ORIGINAL RESEARCH

Medical Evaluation of the Antimicrobial Activity of Rose Oil on Some Standard Bacteria Strains and Clinical Isolates

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ABSTRACT

Introduction • Considerable interest has developed concerning the alternative utilization of aromatic plants rich in essential oils as antibacterial agents in the medical arena. In this study, we investigated the antimicrobial activity for solutions of different concentrations of rose oil on test microorganisms known to potentially have an adverse affect on human health and the environment.

Methods • Research was carried out by the microdilution method. The test microorganisms were standard strains and clinical isolates (CIs) of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 17978, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumonia* ATCC 49619.

Results • *E coli* was inhibited by a minimal concentration of 125 µl/ml rose oil dilution of both the standard strain and CI, *B subtilis* by a minimal concentration of 15.62 µl/ml and 31.25 µl/ml rose oil dilutions of the standard strain and CI, respectively, and *S aureus* by a minimal concentration of 31.25 µl/ml and 125 µl/ml of rose oil dilutions standard strain and CI, respectively. Thus, it was determined that rose oil could exhibit antimicrobial activity in both Gram-positive and Gram-negative bacteria.

Discussion/Conclusions • Different percentages of diluted solutions of rose oil might be used as a preventive and therapeutic treatment for infections caused by *E coli, B subtilis,* and *S aureus*, provided that this is supported by evidence from clinical trials. Consequently, natural antimicrobial rose oil may have beneficial effects on human and environmental health. (*Altern Ther Health Med.* 2022;28(6):52-56)

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INTRODUCTION

Herbal remedies are a valuable and easily available resource for primary healthcare and complementary health systems. Although a large number of plants are being screened for their antimicrobial effects, there are, of course, many species of plants that have medicinal value and are still waiting to be discovered. It may be possible to prove that these plants are rich in compounds with possible antimicrobial effects, but more pharmacologic research is needed.[1]

Antimicrobial agents kill or inhibit the growth of microbes such as bacteria, fungi, protozoa and viruses. The history of antibiotic agents began with the 1877 observations of Pasteur and Joubert, who discovered that a bacterial species can prevent the growth of another species of bacteria.[1]

Rose is a long-lasting, climber plant of the genus Rosa in the family Rosaceae that includes approximately 1350 species worldwide. There are 24 types of roses in Turkey, and *Rosa damascena* Miller is used in the production of rose oil. This pink and pungent species is also known as Isparta rose, Damascus rose, Oil rose, Pink oil rose and Chew rose.[2,3] The Isparta rose is the species most commonly used in the production of rose oil as its essential oil rate is higher than other roses. Rose oil is obtained by water vapor distillation from the collected flowers; 4000 kg of rose blossoms is used to extract approximately 1 kg of rose oil.[3]

Turkey is one of the world's leading producers of rose oil, with approximately 48% of the world's rose oil production.[4]

Rose oil has a clear, light yellow color and characteristic odor, and is mainly composed of acyclic terpenic substances; 40% to 50% of the oil is citronellol and 20% geraniol, which provides the characteristic rose odor. It also contains nerol, which is the cis isomer of geraniol, other terpenic substances, hydrocarbons and 1% to 4% of phenyl ethyl alcohol, an aromatic material.[3]

Genetic and genomic research concerning roses has progressed rapidly in the last 10 years. *Rosa damascena* Mill.

belongs to the Damask group known as rose oil. This species is grown in Europe for rose oil and rose water obtained by distillation of its flowers; rose oil is used in the perfumery and cosmetics sectors.[5]

Rose essential oil is used by the public because of its fragrance, and it is also used for its calming effect and skincare properties.[6] In traditional medicine, it is considered effective in the treatment of abdominal and chest pain, as well as for cosmetic use. It is known to strengthen the heart, and treat menstrual bleeding and digestive system disorders.[7]

It has been reported that rose oil is good for sore throat, headache, eye redness, and pain; increases understanding and improves memory; relieves body pain and reduces heart palpitations due to excitement per the books of Ottoman physicians Ibn-i Sina and Ibn-i Baytar. Also, it has also been stated that rose water sherbet is good for abdominal pain, nausea and indigestion.[2]

Current experimental studies have shown the antibacterial, anti-HIV, anxiolytic, anti-inflammatory, analgesic, hypnotic, antispasmodic, antitussive and antioxidant effects of rose oil.[6,8-11]

Recently, there has been a great deal of interest in the alternative use of aromatic plants rich in essential oils as antibacterial agents in the pharmaceutical industry, although there are a limited number of studies on rose oil. The microdilution method, doses and bacterial strains used in this study are different from those of other studies.

