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Antibacterial Activities of Hydroalcoholic Extract of *Rosa Canina L* against Hospital Acquired Infections

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ABSTRACT

Aims Every year around the world, hospital infections and resistance to antibiotics lead to many complications in hospitalized patients. This study aimed to determine and compare the antibacterial effect of hydroalcoholic extract of *Rosa canina* plant (*Rosa canina L*) on Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus bacteria isolated from surgical wounds in Yasuj hospitals.

Materials & Methods In this cross-sectional study, the effect of *Rosa canina L* hydroalcoholic extract on 20 samples of Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus was investigated. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and zone of inhibition (ZOI) of each clinical sample were investigated and compared with the standard sample. The results were statistically analyzed by SPSS 16 software.

Findings *Rosa canina L* hydroalcoholic extract had no antibacterial effect on Pseudomonas aeruginosa. The lowest inhibitory concentration was related to Staphylococcus aureus 125 µg/ml, Escherichia coli 325 µg/ml and Klebsiella pneumoniae 350 µg/ml, respectively. The difference in minimum MIC, MBC and ZOI between clinical and standard samples was not statistically significant. The ZOI diameter of Klebsiella pneumoniae and Escherichia coli clinical samples were equal (9.8 mm).

Conclusion The hydroalcoholic extract of *Rosa canina L* has a better antimicrobial effect on Staphylococcus aureus than the other 3 investigated bacteria and has no effect on Pseudomonas aeruginosa. There is no difference between the effect of the examined extract on clinical and standard samples, and the effect of this extract on different bacteria is different.

Keywords Plant Extracts; Rosa; Anti-Bacterial Agents; Bacteria

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Introduction

Surgical site infection refers to an infection that occurs within 30 days after the operation and if there is an implant in place, within one year after the operation and involves the incision or the deep tissue of the surgical site. The infection includes superficial or deep tissue of the surgical site and may affect other organs and body parts [1]. This accounts for 14-17% of overall hospital-acquired infections and nearly 38% of hospital-acquired infections in surgical patients post-operatively [2]. Significantly, the rate of hospital-acquired infection at the site of surgery is higher in developing countries, which may be due to a lack of proper management during surgical procedures and inadequate postoperative care [3]. Surgical wound infection is the second most common cause of hospital infections in hospitalized patients [4]. Approximately 0.5% to 3% of patients undergoing surgery will experience infection at or adjacent to the surgical incision site. Compared to patients who do not develop a surgical site infection, those with a surgical site infection are hospitalized approximately 7 to 11 days longer [5]. The presence of pathogenic microorganisms in a surgical incision site causes infection in the skin and subcutaneous fat and muscle-fascial layers or in an organ. Surgical site infection creates a significant clinical and medical burden, including longer hospital stays and increased risk of readmission, increased cost of hospital accommodation and readmission rate [3]. Moreover, the acquired hospital infections cause major illness and even a third of postoperative deaths in patients and impose a high cost of 800 to 7000 dollars [1].

Depending on the surgical site, the type of microorganism is different. For example, in colorectal cancer, these organisms are Enterobacteriaceae and anaerobes. Common non-pathogenic microorganisms at the surgical site, such as Staphylococcus aureus and Staphylococcus epidermidis, have been reported [1]. Moreover, Enterobacteriaceae are gram-negative bacteria and one of the most common causes of hospital infection [6, 7]. Though it varies based on the wound source, the most commonly isolated gram-positive cocci are Staphylococcus aureus. Besides, gram-negative aerobic bacilli such as Escherichia coli, Pseudomonas aeruginosa, and Klebsiella are the most prevailing clinically relevant isolates [8-11].

Multidrug resistance (MDR) is a major cause of human suffering that impairs the trust relationships between doctors and their patients, concomitant with huge economic losses [12]. The impact of antimicrobial resistance (AMR) is a huge concern, which results in the greatest loss to the individual and social economy. Moreover, antibiotic resistance is associated with high morbidity, readmission, long-term care, death, and hospital costs [3]. It is estimated that by 2050, the death rate due to AMR will balloon to 10 million lives per year at an expense of one hundred trillion dollars [13]. Multidrug-resistant microbes may survive for long periods of time and multiply in the presence of minimal nutrients and have the ability to colonize, which is a significant threat to public health worldwide. The epidemiological rate has a significant effect on the resistance pattern of wound bacteria [1].

