Original Article

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Antimicrobial effects of some herbal extracts against infectious bacteria isolated from burn wound

Shahin Mohammadsadeghi[1], Hassan Habibi[2,*], Abdolkhalegh Keshavarzi[3], Abdorrasoul Malekpour[4]

[1]Plastic Surgery Ward, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Agricultural and Natural Resources College, Persian Gulf University, Bushehr, Iran.
[3]Burn and Wound Healing Research Center, Shiraz University of Medical Science, Shiraz, Iran.
[4]Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran.

Abstract

Herbal compounds with antimicrobial effects are of major importance because of increasing antimicrobial resistance of pathogenic bacteria. In this study, we investigated the antimicrobial effects of *Melissa officinalis* L, *Plantago major* L, *Orobancha crenata* Forsk, *Phoenix dactylifera*, *Ziziphus mauritiana*, and *Teucrium polium* seed extracts on some human pathogenic bacteria isolated from burn wounds. Disk-diffusion antibiotic sensitivity testing, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) were applied to assess the antibacterial activity of the extracts in comparison with tetracycline, as a control antibiotic. The extract of the *Orobancha crenata* showed stonger antibacterial effects than the other herbal extracts on *Pseudomonas aeroginosa* and *Kelebsiela ponomoni*. *Staphylococcus aureus* was the most sensitive one to the *Ziziphus mauritiana* nucleus extract compared with other herbs. According to the results of this study, it can be concluded that the extracts of some native plants of Iran can be an appropriate alternative to the existing antibiotics, applicable for prevention of burn infections.

Keywords: Antimicrobial, Burn infections, Burn wound, Herbal extract, Pathogenic bacteria.

1. Introduction

Burns provide a suitable situation for bacterial growth and infection; burn wounds are rich sources of infection in comparison with surgical wounds (1). There are nearly 265,000 deaths caused by burns and its consequences, mainly infections, and with this high incidence, almost half of them occurred in South East Asia (2, 3). The most habitually isolated infectious microorganisms of burn wounds include *Staphylococcus aureus*, *Streptococcus pyogenes*, *E. coli*, *Klebsiella* Spp., *Proteus* spp. and, *Pseudomonas aeroginosa*, which have recently shown antibiotic resistance (4). New enhanced methods of wound healing and tissue repair are offered by herbal medicine and have boosted the quality of life for trauma and burn injuries (5). Herbal medications are applied to heal various diseases because of their cost effectiveness and their easy applications (2). Herbal remedies can accelerate healing the burn wounds because of their different constituents such as flavonoids, oils, alkaloids, saponins, tannins, phenolic compounds, and terpenoids (6) as well as their very few side effects (7). Beside numerous mentioned favorable effects, there are some other stimuluant effect such as immunomodulatory properties and anti-diabetic effects (8). Numerous plants and herbal extracts have shown potent wound-healing properties such as ashwagandha, amla, tulsi, arjuna, aloe vera,

Corresponding Author: Hasan Habibi, Agricultural and Natural Resources College, Persian Gulf University, Bushehr, Iran.
Email: dr.h.habibi@gmail.com

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garlic, turmeric, ginger, shatavari, neem, guduchi, kiwi fruit, tut, kamala, palashlata, kokilaksha, balm mint, teucrium, and common plantain (9-11).

In the present study, we aim to evaluate the antibacterial effects of *Melissa officinalis* L, *Teucrium polium*, *Orobancha crenata* Forsk, *Ziziphus mauritiana*, *Phoenix dactylifera*, and *Plantago major* seed extracts on some pathogenic bacteria isolated from the human burn infections.

2. Material and methods

2.1. Equipment

The seed and leaf of plants (*Melissa officinalis* L, *Teucrium polium*, *Orobancha crenata* Forsk, *Ziziphus mauritiana*, *Phoenix dactylifera*, and *Plantago major*) were purchased from local shops in their natural habitat in Boushehr province, located in the southwest of Iran, and then were dried in a dry and dark environment. These dried seed and leaf were also pulverized to give seed and leaf powder. Species and genus of plants were diagnosed by an expert Botanist (12).

2.1. Extraction

Hydro-alcoholic extract (ethanol 70%) were prepared by seed and leaf soaking for 48 h at room temperature and then filtered with filter paper. The following oils were used: *M. officinalis* L, *T. polium*, *O. crenata* Forsk, *Z. mauritiana*, *P. dactylifera*, and *P. major*.

