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In vitro study of antimicrobial effects of *Rosmarinus officinalis* and *Glycyrrhiza glabra* extracts against some pathogens

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ABSTRACT

Background and aims: Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. The present study was, the antimicrobial effects of *Rosmarinus officinalis* and Glycyrrhiza *glabra* extract against some pathogens.

Methods: In this study, the antibacterial activity using 9 Gram-positive and Gram-negative bacterial strains includes: *Streptococcus pyogenes* ATCC® 19615, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274 and *Staphylococcus aureus* ATCC® 25923 with micro dilution methods was studied. The MIC, MBC were studied also, resistance of these bacteria to standard antibiotics such as erythromycin, cefixime, ceftazidime, tetracycline, ampicillin and amikacin were compared.

Results: In this study, the minimum inhibitory concentration (MIC) was used. The levels of MIC of *R. officinalis* were in ranges from 6.25 to 25 mg/ml. The highest MIC value was observed at 25 ppm against *S. pyogenes*, *S. pneumoniae* and *P. mirabilis* and the levels of MIC of *G. glabra* were in ranges from 6.25 to 12.5 ppm. The highest MIC value was observed at 12.5 ppm against *S. pyogenes*, *S. pneumoniae*, *P. mirabilis* and *S. marcescens*.

Conclusion: In important human pathogens, drug resistance is increasing according to the results of this study, and may be proposed that this plant can be used as a drug. It can be a good way to replace herbs with chemical drugs.

Keywords: Antibacterial activity, Human pathogen, Minimum inhibitory concentration.

Original article

INTRODUCTION

Life and diseases go together where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through their uses as food, clothing and shelter, but also plants have been used to

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control diseases. Therefore, the use of plants as medicines is an ancient and reliable practice.[1] *Glycyrrhiza glabra* is a traditional herb which grows in various parts of the world. The term *Glycyrrhiza* has been derived from ancient Greek word glykos, meaning sweet and rhiza, meaning root.[2] *G. glabra*, commonly called as Licorice, is one of the important traditional medicinal plants grows in the various part of the world and has been used for medicinal purposes for at least 4000 years. The root of this plant has several useful pharmacological properties such as anti-inflammatory, antiviral, antimicrobial, and anticancer activities in addition to immunomodulatory, hepatoprotective and cardioprotective effects.[3] Medicinally, it is used internally for Addison's disease, Asthma, Bronchitis, Peptic ulcer, Arthritis, Allergic complaints and steroid therapy. Externally, liquorices are used for Eczema, Herpes and Shingles. These activities are reported due to two kinds of main constituents, the saponins and flavonoids present in *G. glabra*. Topical applications of such compounds, with free-radical-scavenging properties, have shown to improve[5] significantly wound healing and protect tissues from oxidative damage.[4]

Rosemary (*Rosmarinus officinalis* L.) is an aromatic plant and thus a flavouring agent, widely used in foods. Its extracts have been introduced as preservatives in the food industry). Rosemary extract formulations are the only ones commercially available for use as antioxidants in the European Union and the United States, and they are marketed in an oil-soluble form, as a dry powder, and in water-dispersible or water-miscible formulations.[6] Rosemary is used for flavouring meat and poultry dishes. Thyme adds a pungent taste to meat and vegetables, and is the main ingredient for garnishing soups and stews. Basil is a classic complement to tomatoes, and is used to flavor salads, sauces and vegetables. Sage is widely used for flavouring meat dishes, soups, sausages and canned food.[7] These compounds may protect cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or anti-tumour activity. Essential oils of culinary herbs can inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumour growth.

The present study was, the antimicrobial effects of *R. officinalis* and *G. glabra* extracts against some pathogens.

METHODS

Bacterial strains were obtained from the microbial culture collection of Kerman University of Medical Sciences (KUMS). Evaluating the antibacterial activity of the plant extracts were investigated using a strain of bacteria *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter. baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274. The typed cultures of bacteria were sub-cultured on Nutrient agar (Oxoid, England) and stored at 4oC until required for study.

