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Evaluation of antimicrobial activity of aqueous extract, ethanol, methanol and ashes two species *ramosissimum* and *telmateia* of *Equisetum arvense* on several bacterial species and Yeast

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Abstract

Introduction: Bacterial infections are one of the major health challenges and authorities in this field are faced with many problems especially bacterial resistance to antibiotics. This study aimed to evaluate the antimicrobial effects of aqueous and alcoholic extracts and the ash of two species of horse tail herb against standard strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans* and investigate its effect on eukaryotic cells.

Methods: Aqueous, methanolic and ethanolic extracts and ash were prepared from two horse tail species. In order to evaluate antibacterial function, well diffusion test was used by measuring inhibitory halo diameter on standard strains of *Staphylococcus aureus*, *Candida albicans* and *E. coli*. For determining the minimum inhibitory concentration (MIC) on these strains micro broth dilution method was applied. To evaluate its effect on the eukaryotic cells, blood agar medium was used.

Results: Based on agar diffusion test, the halo of no bacterial growth was associated with extracts in different ranges in standard strains. The MIC was determined using the micro broth dilution. These extracts had no effects on red blood cells and did not produce hemolysis on blood agar medium.

Conclusion: Horsetail extract can be used to treat fungal and bacterial infections either lonely or in combination with other antimicrobial agents. Due to its antimicrobial potential, it can especially be considered in the cases of drug resistance.

Keywords: Horsetail extract, *Staphylococcus aureus, Candida albicans, E. coli*, Antimicrobial activity, Minimum inhibitory concentration

Introduction

Staphylococcus aureus is one of the most common and important pathogens in nosocomial infections and due to the presence of several enzymes including coagulase, hyaluronidase, nuclease, lipase, and hemolysin can cause infection anywhere in the body (1). This bacterium is ranked as the second in producing nosocomial infections after *Pseudomonas aeruginosa*. *Staphylococcus aureus* is also responsible for serious infections such as septicemia, endocarditis and osteomyelitis in hospitalized patients and it is the most common cause of death in hemodialysis patients (2,3). Candidiasis is not just a disease but it is a spectrum of diseases that causes various infections in vulnerable people in an opportunistic way. The range of these diseases is from superficial and simple mucosal infections to dangerous systemic infections and even to fatal infections (4,5). Causal factors of disease are yeast fungi belonging to *Candida* genus (6). *Candida* is an incredible destructive factor of health and it is the main invisible ring in many diseases today. Metabolic toxins of candidiasis are released in all tissues and in case of a defective immune system, it can lead to dangerous complications either directly or indirectly (6). *E. coli* is one of the strains of bacteria that can colonize and survive in various environmental habitats and in animal hosts. These strains are categorized based on serological properties, virulence and virotyping factors. These strains include enterotoxigenic, enterohemorrhagic enteropathogenic, enteroaggregative,

and Enzoinvazive. In addition, they have a specific pattern to create diseases (7). E. coli-sero groups are the causes of a variety of diseases including opportunistic infections, urinary tract infections and diarrhea (8,9). The emergence and spreading of antibiotic-resistant strains in the past decade has became a major concern. This increase in resistant strains is one of the major problems in medicine and it still continues and threatens the public health (3,10,11) Currently, the unchecked spread of antibiotic-resistant strains of *Staphylococcus aureus*, *Candida* and *E. coli* is one of the most important challenges in the society. The imbedded problems in this regard provides further impetus to search for antimicrobial compounds, especially of herb origin (12) of Equisetaceae family (horsetail) with the scientific name of *Equisetum arvense* L. (12-14). Its height is 50-60 cm and its barren stalks which are green and thicker are used in traditional medicine for drug discovery (15). This herb is seen in the north of Iran, Gorgan, Bandar Gaz, Pir Bazarof Rasht, Azerbaijan, Tabriz and Ranjnab wildling (14,15). The existence of silicic acid fatty oil, linoleic acid, Oxytocin, occtic acid is approved in horsetail. In addition, there is a bitter substance and a small amount of oxalic acid, malic acid, a saponin in this herb. 3%-15% of silica is found in fresh herb and up to 70% of it is found in ash herb (15,16). The properties of this herb have been taken into consideration and it is used for the treatment of rheumatoid arthritis and gonorrhea. Ash herb is applied to relieve stomach acid and indigestion. Its tisane is used to relieve fever, stomachache, liver pain, sore throat and swelling. This herb is used for treating prostate disease, urinary tract infections, removal of kidney stones and cysts. This herb has antimicrobial and anti-inflammatory properties as well (17-19). Because of the extended spreading of this plant in our country, investigating the antimicrobial properties of this herb provide a suitable background in order to use natural drugs for controlling and treating bacterial infections. Therefore, this study aimed to evaluate the antibacterial effects of aqueous, ethanolic and methanolic extracts and ash of horsetail on *Staphylococcus aureus*.