Table 1. Microorganisms Used in This Study and Their Sources

Bacteria	Characteristic[a]	Source
E coli	Standard strain ATCC 25922	Süleyman Demirel University, Turkey
P aeruginosa	Standard strain ATCC 27853	Süleyman Demirel University, Turkey
A baumannii	Standard strain ATCC 17978	Süleyman Demirel University, Turkey
B subtilis	Standard strain ATCC 6633	Süleyman Demirel University, Turkey
S aureus	Standard strain ATCC 25923	Süleyman Demirel University, Turkey
S pneumoniae	Standard strain ATCC 49619	Süleyman Demirel University, Turkey
E coli	Ap, Cm, Sm, Sx, Tc	Clinical isolate, Faculty of Medicine, Süleyman Demirel University, Turkey
P aeruginosa	PAO1 standard strain	Clinical isolate, Faculty of Medicine, Süleyman Demirel University, Turkey
A baumannii	Ap, Cip, Cm, Cp, Ctx, Pp, Sx, Tc	Clinical isolate, Faculty of Medicine, Süleyman Demirel University, Turkey
B subtilis	Ap, Cf, Cip, Ctx, Gn	Clinical isolate, Faculty of Medicine, Süleyman Demirel University, Turkey
S aureus	Ap, Cf, Gn, Sm	Clinical isolate, Faculty of Medicine, Süleyman Demirel University, Turkey

[a]Clinical isolate resistance to: Ap (ampicillin); Cf (cephalexin); Cip (ciprofloxacin); Cm (chloramphenicol); Cp (cephoperazone); Ctx (cefotaxime); Gn (gentamicin); Pp (piperacillin); Sm (streptomycin); Sx (sulfamethoxazole); Tc (tetracycline)

METHODS

Collection of Rose Flowers and Preparation of Rose Oil

Rose flowers were collected in the early morning from Guneykent, Isparta. The plant species were identified with the help of regional floras and further confirmed according to Herbarium sheets available in the Rosa Herbarium, Suleyman Demirel University, Isparta, Turkey with the voucher specimen number *Rosa damascena* Mill.-1986.[3,5]

The rose oil used in this study was obtained by distillation. Petals were boiled in water for 30 minutes and the water extract was dried under reduced pressure at 60°C. Drying under reduced pressure was finished when equilibrium humidity was achieved and a concentrated volatile fraction was obtained. Then, 240 g of rose petals were hydrodistilled in a Clevenger type hydrodistillation apparatus for 3 hours to extract the essential oil according to standard procedure as described in the European Pharmacopoeia.[12] After 3 hours of distillation, the essential oil and the water mixture were separated by decantation. The rose essential oil obtained in this way was stored at -5°C prior to analysis.

Gas Chromatography-Mass Spectrometry

The rose oil was analyzed by the Shimadzu GC-MS QP 5050 (Kyoto, Japan) gas chromatograph-mass spectrometer (GC-MS) system. Helium (99.999%) was used as the carrier gas at a constant head pressure of 10 p.s.i. The injection volume was 1 µl. The GC oven was programmed as follows: the initial column temperature was 60°C, the column was heated to 220°C at a rate of 2°C min-1 and held at 220°C for 20 minutes. The GC-MS interface and injector were kept at 250°C and 240°C, respectively. The MS was set to work in the electron impact mode at 70 eV.

Bacterial Strains and Growth Conditions

Bacteria used to determine the antimicrobial activity of rose oil were obtained from the microbial collection of the Department of Microbiology, Faculty of Medicine, Suleyman Demirel University, Turkey (see Table 1). Bacteria were subcultured from stocks maintained in nutrient broth containing 20% glycerol at -80°C.