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI. The procedure followed is briefly described here. *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter. baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274 plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile saline water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over

the medium plate and antibiotic disc was transferred aseptically to the surface of the inoculated medium plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ceftazidim, erythromycin, ceftazidime, ampicillin, amikacin and tetracycline (Padtan Teb Co, Iran).

The leaf of *R. officinalis* and *G. glabra* was collected in the region of Iran (Zabol- southeastern, Iran) and dried at room temperature. Samples were crushed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

For preparing of extracts, plants were properly dried and pulverized into a coarse powder. Each of 40 g grinded powders was soaked in 50 ml ethanol 95%, separately for 20 h (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then, the filtrates were evaporated using a rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 40C in air tight screw-cap tube.

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth (Merck, Germany) supplemented with Tween 80 (Merck, Germany) at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 12.5 mg/ml to 400 mg/ml. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 104 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18-24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extracts at which the incubated microorganism was completely killed.

RESULTS

In this study, the minimum inhibitory concentration (MIC) was used. The levels of MIC of *R. officinalis* were in ranges from 6.25 to 25 ppm. The highest MIC value was observed at 25 ppm against *S. pyogenes*, *S. pneumoniae* and *P. mirabilis* (Table 1).

Table1: Antimicrobial susceptibility, MIC and MBC extract plant for Standard bacteria

Bacterial	MIC/MBC *R. officinalis*	MIC/MBC *G. glabra*	Antibiotic patent
Streptococcus pyogenes	25/50	12.5/25	-
Streptococcus pneumoniae	25/50	12.5/25	E,CE,CF, AM
Hafinia alvei	12.5/25	6.25/12.5	E,TE, AM
S. saprophyticus	12.5/25	6.25/12.5	E,CF,TE, AM
Acintobacter baumannii	6.25/12.5	6.25/12.5	CE,TE
Enterococcus faecalis	12.5/25	6.25/12.5	E,CE, AM
Proteus mirabilis	25/50	12.5/25	E,TE, AM
Serratia marcescens	12.5/25	12.5/25	CE
Staphylococcus aureus	25/50	12.5/25	E,CE, AM

E: Erythromycin, CE: Cefixime, CF: Ceftazidime, TE: Tetracycline, AM:Ampicillin, AN:Amikacin.

The highest MBC for *R. officinalis* leave was 50 ppm, while the lowest MBC was 12.5 ppm and the levels of MIC of *G. glabra* were in ranges from 6.25 to 12.5 ppm. The highest MIC value was observed at 12.5 ppm against *S. pyogenes*, *S. pneumoniae*, *P. mirabilis* and *S. marcescens* (Table 1). The highest MBC for *G. glabra* leave was 12.5 ppm, while the lowest MBC was 25 ppm.

DISCUSSION

The study of Sedighinia, evaluated the antibacterial activity of *G. glabra* against oral pathogens by diffusion methods and determined the minimum inhibitory concentration (MIC) by both broth and Agar dilution methods and minimum bactericidal concentration (MBC) by broth dilution methods and the results show that *G. glabra* extract showed good antibacterial activity against six bacteria. No strain in this study showed resistance against this extract.[8]

The ethanolic extract of *G. glabra* had promising MIC value against all oral bacteria especially *S. mutans*, *A. viscosus*, and *E. faecalis*. Although in some studies, it has been reported that *G. glabra* extract has antibacterial activity against several bacteria such as *S. aureus*, *E. faecalis*, and *E. coli*, but there are a few studies about oral pathogens such as *A. viscosus* and *S. sanguis*.[9]