Medicinal plants are one of the important sources of antimicrobial agents in different countries. Plant natural compounds have been extensively explored for new drug discoveries [14]. About 70-90% of the population in developing countries continue to use ancient medicines based on plant extracts [15]. Plants synthesize secondary metabolites that are not required for their normal growth or development but are essentially required for reproduction and defense mechanism against bacteria, fungus, viruses, vertebrates, etc. These products have a great potential to act as drugs [16, 17].

Antibacterial actions of R. canina have been established against some bacteria [18]. Rosa canina L. is a perennial shrub that belongs to the Rosacea family, which includes 200 species that are distributed worldwide such as Asia, Middle East, Africa, and North America [19]. It grows wild at the margin of forests, puddles of water, shrubberies and pastures. Rosa canina L. has been used for long years as a source of vitamins, medicinal supplements, and food throughout the world. It contains various vitamins (especially vitamin C) and other valuable compounds such as polyphenols, carotenoids, carbohydrates and fatty acids. Moreover, there are evidences of anti-cancer, anti-diabetic, anti-obesity, anti-inflammatory, hepatoprotective, anti-arthritic, neurological, and anti-bacterial properties of this medicinal plant [18, 20]. Different parts of Rosa canina L have been used to treat diseases. For example, the root has been used to treat coughs, hemorrhoids, and heartburn. The leaves are used to treat colds, coughs, influenza and urinary stones. Rose fruit was used to treat asthma, bronchitis and colds, and Rose seeds are used for osteoarthritis, rheumatism and gout [21].

Antimicrobial effects of rose have been attributed to compounds of kaempferol 3-O-(6≤-O-Z-p-coumaroyl)-b-D-glucopyranosid, and kaempferol 3-O-(6≤-O-E-pcoumaroyl)-b-D-glu-copyranoside. These compounds can destroy the cell wall, destabilize the cytoplasmic membrane, inactivate the intracellular enzymes responsible for cellular metabolic processes, and as a result impede duplication and transcript processes through interaction with nucleic acids [22].

Antibacterial effects of four species of Rosa including Rosa pisiformis, Rosa canina, Rosa villosa and Rosa dumalis subsp is analyzed. Analysis found out that the most effective antibacterial agent was found in Rosa canina which inhibited the growth of most of the bacteria tested [23]. In the study, consumption of rose hip tea for three weeks in helpers improved

approximately of the useful bacteria of the bowel [18]. Moreover, in the study by Miklášová *et al.*, the highest antimicrobial effect of the ethanolic extract of Rosa canina was primarily against Pseudomonas aeruginosa CCM 1960 and Escherichia coli CCM 3988, and then counter to the Aspergillus niger, Fusarium culmorum and Alternaria alternata fungi respectively [24]. In the study by Miljković *et al.* antibacterial activity of R. canina dried fruit extract on Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Escherichia coli, Salmonella enteritidis, Enterobacter aerogenes, Pseudomonas aeruginosa, and Candida albicans were indicated [25]. In the mentioned studies, strong antimicrobial activity of R. canina against some microorganisms has been reported, yet, the antibacterial effect of Rosa canina L. on all bacterial strains has not been investigated, especially on the hospital-acquired infections. In addition, the effects of this plant can be different in each geographical region. Moreover, a study conducted in Turkey to examine the antibacterial activities of some rose species revealed that antibacterial activities varied in the ethanolic extracts that had been achieved from different species of rose hips [26].

Therefore, this study aimed to determine and compare the antibacterial effect of the hydroalcoholic extract of *Rosa canina* plant (*Rosa canina L*) on hospital acquired infections including Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus bacteria isolated from surgical wounds in Yasuj hospitals.

Materials and Methods

This was a case control study carried out in Kohgiluyeh and Noyer-Ahmad from June to July, 2020.

Samples

Considering an 8.8% prevalence of the hospital-acquired infections in Iran [27], a 95% confidence interval, and a precision of 5%, a total of 20 samples were collected from hospitals in Yasuj City, Iran. Therefore, 20 samples that consists of 4 groups of bacteria (5 samples in each group), Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli, were prepared for specialized tests. Clinical strains of *P. aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *K. pneumoniae* were collected from the surgical site and trachea.

