

In vitro evaluation of antioxidant and antimicrobial activity of Ziziphus nummularia leaf extract

Asieh Khalili¹, Hassan Barzegar^{2}, Behrooz Alizadeh Behbahani³, Mohammad Noshad²*

1. M.Sc. Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.
2. Associate Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.
3. Assistant Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

*hbarzegar@asnrukh.ac.ir

Abstract

Background and objective: Nowadays, with the increase in the incidence of diseases, the use of natural herbal compounds to treat them is increasing. Among medicinal plants, *Ziziphus nummularia* has been considered for its unique properties. The aim of this study was to investigate the antioxidant activity and antimicrobial activity of *Ziziphus nummularia* leaf extract against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Listeria innocua* and *Bacillus cereus*.

Materials and methods: Extraction of *Ziziphus nummularia* leaves was done by maceration method for 72 hours. The phenol and flavonoid content of the extract was measured. Antioxidant capacity evaluated using DPPH, ABTS and β -carotene/linoleic acid bleaching assay. Agar well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used to evaluate antimicrobial activity of extract.

Results: Total phenolic content and flavonoid of *Ziziphus nummularia* leaf extract were 47.38 mg GAE/g and 28.5 mg QE/g respectively. Antioxidant activity using DPPH, ABTS and β -carotene/linoleic acid bleaching assay were 60.3, 54.55 and 57.44 percent respectively. *Ziziphus nummularia* leaf extract showed a significant antimicrobial effect on the studied microorganisms. The most sensitive and resistant microbial strains to the extract according to the agar well method were *Bacillus cereus* and *Pseudomonas aeruginosa*, respectively.

Conclusion: Due to the considerable antioxidant and antimicrobial activity of *Ziziphus nummularia* leaf extract, this extract can be used in the pharmaceutical and food industries.

Keywords: Extract, *Ziziphus nummularia*, Antimicrobial activity, Antioxidant activity.

Investigating the Antimicrobial Effect of Plant Essential Oils on Standard H37RV and *Mycobacterium Tuberculosis* Clinical Isolates

Mahdieh Shafeghat^{1*}, *Nahid Sepehri Rad*², *Malihe Metanat*³, *Hossein Zati keikha*⁴

¹*Master of Plant Biology, Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan.

²Master Microbiology, Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan.

³Specialist in infectious diseases and tropical medicine, Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan.

⁴Senior Expert in Numerical Analysis, Technical and Vocational University, Zahedan Girls Technical and Vocational College

* Shafaghat.m@gmail.com

Abstract

Background and purpose: *Myrtus Communis* is a shrub and evergreen plant that grows in the plains of Asian countries including Iran and Mediterranean countries. Since ancient times, the essential oil of this plant has been used as a disinfectant. In addition to its antiseptic effect, it is used as a stomach booster and also to cure respiratory and urinary tract diseases. The aim of this study was to determine the antimicrobial effect of the plant essential oil on the H37Rv standard strain and clinical isolates of *Mycobacterium tuberculosis*.

Methodology: The plant essential oil was extracted using a Clevenger Apparatus, then using the Kirby-Bayer standard disk diffusion method, the antimicrobial property of the plant essential oil was determined by determining the minimum inhibitory concentration (MIC) against 4 different strains of *Mycobacterium tuberculosis* compared to the standard strain H37Rv in Four concentrations were evaluated.

Findings: In this study, it was shown that there was a significant difference between the different concentrations determined for microbe cultivation, in the plant essential oil, the minimum inhibitory concentration (MIC) against two bacterial strains was equal to (7.5 mg/ml). It is known that the minimum bactericidal concentration (MBC) of two strains was equal to (15 mg/ml). The highest inhibitory concentration was equal to (15 mg/ml) and one strain was inhibited at this concentration and the lowest inhibitory concentration was The concentration was equal to (3.75 mg/ml). Also, MIC and the MBC against the standard strain were (1.87 and 3.75 mg/ml) respectively. This shows the significant effect of the antimicrobial properties of the essential plant in reducing the growth of bacteria.