The study of Syed was to test the antimicrobial activities of crude chloroform, hexane, ethyl acetate and ethanol extracts of the leaves *G. glabra* (GG) and *Fagonia arabica* (FA) against bacteria (*Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*). Antimicrobial properties of *G. glabra* and *F. arabica* were tested using Agar well diffusion method. Streptomycine was used as standard drug with significant activity values, that is, 23 mm against *E. coli*, 36 mm against *S. epidermidis*, and 34 mm against *S. aureus* and 26 mm against *B. subtilis*. Analysis of data showed that the crude extract of *G. glabra* and *F. arabica* in dichloromethane exhibited superior activity against *E. coli* and *S. epidermidis*.[10]

In the study of Nirmala and Selvaraj, the anti-bacterial activities of the methanol, ethyl acetate, acetone and chloroform extracts of *G. glabra* plant roots were tested against six bacterial species viz., *Bacillus coagulans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhimurium* by the agar disc diffusion method. The results indicated that the extract of *G. glabra* showed various antibacterial activities (9-14mm/20µl inhibition zone) against the bacterial organisms tested. The methanol, ethyl acetate, acetone and chloroform extracts did not inhibit *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* but showed the inhibition effect against *B. coagulans*, *E. coli* and *S. typhimurium*.[9]

In the study of Patil, The antimicrobical potential of *G. glabra* Linn. against certain microorganisms was studied using agar diffusion method. The aqueous and ethanolic extract of *G. glabra* roots significantly inhibited the growth of microorganisms as compared to standard bactericide and fungicide drugs. Diethyl ether fraction exhibited prominent fungicidal activity against *Candida albicans*.[11]

The study of Golshani and Dawoodi showed that *Rosmarinus officinalis* was used to evaluate its antimicrobial effects. The result show that the most efficacy of than extract of rosemary leaves was at concentration of 400 mg/ml against *Pseudomonas aeruginosa* and *Escherichia coli*. minimum inhibitory concentration of the extract on the growth of these bacteria showed changes from 6.25 mg/ml to 100 mg/ml. Also MBC of extract showed range from 12.5 to 200 mg/ml respectively.[12]

The study of Tavassoli and Emam Djomeh revealed antioxidant activity and antimicrobial activity of rosemary leaves extract. Dry rosemary leaf powder was subjected to Soxhlet extraction with pure methanol. The antimicrobial activity of rosemary leaves extract against *Leuconostoc mesenteroides*, *Lactobacillus delbruekii*, *Saccharomyces cerevisia* and *Candida krusei* (*Issatchenikia orientalis*) were determined by minimum inhibitory concentration (MIC).[13]

The effect of rosemary extracts was examined in the study of Klancnik against the foodborne pathogenic bacteria *S. aureus*, *B. cereus*, *C. jejuni*, and *Salmonella Infantis*. Gram positive strains were much more sensitive to rosemary extracts. For example, by the agar dilution method, the MIC for S. aureus and B. cereus was 0.078 to 5.0 mg/ml, whereas for Salmonella, it was 5.0 to 10.0 mg/ml.[14]

The study of Jarrar determines the antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) and investigates the synergistic effects of this extract combined with ceforuxime against methicillin resistant Staphylococcus aureus (MRSA).[15]

The study of Derwich evaluates the antibacterial activities of these aromatic extracts such as Rosmary. There in vitro antibacterial activity was determined by disk diffusion testing and minimum inhibitory concentration (MIC). *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Kellebsiella pneuomonae, Salmonella typhi, Staphylococcus intermedius, Bacillus subtilis, Streptococcus mutans, Micrococcus luteus*, and *Proteus mirabilis* were used as test bacterial strains. The analyses for leaves resulted in the identification of forty seven compounds, representing 65.61% of the total oil and the yields were 0.54%. The major component was α-pinene (18.25%); other predominant components were camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%). The bacterial strains tested were inhibited at minimum inhibitory concentration (MIC) values in the range of 4 at 48.2 µg/mL.[16]

CONCLUSION

The extract were found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics.

CONFLICT Of INTEREST

All authors disclose any financial and personal relationships with other people or organizations and the authors declare that there are not any potential conflicts of interest. I indicate here that any color photo in print is required.

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