Preparation of hydro-alcoholic extract of Rosa canina

Collective samples of *Rosa canina L.* were collected during the post-flowering phases (June and July 2020) from Kohgiluyeh and Boyer-Ahmad Province (in the southwest of Iran). A voucher specimen (herbarium number: 7532 Research Institute of Forests and Rangelands) was deposited at the Herbarium of Yasuj University, Yasuj, Iran. The material was dried in the dark at room temperature before extraction. Plant parts, powdered *Rosa canina L*, were macerated in EtOH-H_2O (70/30, v/v) at room temperature for 48 h, and then, 70% ethanol was added to the remaining material for 24 h and consequently percolated through a column. Finally, the extract solution was concentrated using a rotary evaporator (Hyedolph, type: HeizbadHei-VAP, Germany) under reduced pressure at 40 °C. It should be noted that the extract was stored at -20 °C [28, 29].

Preparation of standard bacterial inoculum

Totally 6 isolates of each bacterium (one standard strain and 5 clinical isolates) were used in this study. The standard strains of Pseudomonas aeruginosa (ATCC27853), Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 13883) and Staphylococcus aureus (ATCC 25923) were purchased from the microbiology department of Tehran University of Medical Sciences. Clinical strains of *P. aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *K. pneumoniae* were collected from the surgical site and trachea. Bacterial isolates were removed from storage, streaked onto Columbia agar plates supplemented with 5% sheep blood (bioMérieux) and incubated for 18-24 h at 35 C in ambient air. A working bacterial suspension was prepared by suspending 4-5 isolated colonies in 5 mL of Mueller-Hinton broth. The turbidity of this suspension was carefully adjusted photometrically (630 nm) to equal that of a 0.5 McFarland standard. For the test, the final inoculum was further diluted in Mueller-Hinton broth to achieve a final concentration of $5*10^5$ cfu/mL.

Determination of the minimum inhibitory concentration (MIC)

To determine the MIC, the broth microdilution method was used according to CLSI standards. In this test, the concentrations of 31.25, 62.5, 250, 500 and 1000 µg/ml of Rosa canina L extract were tested. Accordingly, the created concentration in the first well was equal to that of MIC from each of the substances and other concentrations were obtained from dilution. Finally, 10 µl of bacterial suspensions that prepared was equal to Half McFarland tube (5×106 CFU/ml) added to all wells while the final volume per well was 100 µl. A row of wells was considered to confirm the bacterial growth as a positive control containing only bacteria and bacterial growth medium and a row was considered as the blank which included study materials and the culture medium.

The micro plate was incubated for 18-24 hours in a shaking incubator at 37 °C and the results were read through the eyes (observation of turbidity caused by the growth of bacteria) and the optical density (OD) of plates were read by ELISA plate reader at a wavelength of 620 nm and the percentage of inhibition was measured. To ensure each test was repeated three times [30].

Determination of the minimum bactericidal concentration (MBC)

In order to determine the MIC, 100 microliters of different dilutions of extract were put in ELISA microplate wells in the vicinity of 100 microliters suspension of every bacterium with a concentration of 10^6 CFU/ml. After 24 hours of incubation at 37 °C, all dilutions were put in a Mueller-Hinton agar plate, then again, the incubation of 24 hours at 37 °C was repeated for the second time. Then, by checking whether the colonization was done or not, the minimum bactericidal concentration of the extracts was determined [31].

Determination of zone of inhibition diameter (ZOI)

To determine the zone of inhibition, the Kirby Bauer method was used in bacterial suspension equal to Half McFarland (5×10^6 CFU/ml) was prepared and cultured on Mueller-Hinton agar medium (Pure plate method) using a sterile swab. Then 20 µl of *Rosa canina L* extract in the concentration of MIC was added to a blank disk (Padtan Teb Company) and placed on the culture medium and incubated separately at 37 °C for 18-24 hours. A disc containing 20 µl of DMSO was added to each plate as a blank and a 10 µg Gentamycin antibiotic disc (mast1) was used as a positive control. The tests were repeated three times for each bacterium [32].

Statistical analysis

Statistical analysis was performed using SPSS software version 16. All results are expressed as the mean±standard deviation (SD) of three replicates. Significant differences ($p<0.05$) between average responses were evaluated with the use of one-way ANOVA with the Tukey test.

Findings

The effect of the hydroalcoholic extract of *Rosa canina L* on Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli was compared between two groups of standard samples and collected clinical samples.

Rosa canina L. hydroalcoholic extract has no effect on Pseudomonas aeruginosa. The main difference shown between other groups of bacteria is due to the great difference of Pseudomonas with the others, and the test results with the exclusion of Pseudomonas showed the absence of significant differences between different bacteria in response to the extract of the Rosa canina L. In the MIC method, the lowest inhibitory concentration was related to Staphylococcus aureus 125 µg/ml, Escherichia coli 325 µg/ml and Klebsiella pneumoniae 350 µg/ml, respectively.

