**Evaluation of Antibacterial and Antifungal Activities of Lepidium meyenii (Maca)**

**ABSTRACT**

**Background:** With increasing infectious diseases as well as antimicrobial resistance in pathogens to existing drugs, researchers are now seeking for new drug candidates to be used as alternatives or complementary therapies. Maca is commonly used in traditional medication as herbal medicine.

**Materials & Methods:** In this research, the antibacterial and antifungal activities of maca powder and ethanolic extract were evaluated against *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC29212, and *Candida albicans* ATCC10231 using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and disc diffusion methods.

**Results:** The obtained results showed that there was no significant difference between the MIC and MBC of maca powder and extract against the reference and clinical strains. Also, no strain showed zone of inhibition at 50, 40, 30, and 60 µl of reference concentration.

**Conclusion:** According to the results obtained in this study, maca powder and extract had a poor inhibitory effect on bacterial and fungal growth.

**Keywords:** *L. meyenii; S. aureus; P. aeruginosa; E. coli; E. faecalis; C. albicans*
Introduction
The increasing trend of antimicrobial resistance in pathogens to the traditional antibiotics has reached a warning level [1]. This trend not only complicates the treatment of infectious diseases but also reproduces many diseases. To control and treat the infectious diseases caused by antimicrobial resistant bacteria, the search for new antimicrobial agents is essential and necessary.

The use of natural products with therapeutic properties has a long history dating to human civilization [2].

Recently, the interest in the use of alternative treatments and natural products, especially herbs, has increased [3]. Ginseng (maca) has been used for thousands of years in Asian countries, especially in China, Korea, and Japan, due to a wide range of functional activities such as tonic, immunogenesis, and antiaging activities [4]. Ginseng contains various agents including fatty acids, peptides, and polyacetylene.

Polysaccharides with medical properties are the most important agents in ginseng [5, 6]. Ginseng is an entirely domesticated species belonging to the genus Lepidium of family Cruciferae that is additionally referred to as Brassicaceae. As the source of various vegetables and oil plants of Eurasian origin, this family is of enormous economic importance. Ginseng is the only domesticated species of family Brassicaceae within the New World [7].

Grown up within the late 1980s in its native space to about fifty ha, ginseng has intimate over the last years a meteoric rise from an unnoted biology curiosity to Internet notoriety [8]. In this study, to determine the antibacterial and antifungal properties of ginseng, MIC and disc diffusion methods were used.

Material and Methods
Preparation of herbal extracts: Organic maca powder was provided from Indigo Herbs co, UK. The powder (20 g) was mixed with 200 ml of 70% methanol and placed in the soxhlet apparatus. Methanol extract was filtered and evaporated by vacuum rotary evaporator (Eyela, Tokyo, Japan) at $45^\circ$C. The extract was freeze dried and stored at $4^\circ$C.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): To prepare the reference tube of herbal extract, 1 g of the ginseng extract was dissolved in 3 ml of DMSO. To determine the MIC, ten tubes containing 1 ml of Trypticase Soy Broth (TSB) were used. In the first tube, 1 ml of the reference tube was poured. After mixing, 1 ml of which was removed and poured into the second tube. This process continued to reach the tenth tube, and the last 1 ml of the tenth tube was picked out. In Bacterial tube, $5 \times 10^5 - 10^6$ (CFU)/ml colony forming units were added to each tube, but for fungi, $10^4 - 10^5$ CFU/ml were added to each tube. These tubes were incubated at $37^\circ$C for 18-24 hrs. Gram-positive (S. aureus ATCC25923) and gram-negative (P. aeruginosa ATCC27853, E. coli ATCC25922, and E. faecalis ATCC29212) bacteria and fungi (C. albicans ATCC10231) were used in this part. Subsequently, each tube was shocked and cultured on an agar plate and incubated at $37^\circ$C for 18-24 hrs. Also, three clinical isolates were used to determined MIC and MBC values.

Disc diffusion: To defined inhibition zone for bacteria and fungi used in this study, the disc diffusion method was employed. For this purpose, 0.5 McFarland tubes of bacteria and fungi were prepared and cultured on agar plates. In the next step, the discs were filled with 30, 40, 50, and 60 µl of reference tube. Finally, plates were incubated at $37^\circ$C for 18-24 hrs.
Findings
In this study, serial dilution method was used to determine MIC and MBC values of maca powder and extract. MIC of maca powder was 0.16 g/ml for \( P. \text{aeruginosa} \; \text{ATCC27853} \) and \( C. \text{albicans} \; \text{ATCC10231} \); and 0.083 g/ml for \( S. \text{aureus} \; \text{ATCC25923, E. coli ATCC25922, and E. faecalis ATCC 29212} \). MBC of maca powder was 0.16 g/ml for \( P. \text{aeruginosa} \; \text{ATCC27853, C. albicans ATCC10231, S. aureus ATCC25923, and E. coli ATCC25922; and 0.083 g/ml for E. faecalis ATCC 29212} \) (Chart 1).

MIC of maca extract was 0.16 g/ml for \( P. \text{aeruginosa} \; \text{ATCC27853 and C. albicans ATCC10231}; \) and 0.083 g/ml for \( S. \text{aureus ATCC25923, E.coli ATCC25922, and E. faecalis ATCC 29212} \). MBC of maca extract was 0.16 g/ml for \( P. \text{aeruginosa} \; \text{ATCC27853, C. albicans ATCC10231, S. aureus ATCC25923, and E. coli ATCC25922; and 0.083 g/ml for E. faecalis ATCC 29212} \) (Chart 2).

