Scrutinizing the antimicrobial effect of hydro alcoholic extract of Althaea officinalis (marshmallow) and Matricaria recutita (chamomile) flowers

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Abstract: Introduction: many plants are used to treatment of some diseases for their antimicrobial activities. Sometimes they are even more effective than antibiotics, because every so often the bacteria become resistant to the respective antibiotic and treatment will be failed. Therefore in the present study antimicrobial features of Althaea officinalis and Matricaria recutita flowers against some standard gram negative and positive bacteria were quested. **Methods**: the plants Althaea officinalis and Matricaria recutita were collected for the experience and their hydro alcoholic extract prepared by maserasion method. Antimicrobial effects of them scrutinized by micro dilution and disc diffusion methods. Then their minimum inhibitory concentration and minimum bactericidal concentration determined against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Listeria monosytogenese and Candida albicans. **Results**: outcomes showed both extracts of Althaea officinalis and Matricaria recutita had bactericide and bacteriostatic effects on all cases. Also the hydro alcoholic extract of Althaea officinalis demonstrated more bactericide and bacteriostatic effects than the hydro alcoholic extract of Matricaria recutita as Althaea officinalis in less density illustrated bactericide effect on all except Esherishia coli. **Conclusion**: consequences of the experience confirm the adequacy and analogy of the Althaea officinalis and Matricaria recutita extracts with antibiotics against the relevant bacteria.

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Introduction

Infectious diseases are the most widespread which usually antibiotics should be used for eradicating. But nowadays some microbial Infectious agents have become resistant against relevant antibiotics as a result of irregular use of drugs by people so that the antibiotic's therapy will be failed [1, 2]. However man has found therapeutic effects of herbal extracts, besides unlike many chemical drugs have no side effects [3, 4]. Among these plants, Althaea officinalis and Matricaria recutita can be mentioned for their remedial features.

Matricaria recutita is herbaceous from Asteraceae family which grows wild around bushes and gardens in central and southern area of Iran, this widespread all over the world. Matricaria recutita flowers with lipophilic substances that have anti-inflammatory effects also include prominent hydrophilic materials include Flanoied (Epigenin) and Komarin. Anti-inflammatory activity of Matricaria recutita extract is for the major compounds like Matricin (leading Kamazoln) Bizabobolooxides [5, 6].

Althaea officinalis is also herbaceous and stable from Malvaceae family with yellow branching root and long stem covered with alternating leaves. All parts of this plant have covered by soft villous and it appears with axillary leaves, clusters of white or pink flowers. Its seeds integrated together and make a round and paginal fruit. Root, flowers and leaves are fit for human consumption. This is useful for cough and is softener by making mucus. The root is used against gastro-intestinal diseases. The flower is helpful to eliminating skin agitations [7, 8].

However, Iran is one of the best areas for growing medicinal plants for its weather and its geographic position; it has been the source of producing and consuming herbal medicines. This case of study scrutinized the antibacterial effects of

hydroalcoholic extract of Althaea officinalis and Matricaria recutita, Iranian native plants, against some Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes and Candida albicans.

Selected bacteria are common bacteria in hospital infectious diseases. Pseudomonas aeruginosa is one of the important opportunist pathogens of hospital, which is clearly seen in affected to autoimmune system deficiency and in scald, respiratory diseases, cancerous under chemotherapy, heredity cystisfibrosis, bacterimia, septicemia and many other hospital's infectious [9]. Staphylococcus aureus is the main cause of bacterimia, surgical wound infections and the most common cause of skin and soft tissue infection. In addition, this bacterium is also one of the most common causes of hospital's infections [10]. E. coli is the agent of major urinary tract hospital's infections [15]. Anaerobic Gram-positive bacterium Listeria monocytogenes is isolated from soil, animal feed, water, feces and tissues of a variety of vertebrate and invertebrate animals, including humans. Listeria monocytogenes can cause meningitis, encephalitis and septicemia and it is also cause miscarriages in pregnant women [12]. Candida albicans is one of the most common opportunistic fungal diseases in humans [13].

Materials and Methods

Preparation of plant extracts Matricaria recutita and Althaea officinalis :

Extraction of plants was done for 48 hours by Maserasion method using ethanol (50%). Then Whatman filter paper No.B was used to smooth. The resulting solution was concentrated using a Rotary Heidolph model set and dried at room temperature. Dried extracts were stored in a refrigerator until use.