The minimum bactericidal concentration (MBC) on all bacteria was higher than the standard sample, but none of these differences were statistically significant. The lowest lethal concentration was related to Staphylococcus aureus 225 µg/ml, Klebsiella pneumoniae 425 µg/ml, and Escherichia coli pneumonia 450 µg/ml, respectively. The minimum lethal concentration on all bacteria was higher than the standard sample, but none of these differences were statistically significant (Table 1; Figure 1).

Table 1. Statistical comparison of MBC, MIC of *Rosa canina L* extract

Test	Bacteria	Mean (µg/ml)	SD (µg/ml)	p-value
MIC	Staphylococcus aureus	125.00	76.55	0.55
	Klebsiella pneumoniae	350.00	368.70	
	Escherichia coli	325.00	381.20	
MBC	Staphylococcus aureus	225.00	153.09	0.63
	Klebsiella pneumoniae	425.00	349.11	
	Escherichia coli	450.00	360.12	

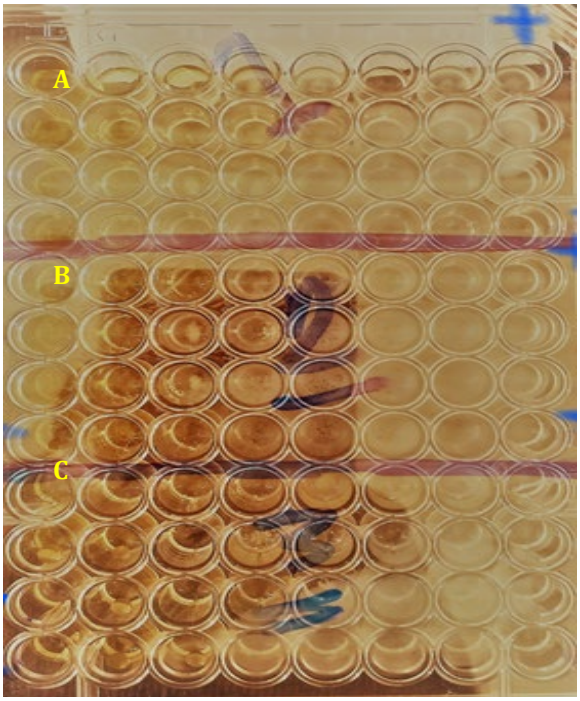

Figure 1. Determination of MIC in µg/ml hydroalcoholic extract of Rosa canina plant in, A) Staphylococcus aureus, B) Pseudomonas aeruginosa, C) Escherichia coli bacteria

The most effective ZOI was observed in Staphylococcus aureus 13.2 mm. ZOI of clinical samples of Klebsiella pneumoniae and Escherichia coli were equal to 9.8 mm. ZOI in the clinical sample of Staphylococcus aureus was higher than in the standard sample, in the rest of the examined bacteria, ZOI in the clinical samples was lower than the standard sample (Table 2).

Table 2. Statistical comparison of Zone of Inhibition (ZOI) diameter of *Rosa canina L* extract

Bacteria	Zone of inhibition (mm)		
	Mean	SD	p-value
Staphylococcus aureus	13.20	1.64	0.48
Klebsiella pneumoniae	9.80	5.12	
Escherichia coli	9.80	5.12	

Discussion

This study aimed to determine and compare the antibacterial effect of the hydroalcoholic extract of *Rosa canina* plant (*Rosa canina L*) on hospital acquired infections including Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus bacteria isolated from surgical wounds in Yasuj hospitals.

Hospital infections have every year financial cost for people and the health system of countries. Due to inappropriate and excessive use of antibiotics, widespread multi-drug and antibiotic resistances have been formed in the world. By replacing antibiotics with plants with antimicrobial properties, it helped a lot to solve this problem. In this study, the effect of *Rosa canina* plant was measured on 4 types of bacteria from infected hospital wounds, including Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus. The results of this study showed that Hydroalcoholic extract of *Rosa canina* plant has no effect on Pseudomonas aeruginosa. It was also indicated that Rosa canina extract had a better effect on Staphylococcus aureus than other studied strains.