2.2. Antibacterial activity by disc diffusion method

Antibacterial properties of plants were assessed on *Pseudomonas aeroginosa*, *Kelebsiela ponomoni* and *Staphylococcus aureus* by disc diffusion method. The discs were sterilized by UV hood. The bacterial suspension was cultured on agar medium. Then, the prepared disks were placed on bacterial culture medium. Tetracycline disk was used as a control antibiotic in this study. Plates then were incubated at 37 °C for 24 h. The diameter of the zone of inhibition was reported in ml (three replicates per plant were considered) (12).

2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

To determine the MIC and MBC, a set of 9 sterile test tubes was used for each extract. The stock solutions (500 mg/ml) were further diluted in a 2-fold serial dilution to obtain the following concentrations: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.91, 1.95, and 0.98 mg/ml. One test tube as a negative control as well as a tube containing tetracycline were used as a positive control. An aliquot of 1 ml of the bacterial suspension was inoculated into each tube. The negative control tubes were inoculated with the same quantity of extracts. All tubes were incubated at 37 °C for 24 h. The lowest concentration that did not permit any visible growth (compared with the negative control tube) was considered as the minimum inhibitory concentration (MIC). The contents of all tubes that showed no visible growth were cultured on Muller Hinton agar and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration that could not produce a single bacterial colony, and the MBC was defined as the lowest concentration of the extract at which 99.9% of the inoculated microorganisms were killed (13, 14).

3. Results

In the present study, *T. polium* extract had the strongest antibacterial effect against *Ps. aeroginosa* (20.20 mm zone of inhibition) (Figure 1). According to the results of this study, *Ps. aeroginosa* with a zone of inhibition of 20.26 mm was affected more than the other bacteria by the alcoholic extract of *M. officinalis* L. The diameter of zones of inhibition for *S. aureus* and *K. ponomoni* were 20.20 and 20.03 mm, respectively (Figure 1). The diameters of zone of inhibition (ZDI) in the presence of *P. dactylifera* extract were 13.43 mm for *S. aureus*, 13.33 mm for *K. ponomoni* and 10.26 mm for *Ps. aeroginosa* (Figure 1).

The diameter of zone of inhibition by *O. crenata* Forsk extract was 20.46, 20.20, and 10.53 mm on *Ps. aeroginosa*, *K. ponomoni* , and *S. aureus*, respectively. It is notable that the diameter of the zone of inhibition by tetracycline on the three studied bacteria was 30.30, 30.10, and 30.05 mm, respectively (Figure 1).

Table 1 demonstrates the results of MIC and MBC of the extracts on cultured bacteria. The obtained data showed the various effects of plant