Preparation of relevant bacteria:

In this case, E. coli bacteria ATTC25922, Pseudomonas aeruginosa ATTC27853, Staphylococcus aureus ATTC25923, 1298 ATTC of Listeria monocytogenes and Candida albicans isolated from clinical specimens obtained from Buali Hospital and were examined. Aseptically opening ampoules of lyophilized bacteria inoculated in TSB liquid medium (Tryptone soy Broth) and was incubated in 35°c for 24 hours. To ensure the purity of the bacteria they were cultured from TSB on blood agar medium and incubated in 35°c for 48 hours. 24 hour before each test, typical colony harvested and inoculated to TSB broth (a 24 hour medium). From fresh medium were transferred to tubes containing normal saline solution and the turbidity of microbial suspension was set using equal to 0.5 McFarland standard of 1.5×10^8 CFU / ml.

Antimicrobial effects assay:

Micro dilution and disk diffusion methods were used to determine the minimum inhibitory concentration (MIC) for bacterial growth [14]. In this method, first of all 100 ml Mueller Hinton Broth was added to wells, and 50 µl of extract was added to the first well, then the final volume of the well reached to 200 µl by adding Mueller Hinton Broth. Afterwards by transferring 100 µl from the first well to the second, from the second to the third, from the third to the forth and …, serial dilution was done. Finally, 100 µl diluted microbial suspension equal to 0.5 McFarland was added to all wells and after 24 hour incubation in 37°c, the lowest concentration of plant extract that showed visible growth was considered as the MIC (Minimum Inhibitory Concentration).

To determine the minimum bactericidal concentrations, from wells without turbidity were cultured on MH agar after 24 hours incubation at 37°C as the lowest concentration of the bacteria were grown in MBC (Minimum Bactericidal Concentration) was considered.

For disk diffusion tests, a suspension with 10^8 bacteria per ml was prepared from 24-hour-culture by McFarland standard solution. Then each disc was saturated with 50µl, 25µl, 12.5µl extract of Althaea officinalis and 50µl extracts of Matricaria recutita prepared with a concentration of 250mg/ml and 125mg/ml for Althaea officinalis and Matricaria recutita respectively. In each experiment used a disk containing 15µl solvent of DMSO and methanol as the negative control and discs containing 10 µg/disc penicillin, 5µg/disc ciprofloxacin,10µg/disc gentamicin as standard antibiotics. Mueller Hinton agar was used in these experiments. After culturing the bacteria on medium, prepared discs were cultured in appropriate distance from each other. Cultures were incubated for 24 h at 35 ° C. then diameter of nonage circles was measured and the average was reported. All antimicrobial effects assay tests were repeated at least 3 times [14].

Results

The results of Anti-microbial activity of hydroalcoholic extracts of Matricaria recutita and Althaea officinalis by using Microdilution and Disk Diffusion tests have shown that Althaea officinalis extracts the concentration of 250mg/ml, 62.5mg/ml, 31.25mg/ml, 31.25mg/ml have bacteriostatic effect on Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes and Candida albicans strains respectively, and in the concentration of 250mg/ml, 125mg/ml, 62.5mg/ml bactericide effect on Staphylococcus aureus, Listeria monocytogenes and fungicide effect on Candida albicans.

Also extract of Matricaria recutita in concentration of 62.5mg/ml, 125mg/ml and 62.5mg/ml have bacteriostatic effect on S. aureus, Listeria monocytogenes and fungi static or fungicidal effect on

Candida albicans, respectively and in concentration of 125mg/ml has bactericidal effect on S. aureus.

Table 1. Results of the minimum inhibitory concentration (MIC) of the hydroalcoholic extract of Althaea officinalis on tested microbial strains

Concentration mg / ml / Microbial strains	125	62.5	31.25	15.62	7.81	3.9	1.9	0.95	extract control	positive control
Escherichia coli	+	+	+	+	+	+	+	+	-	+
Staphylococcus aureus	-	-	-	+	+	+	+	+	-	+
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	-	+
Listeria monocytogenesis	-	-	-	-	+	+	+	+	-	+
Candida albicans	-	-	-	-	+	+	+	+	-	+

Table 2: Results of minimum inhibitory concentration (MIC) of the hydroalcoholic extract of Matricaria recutita on tested microbial strains

Concentration mg / ml / Microbial strains	250	125	62.5	31.25	15.62	7.81	3.9	1.9	extract control	positive control
Escherichia coli	+	+	+	+	+	+	+	+	-	+
Staphylococcus aureus	-	-	+	+	+	+	+	+	-	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	-	+
Listeria monocytogenesis	-	+	+	+	+	+	+	+	-	+
Candida albicans	-	-	-	+	+	+	+	+	-	+