Determination of Minimal Inhibitory Concentration Values

To determine the minimal inhibitory concentration (MIC) values of rose oil, 96-well U-bottom microplates were used. While the MIC value of the rose oil was determined for each bacteria, the first 6 lines of the plate were used from well 1 to 12. 50 µl of cation-adjusted Mueller-Hinton broth (CAMHB) medium was added to all the wells used.

50 µl rose oil was added to the first of the 12 wells used for each bacteria. Via transferring and pipetting, 50 µl was taken from the 1st well, to which the highest concentration of the substance to be tested was added to the 2nd well, transferring and pipetting 50 µl from the 2nd well to the 3rd well, double-fold serial dilutions of substances were made up to the final (12th) well, and 50 µl taken from the final well was thrown ouWt.

Table 2. GC-MS Analysis of Rose Oil

Compound	% Components
Alpha pinene	0.79
Citronellol	35.70
Citronellol acetate	0.72
Eugenol	1.18
Geraniol	21.89
Geranyl acetate	2.00
Germacrene-D	0.40
Heneicosane	4.80
Heptadecane	0.88
Linalool	0.50
Methyl eugenol	1.97
Nerol	10.23
Nonadecane	13.80
Phenylethyl alcohol	2.29
9-Nonadecane	2.85

Abbreviations: GS-MS, Gas Chromatography-Mass Spectrometry.

Table 3. Antimicrobial Effects of Rose Oil and Different Percentages of Diluted Rose Oil Solutions on Standard Strains and Clinical Isolates of 5 Different Bacteria

	Dilutions of Rose Oil Solution											
Bacteria	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	2^{-9}	2^{-10}	2^{-11}	2^{-12}
E coli ATCC 25922	x	x	x	+	+	+	+	+	+	+	+	+
P aeruginosa ATCC 27853	+	+	+	+	+	+	+	+	+	+	+	+
A baumannii ATCC 17978	+	+	+	+	+	+	+	+	+	+	+	+
B subtilis ATCC 6633	x	x	x	x	x	x	+	+	+	+	+	+
S aureus ATCC 25923	x	x	x	x	x	+	+	+	+	+	+	+
E coli (CI)	x	x	x	+	+	+	+	+	+	+	+	+
P aeruginosa (CI)	+	+	+	+	+	+	+	+	+	+	+	+
A baumannii (CI)	+	+	+	+	+	+	+	+	+	+	+	+
B subtilis (CI)	x	x	x	x	x	+	+	+	+	+	+	+
S aureus (CI)	x	x	x	+	+	+	+	+	+	+	+	+

X = no bacterial growth; + = bacterial growth

Abbreviations: CI, clinical isolate.

Well 1H was used for medium control (MC), and 100 µl of CAMHB medium was added to that well. Wells 7H, 8H, 9H, 10H, 11H and 12H were used for *E coli*, *P aeruginosa*, *A baumannii*, *B subtilis*, *S aureus* and *S pneumoniae* reproductive control (RC), respectively, and 50 µl of CAMHB medium was added to all those wells. Rose oil was not pipetted into the MC and RC wells.

Bacteria to be tested were freshly passaged in a 5% sheep blood agar medium and incubated at 3°C for 24 hours. The bacterial suspension was prepared from bacterial colonies to be equal to 0.5 McFarland (approximately 108 CFU/ml) turbidity in 3 cc of CAMHB. This suspension was diluted 1/10 to obtain 107 CFU/ml. Then, 5 µl of the suspension was inoculated into each well, including the RC wells, to set the test concentration of bacteria at 5 × 105 CFU/ml. The bacterial suspension was not pipetted into the MC well. This test was repeated twice more under the same conditions with 96-well U-bottom microplates. Then, the plates were covered and incubated at 37°C. After 24 hours of incubation, the plates were removed from the incubator and the wells were evaluated visually. The lowest non-reproductive concentrations were determined to be the MIC values of the bacteria being studied.[13]

Statistical Analysis

All determinations were performed 3 times and statistical analyses were carried out using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Of the 15 constituents identified by GC-MS analysis of rose essential oil, citronellol was found to be the major compound (35.70%), followed by geraniol (21.89%), nonadecane (13.80%) and nerol (10.23%). Trace amounts of other chemical compounds were also identified. The major components are summarized in Table 2.