Methods

Preparation of herb samples and extracts

Aqueous, ethanolic and methanolic extracts of two species of horsetail named *telmateia* and *ramosissimum* were prepared from the genetic and biologic resources center of herbal medicines research center in north Tehran, Iran. The ashes of two species of horsetail were prepared by burning herbs on the grill, and then a certain amount of ash herb was dissolved in distilled water. In order to sterilize the extracts, they were passed through the filter of 2.0 μm.

Experiment strains

Standard *Escherichia coli* Atcc25922 strain, standard *Staphylococcus aureus* Atcc25923 strain and standard *Candida albicans* Atcc10231 strain, prepared by the microbiology laboratory of Kharazmi University, were used to evaluate the antimicrobial effect of aqueous, ethanolic and methanolic extracts and ash herb. The studied strains were identified using differential, selective, specific media and by biochemical tests.

Determining the antibacterial effect of aqueous and alcoholic extracts on tested strain

The antibacterial activity of aqueous and alcoholic extracts and ash of horsetail herb was determined by agar diffusion (measuring the diameter of inhibitory halo) and broth dilution method (measuring the minimum inhibitory concentration [MIC]) (12).

Determining the sensitivity to extracts

Using disk diffusion test in well diffusion, blank wells were created on microbial plates inoculated with bacteria and *Candida*. Additionally, 50 μL of aqueous, alcoholic extracts and ash were injected in the wells. Then, the plates were incubated for 18 hours at 37°C. The diameter of inhibitory haloes around the wells was measured in millimeters. An injected well with 50 μL of physiologic saline was considered as a technical control. Gentamicin and oxacillin disks were used as a medical record control. All tests were confirmed by repeating three times.

Determining the minimum inhibitory concentration

To determine the MIC of aqueous, alcoholic extracts and ash of the two species of horsetail on bacterial strains, micro broth dilution method was used (12). 100 μL of Mueller Hinton Broth was added to each well of ELISA microplate. Aqueous and alcoholic (ethanolic and methanolic) extracts, and ash herb were prepared and then in the first well, 100 μL of 1:2 dilution of aqueous, alcoholic extracts and ash of both species was added. In this regard, serial dilutions were created in the wells. Then 100 μL of each bacterial suspension (10 per 1 mL) was added separately to wells. Control well containing aqueous, alcoholic extracts and ash herb were without bacteria. After that, the microplate was incubated at 37°C for 18 hours. MIC was defined as the lowest concentration in which bacterial growth inhibited at the end of 18 hours of incubation.

Determining the minimum bactericidal concentration

To determine the (minimum bactericidal concentration) MBC, 10 μL of the well contents at the end of 18 hours of incubation were cultured on Mueller Hinton agar and in order to determine the growth of bacteria, plates were incubated for 18 hours. The lowest concentration of aqueous and alcoholic extracts in which 9/99% of bacteria did not grow was considered as MBC. All tests were repeated three times.

Data analysis

To analyze data, descriptive statistics (mean, frequency, standard deviation) and inferential statistics (analysis of variance [ANOVA] and *t* test) were used. SPSS software version 16 and Microsoft office Excel 2007 were used for data analysis. P value < 0.05 was considered as statistically

significant.

Results

After passing the required time, cultured plates of organisms were tested and the results were recorded. Some strains did not show any reactions to the injected.

The results of MBC by diluting in broth in the standard strain of *E. coli* showed that ramosissimum aqueous extract at a concentration of 90 and ethanol, methanolic and ethanolic extracts at the minimum concentration of 25 mg/mL had a lethal effect. The *telmateia* ash extract at a concentration of 50 mg/mL showed lethal effect.

