

The extract (100 µL) and the bacteria suspensions (100 µL) were added to microtiter plates and incubated at 37℃ for 24 h. In this study, medium without bacteria was as sterility control and medium with bacteria but without extract was as growth control. The growth in each well with that of the growth in the control well was compared. The Minimum inhibitory concentration were visually detected in comparison with the growth in the control well and delineated as the lowest concentration of the components with >95% growth inhibition.

The MIC of vancomycin was determined by agar dilution method using CLSI guidelines [27]. Briefly, gradient plates of Mueller-Hinton agar were prepared with vancomycin (0.5-256 mg/L, Sigma-Aldrich). 0.5 McFarland equivalent inoculum prepared using 24 h old culture was spotted on to gradient plates. Plates were incubated overnight at 37℃ for 24 h before assessing the visible growth.

2.5 Statistical Analysis

The extracts were prepared in triplicate for chemical characterization and antibacterial assays. Data was subjected to analysis of variance following a completely random design to determine the least significant difference (LSD) at $P < 0.05$ using SPSS v. 11.5 (IBM SPSS, New York, USA). In this study values for antibacterial assays were based on Mean ± SD.

3. RESULTS AND DISCUSSION

Among 477 clinical isolates, of the 107 specimens (22.43%) were *S. aureus* that from these 18 specimens (16.82%) were MRSA (Table 1). The preliminary phytochemical analysis of *A. arvensis* leaf extract showed that the plant extract was contained high amounts of saponins alkaloids. Also tannins, flavonoids, glycoside, phenols and resin were observed in methanolic extracts leaf with moderate amounts (Table 2).

The result of disc diffusion method showed that *A. arvensis* leaf methanolic extract recorded different degrees of antibacterial activity on MRSA as evidenced by the inhibited zones at concentrations of 250, 300, 400 and 500 µg/mL (1.3±0.2, 1.5±0.2, 2.2±0.1 and 2.3±0.1 mm, respectively) (Table 3). Inhibition zones of MRSA showed no significant different among extract concentrations of 50,100,150 and 200 µg/mL (P <0.05). Also these concentrations were not effective on the MRSA. The inhibition zone of vancomycin (30-µg disc) was 7.7±0.2 mm that it was significantly higher than all concentrations of the plant extract (P <0.05). The MICs of the plant extract and vancomycin were >100 and 14.5±0.1 µg/mL, respectively. This results showed that the plant extract although have slightly effect on MRSA but it was not sufficient strong.

In our study, all MRSA isolated was entirely sensitive to vancomycin. A similar result was achieved for vancomycin assayed in previous studies [28,29]. The vancomycin has been the most credible therapeutic agent against MRSA. However increased use of vancomycin has set a basis of selection for vancomycin resistance in MRSA [30].

S. aureus is a versatile, robust human pathogen that can cause disease in nearly any organ and tissue in the body. In recent years, the prevalence of MRSA has enhanced worldwide as it is evident from numerous supervision studies [31-39]. However, infection with MRSA changes greatly from one geographic location to another, from hospital to hospital [2]. Recently, some antimicrobial agent such as vancomycin and teicoplanin are exclusively efficient still against MRSA, therefore this pathogen can cause critical infection in different body systems in patients. The emergences of expanding in antibiotic resistance due to many researchers were examined optional accesses to treat staphylococcal infection. Herbal medicines are considered to be an important natural medicine for the treatment of health conditions and diseases [3]. Herbal extracts have been extensively studied as natural products.

Zuo et al. [40] carried out a study on antibacterial activity of aerial parts of the Chinese medicinal plants against clinical isolates of MRSA. The results of this study showed that MIC value ≤ 1.43 mg/mL for *Dendrobenthamia capitata*, *Elsholtzia rugulosa*, *Elsholtzia blanda*, *Geranium strictipes* and *Polygonum multiflorum* plants.

Chomnawang et al. [41] evaluated antibacterial properties of Thai medicinal plants against MRSA. They were demonstrated that *Garcinia mangostana* extract was identified as the most potent plant against MRSA with MIC value of 3.91 µg/mL.

Radji et al. [42] were performed a study on antimicrobial activity of green tea extract against isolates of MRSA. The results of this study showed that inhibited zone19.13±0.25 mm and MIC value 400 µg/mL green tea extract against MRSA.