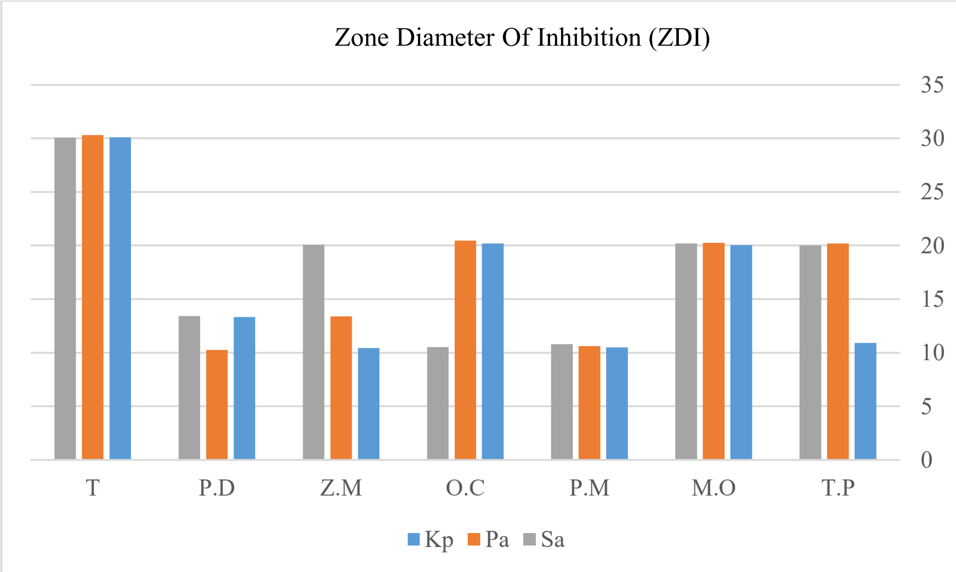

Figure 1. The inhibition zone (mm) of selected herbal extract against bacteria (Herbal: T.P: *Teucrium Polium*, M.O: *Melissa officinalis*, P.M: *Plantago major*, O.C: *Orobancha crenata*, Z.M: *Ziziphus mauritiana*, P.D: *Phoenix dactylifera*, T: Tetracycline.)

extracts on the tested bacteria. The results of MIC and MBC tests showed that *M. officinalis* L extract had the highest effect on *Ps. aeroginosa* (Table 1).

4. Discussion

In this experiment, *P. major* and *Z. mauritiana* had a significant effect on the three studied bacteria and showed a significant difference with the control antibiotic. In summary, the extract of *Z. mauritiana* showed the highest antibacterial activity for these bacteria, while *P. major* extract had the lowest antibacterial activity.

The *T. polium* extracts have shown to be effective on *Bacillus anthracis*, *Bordetella bronchiseptica*, and *Salmonella typhi* (15). The detailed data obtained from a previous study indicated that the growth of pathogenic *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus cremoris*, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* were significantly affected by the *T. polium* extract (16). On the other hand, *T. polium* contains several compounds including α-pinene, β-myrcene, cadinol, myrtenal, limonene; and presumably α-pinene in the essential oil of this plant could play an important role in the antibacterial activity of *T. polium* (17).

The mechanism of action of plant com-

Table 1. MIC and MBC ($mg.ml^{-1}$) results of herbal extracts against bacteria.

Test	Extracts						
	T	P.D	Z.M	O.C	P.M	M.O	T.P
MIC on Kp	0.39	3.125	3.125	0.78	3.125	6.25	3.125
MIC on Pa	0.195	6.25	3.125	0.78	3.125	1.562	1.562
MIC on Sa	0.39	3.125	1.562	3.125	1.562	3.125	3.125
MBC on Kp	0.78	12.5	12.5	3.125	12.5	12.5	12.5
MBC on Pa	0.78	12.5	6.25	1.562	6.25	6.25	6.25
MBC on Sa	1.562	6.25	3.125	12.5	3.125	12.5	25

(Bacteria: Kp: *Kelebsiela ponomoni*, Pa: *Pseudomonas aeroginosa*, Sa: *Staphylococcus aureus*. Herbal: T.P: *Teucrium Polium*, M.O: *Melissa officinalis*, P.M: *Plantago major*, O.C: *Orobancha crenata*, Z.M: *Ziziphus mauritiana*, P.D: *Phoenix dactylifera*, T: Tetracycline.)

pounds on the prevention of bacterial growth involves a destructive effect on the cell wall, which results in the departure of the cell wall components and the exposure of the cell contents leading ultimately to the cell death (18).

The *P. dactylifera* antimicrobial activity can be because of polyphenols (19). Polyphenols seem to have toxicity to microorganisms associated to the site(s) and number of hydroxyl groups on the phenol group, and increased hydroxylation brings more cell toxicities (20).

Abbes *et al*. (2014) reported that the MIC of *O. crenata* against *Listeria* monocytogenes and *Salmonella* enteritidis ATCC 502 were 10 and 25 mm, respectively (21). In our study, *O. crenata* showed a larger diameter of zone of inhibition.

Recent studies have shown that *P. major* extract has a wide range of biological effects, including wound healing, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immunomodulating, and anti-ulcergenic activity (22). *P. major* leave contains a mixture of different polyphenolic antioxidants that may contribute to its wound healing properties (23). Beside its antimicrobial effects, it could be beneficial in burned wound healing.

Conclusion

According to the results of this study, it can be stated that the extract of *T. polium*, *M. officinalis*, *P. dactylifera*, *O. crenata*, *P. major*, and *Z. mauritiana* have high antibacterial effects *in vitro*. More studies (especially *in vivo*) are suggested be done to determine the effective dose, main antibacterial compounds and their structures, and mechanism of action of these plant extracts to introduce them as new antibacterial agents.

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Conflict of Interest

None declared.

5. References

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