The results of evaluating the effect of extracts on eukaryotes

In order to investigate the effect of extracts of two horsetail species on eukaryotic cells, blood agar culture medium containing sheep red blood cells was used. The hemolysis of medium was used to study the effects of extracts. The results showed that the extracts did not have any toxic effects on red blood cells and hemolysis was not observed.

Discussion and Conclusion

Due to the increasing resistance to antimicrobial drugs, researchers try to find new natural compounds with inhibitory properties against microorganisms. In this regard, the antimicrobial effects of various herbs have been reported by many researchers in Iran. In a study conducted by Fathi Azad et al (20), findings showed that aqueous horsetail extract does not have any antibacterial properties. But ethyl acetate extract of horsetail showed a significant inhibitory effect on *Staphylococcus aureus*. Another research by Radulovi et al regarding on microorganisms found that the essence of this herb had antibacterial function against *Pseudomonas aeruginosa* and *Escherichia*. They also highlighted that this herb had antifungal activity (15). Čanadanović-Brunet et al in 2009 found that this herb in comparison with antibiotic control had little antibacterial effects on *Escherichia coli* and *Pseudomonas* species (14). Emin Uslu et al in 2013 contended that aqueous had antibacterial properties against *Staphylococcus epidermis* and *Escherichia coli*, but it was ineffective on *Candida albicans* (16). The presence of fatty oil, silicic acid, linoleic acid, oxytocin, occtic acid and oxitine has been confirmed in horse tail herb. Antimicrobial properties of saponins have been reported in many herbs. In addition, there is a bitter substance and a small amount of oxalic acid, malic acid, a saponin, etc in this herb. Silica forms 3%-15% of fresh herb and 70% of ash herb. Antimicrobial effect of the herb can be associated with saponins and silica and these factors can be effective on the plasma membrane or can inhibit the structural enzymes in the cell wall (15,16).

The results of this study based on agar diffusion test and based on MBC and MIC tests on *Candida albicans* showed that *telmateia* aqueous extract and *ramosissimum* aqueous extract had the highest antimicrobial impact respectively. In a research conducted in 2013, the effectiveness of the mentioned herb on *Candida albicans* was evaluated and it was found that it is ineffective (16).

In the case of *E. coli* in this study, it was found that all extracts had more or less antimicrobial effects on the standard strains of *E. coli*, but the most antimicrobial effect was observed in the ethanolic *telmateia* and *ramosissimum* methanolic extracts. This finding is consistent with the results of the study conducted by Čanadanović-Brunet et al in 2009 (14).

The results of this study on four herb extracts based on agar well diffusion, MIC and MBC test showed that in the presence of ethanolic, methanolic and antibiotics control, was related to *ramosissimum* ethanolic and ethanolic extract. In the second place, it was related to *telmateia* methanolic extract in the case of *Staphylococcus aureus* and the smallest growth was observed for *ramosissimum* aqueous extract. These results suggest that the component which has antimicrobial property is more soluble in alcohol than in water. On the other hand, for two species of horsetail herb, the results of well diffusion, MIC and MBC test did not show any significant difference. The results of two methods were in accordance with the study of Emin Uslu et al (16) conducted on *Staphylococcus epidermidis* in 2013.

Findings also showed that the extracts had no toxic effects on red blood cells and hemolysis was observed. It can be concluded that at this concentration of stock solution, the extracts do not have a toxic effect on eukaryotic cells. Closer examination is required to apply higher concentrations and more complete tests.

According to studies conducted in Iran and abroad, it can be inferred that the minor differences observed may be due to differences in herb species with respect to climate, soil, weather conditions and in the amount of active ingredient. In addition, the laboratory, the company producing consumption substances or researcher techniques can cause partial differences.

Since the bacterial resistance is increasing and transmission of resistance from resistant bacteria to sensitive ones is done easily in different ways and because it can lead to resistance to routine antibiotics, aqueous and alcoholic extracts and ash herb of horsetail herb can be used to treat bacterial infections like herb derivatives as a replacement or as a supplement. Now, one of the major problems in the treatment of infections and in the use of antibiotics is antibiotic resistance which requires special attention.

Ethical issues

No applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper..