Rad et al. [1] investigated *In-vitro* antibacterial activity of *Xanthium strumarium* L. extracts on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MRSA). They reported

Table 1. Number and percentage of isolated MRSA from various clinical specimens

Type of specimens	Number of specimens	*S. aureus*		MRSA	
		No.	%	No.	%
Burn	104	53	50.69	9	16.98
Wound	45	38	84.44	4	10.52
Urine	254	11	4.33	4	36.36
Pus	14	4	28.57	0	0.00
Throat	60	1	1.66	1	100
Total	477	107	22.43	18	16.82

that *X. strumarium* extract affected both methicillin-susceptible *Staphylococcus aureus* and MRSA, though antibacterial activity was more effective on methicillin-susceptible *S. aureus* spp. Also they illustrated that the antibacterial activity exhibited by the methanolic extract may justify the traditional use of this plant as a folk remedy worldwide.

Table 2. Phytochemical analyses of the leaf extract from *Anagallis arvensis*

Chemical compounds	Methanolic extracts leaf
Saponins	++
Tannins	+
Flavonoids	+
Glycoside	+
Alkaloids	++
Phenols	+
Resin	+

The values: High (++), Moderate (+)

Table 3. Anti-MRAS activity of *Anagallis arvensis* leaf extract

Extract concentrations (µg/mL)	MRSA
50	0.0±0.0 d
100	0.0± 0.0 d
150	0.0±0.0 d
200	0.0±0.0 d
250	1.3±0.2 c
300	1.5±0.2 c
400	2.2±0.1 b
500	2.3±0.1 b
DMSO	0±0.0 d
Vancomycin (30-µg disk)	7.7±0.2 a
MIC of extract	>100
MIC of vancomycin	14.5±0.1

Data are expressed as means ± SD of inhibition zone diameter (mm) for different concentration of leaf extracts, controls and minimum inhibitory concentration (MIC) (µg/mL); DMSO: Dimethyl sulfoxide (Negative control). The values with different letters within a column are significantly different ($P < 0.05$; LSD)

Darogha [43] evaluated phytochemical and antibacterial activity of some medicinal plants against MRSA. The results of this study showed that the MIC/MBC of pods aqueous and ethanolic extracts of *Prosopis farcta* against MRSA isolates were 100/25 mg/mL and 25/12.5 mg/mL, respectively. In addition, Darogha [43] reported that pods aqueous and ethanolic extracts of *P. farcta* was included alkaloids, tannins, glycosides, flavonoids, saponins, phenols and resins. The result of our study also showed that these compounds were available in the plant extract.

Voravuthikunchai and Kitpipit [44] examined aqueous and ethanolic extracts of ten traditional Thai medicinal plants for their ability to inhibit 35 hospital isolates of MRSA. The results showed that nine medicinal plants displayed activity against all isolates tested. Ethanolic extracts of *Garcinia mangostana, Punica granatum* and *Quercus infectoria* were most effective, with MICs for MRSA isolates of 0.05–0.4, 0.2–0.4 and 0.2–0.4 mg/mL, respectively, and for *S. aureus* ATCC 25923 of 0.1, 0.2 and 0.1 mg/mL, respectively. MBCs for MRSA isolates were 0.1–0.4, 1.6–3.2 and 0.4–1.6 mg/mL, and for *S. aureus* ATCC 25923 were 0.4, 3.2 and 1.6 mg/mL, respectively.

The basis of varying degree of sensitivity of tested MRSA to plant extract may be due to the natural tolerance of MRSA and the nature and combinations of phytochemical present in the plant extract. This effect can be related to highest concentrations of alkaloids and saponins in the plant extract. In previous studies reported that these phytochemicals are known to have antimicrobial activity [1]. The mechanisms concept to be responsible for the activity of these phytochemicals against MRSA may include enzyme inhibition by the oxidized compounds which act as an origin of stable free radical and often lead to deactivation of the protein and loss of function. They have the ability to compound not only with extracellular and soluble proteins but also bacterial cell walls and interrupt microbial membranes [30]. However, the search for new antibacterial factors should be continued by screening many other plant families. The antimicrobial and phytochemical studies would provide valuable knowledge to the understanding media worldwide [30].