Table 3: Minimum bactericidal concentration (MBC) of hydroalcoholic extract of Althaea officinalis on tested microbial strains

Concentration mg / ml / Microbial strains	125	62.5	31.25	15.62	7.81	3.9	1.9	0.95	Positive control
Escherichia coli	+	+	+	+	+	+	+	+	-
Staphylococcus aureus	-	+	+	+	+	+	+	+	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	-
Listeria monocytogenesis	-	+	+	+	+	+	+	+	-
Candida albicans	-	-	-	-	+	+	+	+	-

Table 4: Minimum bactericidal concentration (MBC) of hydroalcoholic Matricaria recutita extract on tested bacterial strains

Concentration mg / ml / Microbial strains	250	125	62.5	31.25	15.62	7.81	3.9	1.9	negative control
Escherichia coli	+	+	+	+	+	+	+	+	-
Staphylococcus aureus	-	+	+	+	+	+	+	+	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	-
Listeria monocytogenesis	+	+	+	+	+	+	+	+	-
Candida albicans	+	+	+	+	+	+	+	+	-

Table 5: Average diameter of growth inhibition of the tested plant extracts on microbial strains * Dash means the absence of the growth inhibition zone

The average inhibition diameter zone (mm)					
Microbial strains / extract	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Listeria monocytogenes	Candida albicans
Matricaria recutita (mg/ml50)	9	-	-	14	17
Althaea officinalis (mg/ml50)	-	-	-	-	-
Althaea officinalis (mg/ml25)	-	-	-	-	-
Althaea officinalis (mg/ml5/12)	7.5	-	-	9	-
10 µg/disc penicillin	17 ±1				
5µg/disc ciprofloxacin		29		22.5±1	
10 µg/disc gentamicin			21±1		
Negative control	-	-	-	-	-

Discussion

Microorganisms play an important role in human diseases. They can cause death so man ever has tried to repel them. Extracted chemicals of plants are considered as antimicrobial compounds which can replace synthetic drugs, because they have fewer side effects. The importance of studies on medicinal plants has been identified [15].

As discussed later, hydroalcoholic extract of Althaea officinalis dosent have bactericidal activity on Gram-negative bacteria like E.coli and pesudomonas, but it shows more bactericidal effect on Gram-positives. Also Naovi and colleagues in 1991 has been reported anti-fungal properties of ethanolic extract of this plant on Microsporum canis, Phialophora jeanselmei, Piedraia and Candida albicans and anti-bacterial effect on E.coli, Staphylococcus aureus, and Streptococcus pyogenes Corynebacterium diphtheriae [16]. However, in a survey conducted by A. Naini and colleagues, the results have shown that an ethanolic, methanolic, and ketonic extract of Althaea officinalis that was done by disc diffusion method has no antifungal effect on Candida albicans [17], Bassam and colleagues also reported that the ethanol extract of Althaea officinalis shoots against methicillin-resistant Staphylococcus aureus, has no antibacterial effect [18].

Also, the results of the bacteriostatic effect of hydroalcoholic extracts of Matricaria recutita and Althaea officinalis was similar, because no one had bacteriostatic effect on Gram-negative bacteria Althaea officinalis extract had more bacteriostatic effect on Candida albicans than Gram-positive bacteria, in addition Matricaria recutita extract had no bactericide effect on none of the strains. The interesting point of the previous studies by Kedzia in 1991 on effect of Matricaria recutita essential oil on Staphylococcus aureus and Candida albicans was that isolated compounds of Matricaria recutita essential oil such as flavonoids and α-bisabolol had strong antibacterial effect on Gram-positive and Gram-negative bacteria and Candida albicans separately [19]. While the study of A. Naini, the ethanolic extract of Matricaria recutita had no effect on candida albicans [17]. In another study conducted in 2005 and 2008 by Weseler and Shikov respectively concluded that Matricaria recutita essential oil had strong antibacterial effect on H. pylori [20, 21].

These differences may be due to the extraction method, where the plants are collected and parts of the plant that has been studied.

As for the extracts are derived of plant by different methods and solvent, can show different antibacterial effects. It is suggested that other extracts are examined. Also, the results of the before studies show that the antibacterial effect of the essential oils may be stronger than hydroalcoholic extract. This issue should be scrutinized in later studies.

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