Conclusion: The results of this study indicate the positive effect of the essential oil of the case in the treatment of tuberculosis. Therefore, the compounds in the essential oil of this plant can be used to develop and produce new Tuberculosis drugs.

Keywords: Medicinal Plants, *Mycobacterium Tuberculosis*, Microorganisms, Case Plants

Frequency of agr types and resistance to aminoglycosides among biofilm producing *Staphylococcus aureus* strains isolated from patients with urinary infection in Isfahan during 2017

*Narges Sadat Mostafavi*¹, *Fateh Rahimi*^{2*}

1- MS.c of Microbiology-Pathogenic Microbes, Department of Microbiology, Faculty of Biological Science and Technology, University of Isfahan

2- Associate Professor of Bacteriology, Department of Microbiology, Faculty of Biological Science and Technology, University of Isfahan

* f.rahimi@sci.ui.ac.ir

Abstract

Background and Aim: *Staphylococcus aureus* is one of the most common nosocomial pathogens which can cause a broad spectrum of infections such as urinary infection (UI). These bacteria are able to produce biofilm that results in recurrent and chronic infections. *S. aureus* have the high potential to acquire resistance to broad spectrum of antibiotics such as methicillin, vancomycin and aminoglycosides which results in emergence of multidrug resistant strains and making infections by these bacteria difficult to treat. The aim of this study was to characterize the frequency of different agr types and resistance to aminoglycosides among biofilm producing *S. aureus* strains isolated from UI in Isfahan, Iran.

Material and Methods: During 2017, a total of 119 suspected *S. aureus* isolated from patients with UI were collected from a hospital laboratory in Isfahan and confirmed using specific primers for nucA gene. The ability of the strains to form biofilm was evaluated using qualitative Congo red agar (CRA) and quantitative microtiter plate (MTP) assay and resistance of biofilm producing strains to 8 antibiotics was tested by disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). The presence of different aminoglycoside resistance genes and also agr types among strains was determined by separate polymerase chain reaction (PCR) tests and a multiplex-PCR, respectively.

Results: All 119 isolated strains were confirmed as *S. aureus* and using combination of qualitative and quantitative biofilm assays a total of 78 (66%) strains were selected as strong and moderate biofilm producing strains, in which 12% of *S. aureus* strains were slime positive. The results of antibiotic susceptibility testing showed that 43, 35, 36, 73, 32, 41, 46 and 43% of strains were resistant to ceftazidime, gentamicin, kanamycin, streptomycin, amikacin, tobramycin, azithromycin and erythromycin respectively. Furthermore, 44, 32, 17 of resistant strains harbored aac(6')-Ie-aph(2'')-Ia, ant(6')-Ia and aph(3')-IIIa genes, respectively. On the other hand, 40% of strains were negative for agr locus and agr type I was the most frequent type.

Conclusion: The results of the presents study revealed the high prevalence of biofilm producing and antibiotic resistant *S. aureus* strains among patients in the studied hospital in Isfahan, which dissemination of such strains could be a major threat to public health.

Key words: *S. aureus*, UTI, biofilm, aminoglycoside, agr types

Evaluation of Antibacterial activity of methanolic extract of *Hippophae rhamnoides* leaf on *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Bacillus cereus*

Mohammad Noshad^{*1}, Behrooz Alizadeh Behbahani², Mostafa Rahmati-Joneidabad³

1- Associate Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

2- Assistant Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

3- Assistant Professor, Department of Horticultural Science, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

* Noshad@asnrukh.ac.ir

Abstract

Background and objectives: Infectious diseases caused by pathogenic bacterial strains are increasing in many countries, including Iran. *Hippophae rhamnoides* L. is an important source of natural antimicrobial agents. The aim of this study was to extract methanolic extract of *H. rhamnoides* leaf and to evaluate its antimicrobial activity against *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Bacillus cereus*.

