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Investigation of microbiological contamination and antibacterial properties of Rose water (*Rosa damascena*) against *Staphylococcus aureus, Staphylococcus epidermidis,* and *Bacillus cereus*

Serra AGIRMAN YILMAZ[1,2*], Okan AYDOGAN[3], Ayten ALTINTAS[4]

1 Istanbul Medipol University, School of Medicine, Department of History of Medicine and Ethics, Istanbul, Türkiye
2 Istanbul Medipol University, Graduate School of Health Sciences, Istanbul, Türkiye
3 Istanbul Medipol University, School of Medicine, Department of Medical Microbiology, Istanbul, Türkiye
4 Istanbul Medipol University, School of Medicine, Department of History of Medicine and Ethics, Istanbul, Türkiye

ABSTRACT

The study investigates the antibacterial potential of rose water (*Rosa damascena*), a widely used hydrosol in both medicinal and cosmetic applications. The primary objective was to assess the microbiological contamination and antibacterial activity of rose water against *Staphylococcus epidermidis* (ATCC 49461), *Staphylococcus aureus* (ATCC 29213), and *Bacillus cereus* (ATCC 9634). Three different rose water samples were tested. The antibacterial effects were evaluated using both disc diffusion and microdilution methods. The results revealed that none of the tested rose water samples exhibited antibacterial activity against the selected bacteria. No bacterial growth was detected in the rose water samples, indicating the absence of intrinsic microbial contamination. The study showed that the rose water doesn't demonstrate antibacterial properties against the tested bacterial strains. In addition, it suggests that further research should be conducted on distillation techniques rather than additional chemicals to extend the storage period of rose water.

* Corresponding author: Serra AGIRMAN YILMAZ
E-mail: serra.agirman@medipol.edu.tr
ORCIDs:
Serra AGIRMAN YILMAZ: 0000-0002-4104-8479
Okan AYDOGAN: 0000-0001-7275-8724
Ayten ALTINTAS: 0000-0003-0531-0035

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INTRODUCTION

Rose is a culturally important plant that has been widely prescribed for medicinal and has a special place in cosmetics due to its fragrance from ancient times to the present day. This privileged plant, which has also been the subject of literature due to its symbolic value. *Rosa damascena* Mill. is one of the most famous rose species and is also known as *Damascus rose*, oil-bearing rose, pink rose, and is the rose species with the highest economic value[1]. Türkiye is one of the most important producers of roses and rose oil[1,2].

Rose water, also known as "gulab" or "jallab", is one of the herbal products frequently used in ancient medicine. As a by-product of the process of extracting rose oil through traditional distillation methods, it is one of the most widely used products of roses. These by-products, which are also called hydrosol and obtained especially as a result of single distillation process, contain some of the essential oil components[3]. Previously reported that the rose water obtained by traditional distillation methods contains 0.025% rose oil[4]. But also reported in the literature that rose waters grown and produced in Iran contain 10-50% rose oil[5]. In general, hydrosols are used in aromatherapy due to their antibacterial, antifungal, antiseptic, analgesic, antioxidant, etc. properties[3]. Rose petals, which are rich in geraniol and phenyl ethyl alcohol, have been found to have antimicrobial activity[6]. Rose water, like rose oil, is one of the herbal products with high economic profit. According to Gülbirlik's information note, the annual income from rose water in 2022 is stated as € 2,000,000[1]. Rose water is preferred for its moisturizing, antiseptic, and anti-inflammatory effects in the skin-care routine as well as in the food industry[7,8]. Unlike rose oil, there are a limited number of studies in the literature on the shelf duration of rose water and the possibility that it may contain microorganisms over time and it's *in vitro* antimicrobial effect[7-10].

Staphylococcus epidermidis used in the study is one of the most common members of human skin microbiota[11]. This generally harmless microbiota member may be encountered especially in foreign body-mediated infections[12]. It is also known that staphylococci have mechanisms that facilitate their colonization of the skin microbiota in an evolutionary cooperation with antimicrobial peptides[13]. *Staphylococcus aureus* is one of the most common pathogenic bacterial species in humans. The bacteria which can be found

naturally in body parts such as the skin, respiratory tract, and digestive system, can lead to serious infections in cases where the immune system is weakened. Especially methicillin-resistant *Staphylococcus aureus* (MRSA) strains are known as one of the main causative agents of nosocomial infections[14,15]. *S. aureus* is involved in a wide range of diseases including skin infections, pneumonia, blood infections, and sepsis[14]. In addition, resistance to antibiotics makes it difficult to treat *S. aureus* and this poses a major clinical threat[12,13]. *Bacillus cereus* can be found in natural environments such as soil and air and has been reported as the causative agent of foodborne infections[16]. Apart from foodborne infections, *B. cereus* may cause infections in individuals with weak immune systems, after injuries or surgical interventions[17].