4. CONCLUSION

In recent years, the use of and look for drugs and dietary supplements derived from plants have accelerated. The obtained results clearly indicated (for the first time, to the best of our knowledge) that methanolic extract of *Anagallis arvensis* L. (Scarlet pimpernel) leaf may be used for treatment of MRSA. In addition, it can be used as food supplement and material for food in food industries. In addition, screening for bioactive phytochemicals from extracts of *Anagallis arvensis* L. leaf should was performed in search of novel chemotherapeutic agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rad JS, Alfatemi SMH, Rad MS, Iriti M. *In-vitro* antioxidant and antibacterial activities of *Xanthium strumarium* L. extracts on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. Ancient Science of Life. 2013;33:107-11.
2. Hoseini Alfatemi SM, Motamedifar M, Hadi N, Saraie HSE. Analysis of virulence genes among methicillin resistant *Staphylococcus aureus* (MRSA) strains. Jundishapur Journal of Microbiology. 2014; 7(6):e10741.
3. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Sharifi-Rad M, Iriti M, Sharifi-Rad M, Sharifi-Rad R, Raeisi S. Phytochemical compositions and biological activities of essential oil from *Xanthium strumarium* L. Molecules. 2015;20(4):7034-7047.
4. Alfatemi SMH, Rad JS, Rad MS, Mohsenzadeh S, Teixeira da Silva JA. Chemical composition, antioxidant activity and *in vitro* antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* spp. 3 Biotech. 2015;5(1):39-44.
5. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Setzer WN. Chemical composition, antifungal and antibacterial activities of essential oil from *Lallemantia royleana* (Benth. in Wall.) benth. Journal of Food Safety. 2015;35(1):19–25.
6. Miri A, Rad JS, Alfatemi SMH, Rad MS. A study of antibacterial potentiality of some plants extracts against multidrug resistant human pathogens. Annals of Biological Research. 2013;4(8):35-41.
7. Sharifi-Rad J, Miri A, Sharifi-Rad M, Sharifi-Rad M, Hoseini-Alfatemi SM, Yazdanpanah E. Antifungal and antibacterial properties of grapevine (*Vitis vinifera* L.) leaves methanolic extract from iran- in vitro study. American-Eurasian Journal of Agricultural & Environmental Sciences. 2014;14(11):1312-1316.
8. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Teixeira da Silva JA. Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from Nitraria schoberi fruits. 3 Biotech. 2015;5:677-684.
9. Rad JS, Alfatemi MH, Rad MS, Sen DJ. Phytochemical and antimicrobial evaluation of the essential oils and antioxidant activity of aqueous extracts from flower and stem of *Sinapis arvensis* L. American Journal of Advanced Drug Delivery. 2013;1(1):001-010.
10. Rad JS, Alfatemi SMH, Rad MS. *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria. International Journal of Biosciences. 2014;4(6):217-222.
11. Kayani SA, Masood A, Achakzai AKK, Anbreen S. Distribution of secondary metabolites in plants of Quetta-Balochistan. Pakistan Journal of Botany. 2007;39(4):1173-1179.
12. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Iriti M. Antimicrobial synergic effect of allicin and silver nanoparticles on skin infection caused by methicillin-resistant *Staphylococcus aureus* spp. Annals of Medical and Health Sciences Research. 2014;4:863-8.
13. Sharifi-Rad J, Miri A, Hoseini-Alfatemi SM, Sharifi-Rad M, Setzer WN, Hadjiakhoondi A. Chemical composition and biological activity of *Pulicaria vulgaris* essential oil from Iran. Natural Product Communications. 2014;9(11):1633-1636.
14. Rad JS, Alfatemi SMH, Rad MS, Iriti M. Free radical scavenging and antioxidant activities of different parts of *Nitraria schoberi* L. Journal of Biologically Active Products from Nature. 2014;4:44‑51.
15. Al-Snafi AE. The chemical contents and pharmacological effects of *Anagallis arvensis* - a review. International Journal of Pharmacy. 2015;5(1):37-41.
16. Us Salam I, Ahmed M, Tariq-Ali S. Allelopathic effects of scarlet pimpernel (*Anagallis arvensis*) on seed germination and radical elongation of Mung bean and Pearl millet. Pakistan Journal of Botany. 2011;43(1):351-355.
17. PDR for herbal medicines. Medical Economic Co. Montvale, New Jersey. 2000;668-669.
18. Sundaram EN, Singh KP, Reddy KP, Reddy PU, Nair KRJ, Raveendard CH, Nayak C. A preliminary study to evaluate analgesic and behavioural activities of the homoeopathic drug, *Anagallis arvensis* in