Table 4. MIC Values and Ranges for Standard Strains and Clinical Isolates of Different Bacteria Inhibited by the Effects of Rose Oil Solutions

Bacteria	MIC value (µl/ml)	MIC value (dilution)	MIC range (µl/ml)	MIC range (dilution)
E coli ATCC 25922	125	1/8	125-500	1/8-1/2
B subtilis ATCC 6633	15.62	1/64	15.62-500	1/64-1/2
S aureus ATCC 25923	31.25	1/32	31.25-500	1/32-1/2
E coli (CI)	125	1/8	125-500	1/8-1/2
B subtilis (CI)	31.25	1/32	15.62-500	1/32-1/2
S aureus (CI)	125	1/8	125-500	1/8-1/2

Abbreviations: CI, clinical isolate; MIC, minimal inhibitory concentration.

Standard strain testing

This study was carried out in 2 consecutive stages. In the first stage, standard strains were evaluation. A row from A to F was reserved on the microplates for each of the 6 bacteria. *E coli* was tested in row A, *P aeruginosa* in row B, *A baumannii* in row C, *B subtilis* in row D, *S aureus* in row E and *S pneumoniae* in row F.

A total of 5 bacteria (*E coli, P aeruginosa, A baumannii, B subtilis* and *S aureus*) were considered test microorganisms for investigation of the antimicrobial effects of rose oil and different percentages of diluted rose oil solutions. *S pneumoniae* was excluded from the study due to the fact that no growth was observed at any concentration in row F.

No growth was observed in the MC wells. In the RC wells, there was growth of 5 bacteria, but there was no growth in the *S pneumoniae* wells.

The resulting mean values were obtained from 3 repeated tests performed under the same conditions with 96-well U-bottom microplates (see Tables 3 and 4).

Clinical isolate testing

In the second step, CIs were evaluated. A row from A to E was reserved on the microplates for each of the 5 bacteria. *E coli* was tested in row A, *P aeruginosa* in row B, *A baumannii* in row C, *B subtilis* in row D and *S aureus* in row E.

No growth was observed in the MC wells. In the RC wells, there was growth of all 5 bacteria.

The reulting mean values were obtained from 3 repeat tests performed under the same conditions with the 96-well U-bottom microplates (see Tables 3 and 4).

Plant extracts have been used for food preservation, medicine, alternative medicine and natural therapies for thousands of years.[14-16] To improve the quality of health services, the plants used in traditional medicine should be investigated scientifically. Plant extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens.[17]

GC-MS analysis of rose essential oil in this study is consistent with the findings of previous studies.[18-22] In 2019, Thakur et al. reported citronellol, geraniol and nonadecane as the major constituents of the essential oil of damask rose.[23] Hindumathy et al., in their study investigating the antibacterial effect of *Rosa indica* extracts,[1] found that Gram-negative bacteria are more sensitive than Gram-positive bacteria. They postulated that this was caused by the different chemical content of the cell wall of various microorganisms.

As a result of these studies, it was understood that plant extracts decreased bacterial growth and that the decrease varies according to the concentration of the extract used. The bacteriostatic effect of 70% ethanol extracts was most prominent in *S aureus*. Studies have shown that plant extracts could be used as antimicrobial agents in the treatment of some diseases caused by pathogens, provided that this is supported by clinical studies.[1]

In another study, the antibacterial effect of *Rosa indica* was investigated with the Agar diffusion method. The study found that methanolic extracts of red roses were more effective against the pathogens used. An inhibitory effect in a 27-mm area against *E coli*, 26-mm area against *P aeruginosa* and 25-mm area against *Staphylococcus aureus* were seen.[24] Acetone extract of rose showed a more effective antimicrobial effect in Gram-positive and Gram-negative bacteria than aqueous extract.[25]