The test microorganisms have been included in the study in terms of having clinical importance and capability to develop resistancy against antibiotics. The primary aim of this study was to determine whether rose water has been contaminated over time by microbiological methods. Secondarily, it was aimed to determine the antibacterial activity of rose water on *S. epidermidis* in the skin microbiota, *S. aureus*, one of the most important opportunistic pathogens, and *B. cereus*, whose natural habitat is soil and can form spores in this environment.

METHODOLOGY

The properties of rose water samples

Agents: Rose water was obtained from two different brands, Rose & Cure, which grows organic roses in Isparta, and Sebat LTD, which grows organic roses in Afyonkarahisar. Two rose waters from Rose & Cure brand, one of which was a new harvest rose water 1 and one of which was the previous year's harvest rose water 3, and a total of three rose waters obtained by single distillation method, named as rose water 2 from Sebat brand, were used in the study.

The chemical analysis of the rose waters was given in Table 1. The analyses were not performed by us but were requested from the companies.

Table 1. Chemical analysis results of rose waters

	Percentage Amount in Volatile Composition	
Composition	**Rosewater 1**	**Rosewater 2**
Hexyl Alcohol	0.134	NA
Menthone	0.413	NA
Linalool	1.074	0.542
4-Terpineol	0.273	0.317
Menthol	0.442	NA
Citral	0.137	0.242
Alpha Terpineol	0.377	0.250
Geranial	0.433	0.730
Citronellol	13.949	13.573
Cis-Geraniol	6.101	6.574
Phenethyl Acetate	0.181	0.182
Trans-Geraniol	17.812	18.673
Benzyl Alcohol	0.823	0.868
Phenethyl Alcohol	52.081	53.095
Methyl Eugenol	1.318	1.274
Eugenol	2.891	3.681
Nerolic Acid	0.606	NA
Others	0.956	NA

NA: Not applicable.

Isolation of bacterial strains

The three rosewaters included in the study were inoculated on Brain Heart Infusion Agar (Biolife Italiana, Italy) medium in 1 mL, 0.5 mL, and 0.1 mL volumes. The media were incubated at 37°C for 24-48 hours and bacterial growth were assessed.

Disc diffusion (Kirby-Bauer) test

0.5 McFarland suspensions were prepared and inoculated on Mueller Hinton Agar (MHA) medium for disc diffusion test. Blank antibiotic disks were placed on the media. Each blank disk was impregnated with 30 µL of the tested rose water. After incubation at 37°C for 24 hours, the test results were analyzed.

Minimum inhibitory concentration (MIC) test

Staphylococcus aureus (ATCC 29213), *Staphylococcus epidermidis* (ATCC 49461), and *Bacillus cereus* (ATCC 9634) used in the study. The EUCAST

criteria were followed for the MIC test. A sterile 96-well microplate was used in the antibacterial susceptibility test based on the microdilution method. From Mueller Hinton Broth (MHB) medium 50 µL were added to each well of the microplate. Vancomycin was used as control. Serial dilution of all tested rose waters was performed starting from a concentration of 1000 µg/mL (the dilution concentrations to be obtained are 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/mL) respectively. A stock solution was prepared by adding MHB from the prepared bacterial strains to $1x10^8$ (CFU/mL) cells per mL. 10 µL of the prepared bacterial stock solution was added to all wells and the microplates were incubated at 37°C for 24 h. At the end of the incubation, the sensitivity expressed as MIC values of the tested rose waters were compared with that of vancomycin detected manually by the naked eye.

RESULTS and DISCUSSION

Bacterial growth in rose water samples

The rose water samples and their chemical compositions were given in Table 1. Brain Heart Infusion Agar (BHI) plates were inoculated with 1 mL, 0.5 mL, and 0.1 mL of the three tested rose water samples and incubated at 37°C for 24 and 48 hours. No bacterial growth was observed on any of the BHI plates after both 24 and 48 hours of incubation, confirming the absence of intrinsic bacterial contamination in the tested rose water samples.