References

1. Archer GL, Climo MW. Staphylococcus aureus bacteremia--consider the source. N Engl J Med 2001; 344(1): 55-6.
2. Kaplan SL, Hulten KG, Gonzalez BE, Hammerman WA, Lamberth L, Versalovic J, et al. Three-year surveillance of

community-acquired Staphylococcus aureus infections in children. Clin Infect Dis 2005; 40(12): 1785-91.
3. Shajari GR, Moniri R. Pattern of Staphylococcous aureus susceptibility and resistance to antibiotics in Kashan. J Kashan Univ Med Sic (Feyz) 2002; 6(3): 31-6. [In Persian].
4. Perumal P, Mekala S, Chaffin WL. Role for Cell Density in Antifungal Drug Resistance in Candida albicans Biofilms. Antimicrob Agents Chemother 2007; 51(7): 2454-63.
5. Seneviratne CJ, Jin LJ, Samaranayake YH, Samaranayake LP. Cell density and cell aging as factors modulating antifungal resistance of Candida albicans biofilms. Antimicrob Agents Chemother 2008; 52(9): 3259-66.
6. Al-Fattani MA, Douglas LJ. Biofilm matrix of Candida albicans and Candida tropicalis: chemical composition and role in drug resistance. J Med Microbiol 2006; 55(Pt 8): 999-1008.
7. Johnson JR. Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev 1991; 4(1): 80-128.
8. Lemonnier M, Landraud L, Lemichez E. Rho GTPase-activating bacterial toxins: from bacterial virulence regulation to eukaryotic cell biology. FEMS Microbiol Rev 2007; 31(5): 515-34.
9. Davis JM, Carvalho HM, Rasmussen SB, O'Brien AD. Cytotoxic necrotizing factor type 1 delivered by outer membrane vesicles of uropathogenic Escherichia coli attenuates polymorphonuclear leukocyte antimicrobial activity and chemotaxis. Infect Immun 2006; 74(8): 4401-8.
10. Norouzi J, Vali GR, Yousefi H. Surveying the effects of different methods of motations on the antibiotic resistance patterns and plasmids in E. coli and S. aureus. J Kashan Univ Med Sic (Feys) 2004; 8(1): 1-8. [In Persian].
11. Makovee JA, Ruegg PL. Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994-2001). J Am Vet Med Assoc 2003; 222(11): 1582-9.
12. James TK, Rahman A. Chemical control of field horsetail (Equisetum arvense). Biology and Control of Weeds 2010; 63: 102-7.
13. Beckert C, Horn C, Schnitzler JP, Lehning A, Heller W, Veit M. Styrylpyrone biosynthesis in Equisetum arvense. Phytochemistry 1997; 44(2): 275-83.
14. Čanadanović-Brunet JM, Ćetković GS, Djilas SM, Tumbas VT, Savatović SS, Mandić AI, et al. Radical scavenging and antimicrobial activity of horsetail (Equisetum arvense L.) extracts. Int J Food Sci Technol 2009; 44(2): 269-78.
15. Radulovi N, Stojanović G, Palić R. Composition and Antimicrobial Activity of Equisetum arvense L. Essential Oil. Phytother Res 2006; 20(1): 85-8.
16. Emin Uslu M, Erdogan I, Bayraktar O, Ates M. Optimization of extraction conditions for active components in Equisetum arvense extract. Romanian Biotechnological Letters 2013; 18(2): 8115-31.
17. Vesna M, Niko R, Zoran T. Miroslavast, gordanast, antioxidant, antimicrobial and genotoxicity screening of hydro-alcoholic extracts of five Serbian Equisetum species. Plant Foods Hum Nutr 2007;62:113-9.
18. Stajner D, Popović BM, Canadanović-Brunet J, Anackov G. Exploring Equisetum arvense L., Equisetum ramosissimum L. and Equisetum telmateia L. as sources of natural antioxidants. Phytother Res 2009; 23(4): 546-50.
19. Mimica-Dukic N, Simin N, Cvejic J, Jovin E, Orcic D, Bozin B. Phenolic compounds in field horsetail (Equisetum arvense L.) as natural antioxidants. Molecules 2008; 13(7): 1455-64.
20. Fathi Azad F, Lotfipour F. Study on the invitro antimicrobial activity of Achillea millefolium and Equisetum arvense. Pharm Sci 2004; 1: 37-46.