In 2014, Shohayeb et al. found that Gram-positive bacteria, *S aureus*, *B subtilis* and *S pyogenes* were more sensitive than Gram-negative bacteria and had MICs and minimum bactericidal concentrations (MBCs) in the range of 0.125 to 2 mg/ml and 0.5 to 4 mg/ml, respectively.[13]

Ulusoy et al. reported that rose absolute and essential oil contained high levels of phenolics and demonstrated strong antibacterial activity against *E coli* (ATCC 25922), *P aeruginosa* (ATCC 27853), *B subtilis* (ATCC 6633), *S aureus* (ATCC 6538), *C violaceum* (ATCC 12472) and *Erwinia carotovora* (ATCC 39048) strains.[26]

The results of this research showed that rose oil has an inhibitory antimicrobial effect on both Gram-positive (*B subtilis*, *S aureus*) and Gram-negative (*E coli*) bacterial groups.

Different percentages of diluted solutions of rose oil were highly effective on 3 of the bacteria studied (*E coli*, *B subtilis* and *S aureus*) and inhibited the growth of both the standard strains and CIs (see Table 3). The antimicrobial effects of rose oil against the standard strains and CIs of *E coli* were the same. Nevertheless, the resistance of CIs of *B subtilis* and *S aureus* to rose oil solutions were higher compared with the standard strains (see Table 4). Due to their pathogenicity and virulence factors, the antimicrobial effect of rose oil on these bacteria is highly important.

In particular, considering the presence of significant amounts of *S aureus* bacteria in the oral cavity and dental plaque, a diluted solution of rose oil could be evaluated and produced as a mouthwash to be used in the treatment of oral and dental diseases.[27-29] Because Turkey and especially Isparta city produces a significant percentage of the worldwide production of rose oil, a patented gargle obtained from oily rose water would create an important economic market for Turkey and the Isparta region.[4]

In addition, the inclusion of rose oil extract in toothpaste and other oral-dental products may help prevent bad breath and also protect oral and dental health. Thus, it would be useful to investigate this rose oil feature, which seems to be an important antimicrobial agent for oral flora, with dental health examinations.

B subtilis, which was inhibited by a minimal concentration of 15.62 µl/ml and 31.25 µl/ml of rose oil dilutions for its standard strain and CI, respectively (see Table 4), is a saprophytic bacterium, but it may cause eye inflammation such as panophthalmia and iridocyclitis as a result of direct penetration into the tissue and especially into the eye.[30] Also, microorganisms of the genus *Bacillus* are one of the most important factors in aggressive endophthalmitis, which develops after trauma. The culture results in cases of endophthalmitis due to the genus *Bacillus* show *B subtilis* with different *Bacillus* species.[31,32] In this study, *B subtilis* was found to be inhibited by microdilutions of 2-6 and 2-5 of rose oil for its standard strains and CIs, respectively, and was found to have a high antimicrobial susceptibility to rose oil (see Table 3).

In this context, it has been theorized that rose oil may be used as eye drops in *B subtilis*-induced eye inflammations and especially in the early stages of post-traumatic endophthalmitis (provided that this is supported by clinical trials). It has been predicted that rose oil solution eye drops may be a 100% natural, high value-added patented new product if it is supported by epidemiologic data and clinical findings.

CONCLUSIONS

In conclusion, it has been shown that rose oil has high antimicrobial activity against *E coli, B subtilis* and *S aureus* bacteria and may have the potential to be used as an alternative to antibiotics in infections caused by these bacteria. In addition to its natural therapeutic effect, it has been found that rose oil could be used in the prevention of infections caused by pathogenic bacteria, especially with its natural composition and high antibacterial effect. Consequently, natural antimicrobial rose oil, which could be considered an alternative to the usual prevention and treatment of bacterial infections, may have beneficial effects in human as well as environmental health by preventing the increase of antibiotic-resistant bacterial species.

This study is preliminary and it's aim was to investigate the effect of rose oil on different bacterial species. The mechanism of the antimicrobial activity of rose essential oil on different microorganisms should be evaluated in further studies.

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CONFLICT OF INTEREST

None.

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