



## Research Article

# CONTROL OF OPPORTUNISTIC BACTERIA USING AQUEOUS AND ETHANOL EXTRACTS OF *Rosa damascena*

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**Abstract-** Multidrug resistance in certain bacteria that has clinical significance has seen a tremendous increase in recent times due to abuse or improper use of antibiotics in recent times. Certain species of *Staphylococcus* that cause skin and soft tissue infections are of a great challenge to researchers to design antibiotics for their management such as Methicillin Resistant *Staphylococcus aureus* [MRSA] and Methicillin Resistant *Staphylococcus epidermidis* [MRSE]. Such antibiotic resistance in opportunistic bacteria has inspired our research group to extract potential bioactive compounds from natural resources to manage such bacterial infections. In this study the antibacterial activities of aqueous and ethanol extracts of from the petals of *Rosa damascena* against opportunistic bacteria MRSA and MRSE was evaluated. Our findings revealed that aqueous extract of *R. damascena* petals has shown a greater antibacterial activity against *S. aureus* and *S. epidermidis* at 0.1 g/mL when compared to ethanol extracts. Thus, assuring that bioactive compounds from *R. damascena* petals have a potential to serve as alternatives to chemical antibiotics against MRSA and MRSE.

**Keywords-** Opportunistic Bacteria, Methicillin Resistant Streptococcus, Natural Antibiotics, *Rosa damascena* extracts, Natural antimicrobials

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## Introduction

It is widely known that medicinal plants have been used for the treatment of diseases since the early civilizations. Although synthetic chemistry is still considered to be dominant as a method for the discovery and production of novel products for disease prevention and treatment, traditional folk medicine is still used and relied on by nearly 80% of the world's inhabitants [1].

Due to their beauty and fragrance, the members of the genus *Rosa* (Family Rosaceae) are considered to be one of the world's most popular ornamental plants [2]. In specific, *Rosa damascena* Mill. is regarded as one of the most important *Rosa* species because of its beauty, flavor and fragrance industry. *R. damascena* species originally existed as a wild plant in Damascus, Syria, where its name was based on. However, it is now cultivated in various countries around the world [2], one of which is the region of Kurdistan, located in the country of Iraq, the Middle East, where this research was conducted in. One of the major problems that concerns the public health in the present, is bacterial resistance against antibiotics [3]. Henceforth, researchers have been investigating and screening for the discovery of more antimicrobial agents in natural sources [4]. The realization that many infectious pathogenic organisms are developing fast resistance(s) against the currently used drugs has necessitated a search for new, preferably natural sources of antibacterial compounds. The genetic changes in human pathogens during 1990s and in recent times have massively contributed for the outburst of multidrug resistant (MDR) human bacterial pathogens that resulted in the impairment of the control and management strategies for the infections caused by these pathogens [5]. Most importantly, the rise of antibiotic resistant strains is due to the mis-use of antibiotics [6]. In recent times it has been evident that there is a continuous increase in the reports citing the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) in clinical isolates globally which poses a greatest threat for the existing therapeutic approaches as there is a scarcity of new antimicrobial agents for the treatment of these infections [7-8].

Plants and plant parts have been effectively serving as alternative resources for isolation of bioactive components which have therapeutic effects. Phyto-chemicals that are produced exclusively by plants have several medicinal properties such as antimicrobial effects. Phyto-chemicals exhibit structural diversity and biological functionality that is crucial for a potential lead for drug designing [9-11].

Therefore, in this research, we evaluated the antimicrobial activity of aqueous and ethanol extract of *Rosa damascena*, in order to take part in the process of isolating the bioactive products against such infections, that are both more effective, and have less to non-probability of creating any side-effects.

After numerous trials and errors, results were obtained which encompasses the antibacterial activity of *R. damascena*, and its effects on three species of bacteria, which were:

*Escherichia coli*, which refers to a wide range of bacteria that can cause various diseases, such as pneumonia, diarrhea, and urinary tract infections.

*Staphylococcus aureus*, which can cause skin infections, pneumonia, and etc.

*Staphylococcus epidermidis*, which can cause skin infections, endocarditis, pneumonia, and etc. The determination of the antibacterial activity was performed using well diffusion method, and was then compared with specific antibiotics using disc diffusion method. The antibiotics differed according to the bacteria in use; i.e. third generation cephalosporin for *E. coli*. The results are shown in the tables and charts for a better understanding.