Mohammadi *et al.* show that all the hydroalcoholic extracts used in their study have antimicrobial properties and were able to prevent the growth of resistant strains of Klebsiella pneumoniae. *Thyme* and *Adiantum capillus-veneris* extracts have the most antimicrobial properties [33]. In the study of Miklášová *et al.*, it is shown that Escherichia coli and Pseudomonas aeruginosa are more affected by *Rosa Canina* extract than gram-positive Enterococcus faecalis Bister [24].

Contrary to Cendrowski *et al.*'s [22] study, in the current study, the hydroalcoholic extract of *Rosa Canina* had no effect on Pseudomonas aeruginosa, and the most effective was related to Staphylococcus aureus bacteria and then Escherichia coli. In the study of Yazdanpanah & Shirvani, *Rosa Canina* extract causes a significant decrease in the count of total microbial load, Staphylococcus bacteria and Escherichia coli compared to the control sample. The fruit extract of Rosa canina has a high potential to increase the durability of chicken meat stored in the refrigerator and as a suitable substitute for chemical preservatives, it will be able to be used in industrialization [34]. The hydroalcoholic extract of the *Rosa canina* plant had a good effect on Escherichia coli and Staphylococcus aureus, which shows the potential of medicinal plants, including Rosa canina, to increase the durability of food and its antimicrobial properties. It is expected that with more studies on this medicinal plant, it will be possible to create suitable medicines based on the ingredients in the plant extract.

As a result of the study of Selahvarzian *et al.*, the greatest antimicrobial effect of the ethanolic extract of *R. canina*. is discovered to be against Pseudomonas aeruginosa CCM 1960 and Escherichia coli CCM 3988 [18]. In the study by Moustafa *et al.*, the antimicrobial activity of ethanolic extract of Rosa Canina with and without irradiation has antimicrobial activity against gram-negative and gram-positive bacteria and Candida spp. Gram-positives were sensitive to all extracts, however, the antimicrobial effect of these extracts on the activity of gram-negative bacteria such as *P. aeruginosa*, *E. coli* and *K. pneumoniae* is moderate. The antimicrobial effect of the extracts was less on *S. typhi*. Of course, the *R. Caninaat* 1.0 kGy shows significant inhibitory activity against all the tested pathogens [35]. Moreover, the findings of the study by Hacioglu *et al.* show that rosehip tea bags can be used as a safe adjunct to ampicillin treatment for bacterial infections [36]. Also, Research by Cendrowski *et al.* showed that Bacillus cereus among gram-positive bacteria and Escherichia coli and Klebsiella pneumoniae among gram-positive bacteria are the most sensitive to freeze-dried extracts of rose fruits (Rosa rugosa). However, among gram-positive bacteria, Staphylococcus aureus and Enterococcus faecalis, and in gram-negative bacteria, Pseudomonas aeruginosa and Proteus mirabilis are most resistant to the extract [22]. The current study was done on rose fruits (Rosa rugosa), while in the present study, the extract derived *Rosa canina L*. Moreover, in the present study, the effect of Rosa *canina L* was studied against hospital acquired infections, while in the above study, bacteria were derived from model fluids imitating protein food.

In the present study, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae were the most sensitive to the performance of *Rosa Canina* extract. The minimum inhibitory concentration was related to Staphylococcus aureus, Escherichia coli, and Klebsiella pneumonia, respectively, and the minimum bactericidal concentration was related to Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli, respectively, from the lowest to the highest. The highest ZOI was observed in Staphylococcus aureus, followed by Escherichia coli and Klebsiella pneumonia. It can be said that the effectiveness of this extract as an inhibitor and killer is different in different bacteria.

This study has limitations because it was unable to identify specifically which of the active compounds in Rosa canina L. is most effective at preventing nosocomial infections. In this research, the low sample size may also affect the findings of the research and generalizability of the result. Furthermore, one of the main limitations is that the study did not perform disc diffusion method to assess the zone inhibition for the MIC of Rosa canina L. extract. The study was also lacking the uses of antibiotics susceptibly test. It is suggested that this study be done with a larger sample. It is also suggested that in future studies, a comparison between antibiotics and the extract of this plant

against more microorganisms can be made in order to understand its use and special properties.

Conclusion

The hydroalcoholic extract of *Rosa canina L.* has a better antimicrobial effect on Staphylococcus aureus than the other 3 investigated bacteria and has no effect on Pseudomonas aeruginosa. There is no difference between the effect of the examined extract on clinical and standard samples.

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