MIC of maca powder was 0.16 g/ml for clinical isolates of \( P. \text{aeruginosa} \; \text{and C. albicans}; \) and 0.083 g/ml for clinical isolates of \( S. \text{aureus, E. coli, and E. faecalis} \). MBC of maca powder was 0.16 g/ml for clinical isolates of \( P. \text{aeruginosa, C. albicans, S. aureus, and E. coli}; \) and 0.083 g/ml for clinical isolates of \( E. \text{faecalis} \) (Chart 3).

MIC of maca extract was 0.16 g/ml for clinical isolates of \( P. \text{aeruginosa and C. albicans}; \) and 0.083 g/ml for clinical isolated of \( S. \text{aureus, E. coli, and E. faecalis} \). MBC of maca extract was 0.16 g/ml for clinical isolates of \( P. \text{aeruginosa, C. albicans, S. aureus, and E. coli}; \) and 0.083 g/ml for clinical isolates of \( E. \text{faecalis} \) (Chart 4).

The disc diffusion method results showed that all the strains had a borderline inhibition zone diameter, but obtained results by statistical analysis showed no significant difference between the isolates inhibition zone diameters at 30, 40, 50, and 60 µl of reference concentration.

Discussion
Infections and infectious diseases are considered as a serious threat for human health. Today’s, resistance to antimicrobial drugs is rising. Given the increasing trend, researchers have turned to herbal medicines to replace antibiotic treatment. Until now, different antimicrobial agents...
**Chart 2)** MIC and MBC of ginseng extract against the reference strains

**Chart 3)** MIC and MBC of ginseng powder against the clinical isolates

**Chart 4)** MIC and MBC of ginseng extract against the clinical isolates
have been isolated from several herbaceous plants. Since these compounds have relatively novel chemical structures and antimicrobial mechanisms, there has been a growing interest in using antimicrobial herbaceous plants \cite{9}. The present study results revealed that there was no significant difference between the MIC and MBC of maca powder and its alcoholic extract against the reference and clinical strains. Also, all the strains showed a borderline inhibition zone diameter at 30, 40, 50, and 60 µl of reference concentration. Peng Xue \textit{et al.} used MIC and MBC methods to evaluate the antibacterial effect of ginseng extract on \textit{Fusobacterium nucleatum}, \textit{Clostridium perfringens}, and \textit{Porphyromonas gingivalis}, consistent with this study using these methods \cite{10}. Eun-Ah Bae \textit{et al.} (2001) showed anti-Helicobacter pylori activity of ginseng. In their study, ginseng MIC against \textit{H. pylori} was reported as 50 µg/ml, but in the present research, the obtained MIC was higher than that reported in Eun-Ah Bae \textit{et al.}'s research \cite{11}. In Soyoung Na \textit{et al.}'s research, methanol extract of heated ginseng showed higher antimicrobial activity against \textit{Bacillus cereus} and \textit{S. aureus} than ethanol extract \cite{12}, but the obtained results in the present study showed no significant difference between the ginseng powder and its methanolic extract regarding the antibacterial properties. This difference could be attributed to the different concentrations of ethanolic and methanolic extracts used. Singriya \textit{et al.} showed that Indian ginseng had antibacterial and antifungal activity against \textit{P. aeruginosa}, \textit{B. subtilis}, and \textit{E. aerogens} \cite{13}. In the present research, ginseng showed weak antibacterial and antifungal activities against \textit{P. aeruginosa}, \textit{S. aureus}, \textit{E. coli}, \textit{E. faecalis}, and \textit{C. albicans}. In another research done by Sung \textit{et al.} (2008), it was shown that ginseng had antifungal activity against \textit{C. albicans} \cite{14}, consistent with the present research, showing that ginseng powder and methanolic extract had antifungal activity against \textit{C. albicans}. In Sung \textit{et al.}'s study, Korean red ginseng saponins had a weak antimicrobial effect on methicillin-resistant \textit{S. aureus}, confirming the present study results for \textit{S. aureus} strains \cite{15}. In Song \textit{et al.}'s investigation, gerimax ginseng had a strong antibacterial effect on \textit{P. aeruginosa} associated lung infection in chronic phase, whereas in the current study, ginseng powder and ethanol extract had a weak antibacterial effect on \textit{P. aeruginosa} strains. This difference might be due to the presence of different ginseng species with different properties \cite{16}. In another study done by Xue \textit{et al.}, it was found that ginseng extract had antibacterial effects on \textit{F. nucleatum}, \textit{C. perfringens}, and \textit{P. gingivalis}. The difference in results may be due to the selection of anaerobic bacteria, and ginseng may have an antibacterial property only on anaerobic bacteria \cite{10}. According to the results obtained in this study, ginseng powder and extract had a poor inhibitory effect on bacterial and fungal growth.  

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\textbf{Conflict of Interests:} No conflict of interested.  

\textbf{Ethical Permissions:} This study doesn't have any ethical permissions and we just used five MDR resistant isolates to figure out that maca has antibacterial properties or not.  

\textbf{Authors’ Contributions:} Maryam Mohammadbeigi, Samira Alimoradi, and Seyyed Reza Hashemi conducted lab experiments, analyzed data, and wrote manuscript. Maryam Meskini designed the study, analyzed data, and wrote manuscript.  

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References