Disc diffusion (Kirby-Bauer) test

After 24 hours of incubation at 37°C, none of the three tested rose water samples produced any inhibition zones against *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 49461), and *B. cereus* (ATCC 9634) on MHA plates. The absence of inhibition zones suggests that the rose water samples tested did not exhibit antibacterial activity under the conditions of the disc diffusion assay.

Minimum inhibitory concentration (MIC) test

In the microdilution assay, serial dilutions of the rose water samples (ranging from 1000 µg/mL to 3.9 µg/mL) were tested against the bacterial strains. The wells containing the bacterial suspensions in the presence of rose water exhibited no inhibition compared to the growth control. Visual inspection confirmed bacterial growth at all tested concentrations, indicating that none of the rose water samples demonstrated a detectable MIC value against *S. aureus, S. epidermidis,* or *B. cereus*. Conversely, the positive control (vancomycin) effectively inhibited bacterial growth, confirming the validity of the assay.

The study results indicate that none of the tested rose water samples exhibited antibacterial activity against the selected bacterial strains. These findings suggest that under the experimental conditions applied in this study, rose water does not possess intrinsic antibacterial properties against the tested bacterial species.

Maruyama et al. reported that rose water inhibited the mycelial growth of *Candida albicans* at a concentration of approximately 2.2% and reduced the viability of MRSA within 1 hour[10]. The antimicrobial effect may be affected by factors such as the active ingredients in the composition of the herbal hydrosol used, the method of application, the susceptibility of bacterial strains, the preparation process of the hydrosol, the way the hydrosol is prepared, the concentration of its components and the extraction method used. The essential oil content in rose water used in the mentioned study was 0.095% and the main components were phenethyl alcohol (43.65%), citronellol (18.24%) and geraniol (14.8%)[10]. The contradictory findings with our study may have been due to the content of the rose water used.

In a study conducted by Aliasgari et al. similarly with our study, it was found that rose water reduced the adhesion of bacteria however had no effect on their growth and had no bactericidal effect[9]. In an *in vivo* study by Bayhan et. al.[7] found no antibacterial activity of rose hydrosol after hand-rubbing. In the study conducted by Ulusoy et al. in which Sebat brand products were used, no antimicrobial activity of rose water against *Chromobacterium violaceum* (ATCC 12472), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Erwinia carotovora* (ATCC 39048) was found in contrast to rose oil[17,18]. This study, which found similar results with a rose water found in our study, supports our findings.

Furthermore, all tested rose water samples were free from bacterial contamination. In the literature, it has been reported that rose waters with low essential oil content are sensitive to contamination and storage conditions should be considered[6,8]. It has been reported that rose waters obtained by double distillation method can be used for at least one year and manufacturers use antimicrobial additives for a 12-month shelf duration[6,8]. It is known that the water-soluble components of rose oil are also separated in rose waters obtained by double distillation method[8]. In our study, no difference was observed in terms of bacterial contamination between the one-year product and the newly harvested product of the same brand obtained by single distillation method, but the odor change was subjectively noticed by the researchers, although no odor scale was used. This shows that rose waters obtained by single distillation

method and containing an average of 0.3% rose oil can be used for more than one year despite the odor change.

Baydar et al. showed that physical or chemical preservation methods did not have a negative effect on the components of rose water, however had a positive effect on shelf life[8]. Indeed, Labadie et. al. observed microbial growth in orange blossom (*Citrus aurantium*) and rose flower (*Rosa damascena* and *Rosa centifolia*) hydrosols at ambient temperature and also at 5°C when stored in a non-sterile container and suggested that additional barriers such as chemical preservatives or aseptic packaging would be necessary to ensure microbial stability[4,19].

The findings obtained in our study suggest that the addition of extra chemicals is not necessary in rose waters obtained as a single distillation product. Although the results of the study indicate a lack of antibacterial activity of rose water against the tested bacteria, its originality lies in filling a gap in the existing literature regarding the antibacterial potential of rose water. This result highlights the need for further investigation to understand the true potential of rose water in antimicrobial applications. Our study provides valuable insights, particularly for the cosmetic and herbal product industries, and underscores the importance of understanding the shelf life and intended use of rose water. Future studies may provide a more comprehensive understanding of its properties and contribute to the development of guidelines for its use in various industries.

STATEMENT OF ETHICS

This study does not require any ethical permission.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

The main scheme of the research was proposed by AA, and she provided the rose water. The experiments were conducted by OA, and the abstract and methodology part was written by him. The introduction and discussion sections of the manuscript were written by SAY and checked by AA. The final version of the manuscript was checked by all authors.

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