Methods: In this study, methanolic extract of *H. rhamnoides* leaf was extracted by the maceration method and then its antibacterial effect against bacterial pathogens *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Bacillus cereus* was evaluated using disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration.

Results: The results of disk/well diffusion agar tests showed that increasing the extract concentration from 5 to 35 mg/ml caused a significant increase in the diameter of the growth inhibition zone for all microbial strains. *Pseudomonas aeruginosa* were the most resistant strains and *Streptococcus pyogenes* was found to be the most sensitive strains to the extract. The minimum inhibitory concentration for Gram-positive bacteria (4 mg/ml) was lower than that for Gram-negative bacteria (16 mg/ml). Also, the minimum bactericidal concentration for *Streptococcus pyogenes* and *Pseudomonas aeruginosa* was 256 and 512 mg/ml, respectively.

Conclusion: The results of the antimicrobial activity of the methanolic extract of *H. rhamnoides* leaf showed that it is possible to use the extract for medicinal purposes and food preservation.

Keywords: Hippophae rhamnoides; Methanolic extract; Antibiotic resistant; Natural antimicrobial; Foodborne diseases.

Frequency of prophage types and genes encoding β -lysin and staphylokinase among methicillin resistant *Staphylococcus aureus* strains isolated from patients in Tehran during 2017

Fatemeh Mohaghegh¹, Fateh Rahimi^{2*}

1- MS.c of Microbiology-Pathogenic Microbes, Department of Microbiology, Faculty of Biological Science and Technology, University of Isfahan

2- Associate Professor of Bacteriology, Department of Microbiology, Faculty of Biological Science and Technology, University of Isfahan

* f.rahimi@sci.ui.ac.ir

Abstract

Background and Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major opportunistic pathogen associated with nosocomial and community-acquired infections that causes significant morbidity and mortality. These bacteria have a high ability to acquire mobile genetic elements, such as bacteriophages, via horizontal gene transfer phenomenon. Bacteriophages are able to convert non-virulent strains to virulent ones through phage conversion which results in production of virulence factors such as β -lysin, staphylokinase, enterotoxins, toxic shock syndrome toxin-1 (TSST-1), lipase, exfoliative toxin A, and Panton-Valentine leukocidin (PVL). In this study we aimed to characterize the presence of different prophage types, β -lysin and staphylokinase virulence genes among MRSA strains isolated from patients in Tehran, Iran.

Material and Methods: During 2017, a total of 50 suspected MRSA isolates were collected from patients in two referral hospitals in Tehran. All isolates were identified as *S. aureus* using specific primers for *nucA* gene and resistance to methicillin was determined by combination of disk diffusion method, according to the guidelines of Clinical Laboratory and Standard Institute (CLSI), and PCR by specific primers for *mecA* gene. A multiplex-PCR assay was employed for prophage typing of strains and all MRSA strains were tested for presence of *hly* and *sak* virulence genes.

Results: All 50 isolated strains from patients in 2 referral hospitals were confirmed as MRSA. Except for SGD and SGL all prophage types were identified among the strains in which SGF, SGFa and SGFb prophage types were present in all strains and the frequency of SGA and SGB prophage types was limited to 16 and 64% of strains, respectively. Moreover, 4 different prophage patterns were also identified among all, in which pattern 3 (consisted of SGB, SGF, SGFa and SGFb prophage types) was the dominant one. Furthermore, 100 and 82% of MRSA strains were positive for *hly* and *sak* genes, respectively.

Conclusion: The results of the current study indicated the high prevalence of prophage types and virulence genes among MRSA strains in the studied hospitals. Such strains have a high potential to produce a variety of virulence factors and also different diseases.

Keywords: MRSA, prophage type, β -lysin, staphylokinase, multiplex-PCR