rats. Indian Journal of Research in Homoeopathy. 2010;4(2):41-44.
19. Gulshan AB, Dasti AA, Hussain S, Atta MI, Amin-ud-Din A. Indigenous uses of medicinal plants in rural areas of Dera Ghazi Khan, Punjab, Pakistan. ARPN Journal of Agricultural and Biological Science. 2012;7(9):750-762.
20. Fadeyi MG, Adeoye AE, Olowokodejo JD. Epidermal and phytochemical studies with genus of *Boerhavia* (Nyetanginaceae). Crude Drug Research.1989;29:178-84.
21. Odebiyi A, Sofowora AE. Phytochemical screening of Nigerian medicinal plants. Part III. Lloydia. 1990;41:234-46.
22. Harborne JB. Phytochemical methods. London, UK: Chapman and Hall Publications. 1992;7-8.
23. Abulude FO. Phytochemical screening and mineral contents of leaves of some nigerian woody plants. Research Journal of Phytochemistry. 2007;1:33-9.
24. Vandepitte J, Engback K, Piot P, Heuck G. Basic Laboratory procedures in clinical bacteriology. W.H.O. Switzeriand; 1991.
25. Clinical and Laboratory Standard Institute (CLSI). Performance standard for antimicrobial susceptibility testing. 12th edition. Information Supplement. 2010; M100-520.
26. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1996;45(4): 493-496.
27. Performance standards for antimicrobial susceptibility testing, 17th informational supplement (M100-517) Wayne, Pa: Clinical and Laboratory Standards Institute; 2007.
28. Adwan K, Abu-Hasan N, Adwan N, Jarrar N, Abu-Shanab B, Abu-Zant A. Nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* in Palestine. Microbial Drug Resistance. 2005;2:74-76.
29. Kumari N, Mohapatra TM, Singh YL. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary-care hospital in Eastern Nepal. Journal of Nepal Medical Association. 2008;47:53-56.
30. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Miri A, Sharifi-Rad M. Phytochemical screening and antibacterial activity of *Prosopis farcta* different parts extracts against methicillin-resistant *Staphylococcus aureus* (MRSA). Minerva Biotecnologica. 2014;26(4):287-93.
31. Simor AE, Ofiner-Agostini M, Bryce E, Green K, McGeer A, Mulvey M, Paton S. The evolution of methicillin-resistant *Staphylococcus aureus* in Canadian Hospitals: 5 years of national surveillance. Canadian Medical Association. 2001;165: 21-26.
32. Tiemersma EW, Bronzwaer LAM, Lyltikalinen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundmann H. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999-2002. Emerging Infectious Diseases Journal. 2004;10:1627-1634.
33. Dancer SJ. Considering the introduction of universal MRSA screening. Journal of Hospital Infection. 2008;69(4):315-320.
34. Cookson B, Bonten MJM, MacKenzie FM, Skov RL, Verbrugh HA, Tacconelli E. Meticillin-resistant *Staphylococcus aureus* (MRSA): Screening and decolonization. International Journal of Antimicrobial Agents. 2011;37(3):195-201.
35. Wan MT, Lauderdale TL, Kobayashi N, Urushibara N, Chou CC. Population deviation of piggery-associated methicillin-resistant *Staphylococcus aureus* based on mec-associated direct repeat unit analysis. Infection, Genetics and Evolution. 2013;16: 349-354.
36. Chuang YY, Huang YC. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Asia. The Lancet Infectious Diseases. 2013;13(8):698-708.
37. Moody J, Septimus E, Hickok J, Huang SS, Platt R, Gombosev A, Terpstra L, Avery T, Lankiewicz J, Perlin JB. Infection prevention practices in adult intensive care units in a large community hospital system after implementing strategies to reduce health care-associated, methicillin-resistant *Staphylococcus aureus* infections. American Journal of Infection Control. 2013;41(2):126-130.
38. Abbasi-Montazeri E, Khosravi AD, Feizabadi MM, Goodarzi H, Khoramrooz SS, et al. The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns. 2013;39(4):650-654.
39. Aklilu E, Zunita Z, Hassan L, Cheng CH. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA)

among veterinary students and personnel at a veterinary hospital in Malaysia. Veterinary Microbiology. 2013;164(3-4): 352-358.

40. Zuo GY, Wanga GC, Zhaoa YB, Xua GL, Haob XY, Hanc J, Zhaoc Q. Screening of Chinese medicinal plants for inhibition against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Journal of Ethnopharmacology. 2008; 120(2):287-290.
41. Chomnawang MT, Surassmoa S, Wongsariyaa K, Bunyapraphatsara N. Antibacterial activity of thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. Fitoterapia. 2009; 80(2):102-104.
42. Radji M, Agustamaa RA, Elyab B, Tjampakasaric CR. Antimicrobial activity of green tea extract against isolates of methicillin–resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa*. Asian Pacific Journal of Tropical Biomedicine. 2013;3(8): 663–667.
43. Darogha SN. Phytochemical analysis and antibacterial activity of some medicinal plants against methicillin-resistant *Staphylococcus aureus*. Tikrit Journal of Pharmaceutical Sciences. 2009;5(2): 116-126.
44. Voravuthikunchai SP, Kitpipit L. Activity of medicinal plant extracts against hospital isolates of methicillin-resistant *Staphylococcus aureus*. Clinical Microbiology and Infection. 2005;11(6): 510-512.

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