## Materials and Methods

### Sample collection

The fresh rose petals of *R. damascena* were collected, washed under tap water and placed in a clean area where the sunlight can reach to dry the petals, for 3 days. The dried petals were then grounded using mortar and pestle and powder was produced. The powder was then placed inside an oven for 20 minutes for reassurance of complete drying. Afterwards, extraction was performed, which was performed using water and ethanol.

### Extract Preparation

For the preparation of aqueous extracts, 5 grams of the rose petal powder mixed with 75 mL of double distilled water and subjected to Soxhlet extraction. The extract was then placed under UV light for elimination of any possibility of contamination. Following which, the extract was concentrated by subjecting to evaporation using Rotavapor. For the preparation of ethanol extracts, 5 grams of the rose petal powder was added to 50 mL of 70% ethanol, and incubated for 72 hours at room temperature. Later, the homogenate was subjected to evaporation using the Rotavapor. Afterwards, both the extracts were stored at 4 degrees Celsius until further use.

### Antibacterial Susceptibility Assay

The antibacterial activity of the two extracts was tested using well diffusion method on Mueller Hinton media. Three species of bacteria, which were *E. coli*, *S. aureus*, and *S. epidermidis* were cultured into the plates respectively. Bacteria that were used in this research were obtained from the laboratory of Komar University of Science and Technology, Sulaymaniah, Kurdistan. Bacteria were sub-cultured from stocks maintained in nutrient broth. All the bacterial samples were maintained according to standard laboratory conditions. The antibacterial activities of the extracts were tested using well diffusion method, which was later compared to antibiotics using disc diffusion method (the antibiotics differed according to the bacteria in use; e.g. third generation Cephalosporin for *E. coli*).

### Results

Our scientific investigation results include the results of the measurement of the inhibition zones of each extract on three species of bacteria which were *E. coli*, *S. aureus*, and *S. epidermidis*. The results of this measurement allow for the evaluation of the quality of each extract as an antibacterial source. In this study, both of the aqueous and ethanol extracts of *R. damascena* using the Soxhlet extraction method showed considerably good results against the two species of bacteria; *S. aureus* and *S. epidermidis* [Table-1] and [Table-2], but with no effect on *E. coli*. Graphical representations are shown in [Fig-1] and [Fig-2].

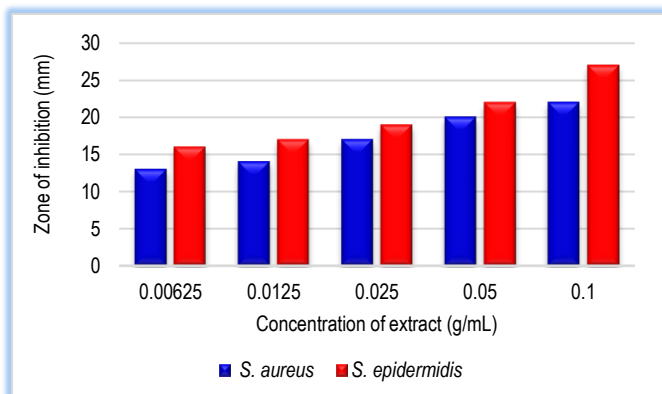


Fig-1 Antibacterial effects of aqueous extract of *R. Damascena* on *S. aureus* and *S. epidermidis*

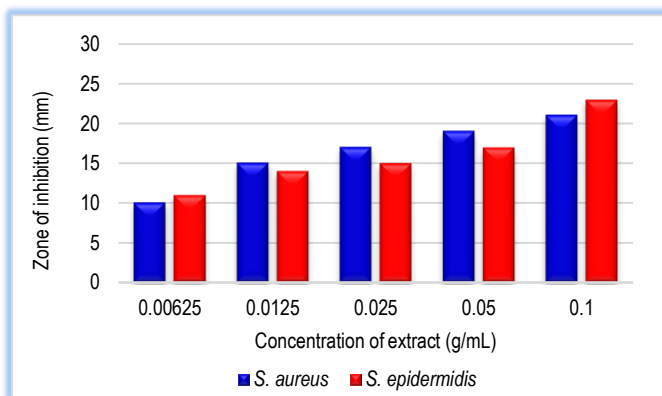


Fig-2 Antibacterial effects of ethanol extract *R. damascena* on *S. aureus* and *S. epidermidis*

Table-1 Antibacterial activity of aqueous extracts of *R. damascena* against *S. aureus* and *S. epidermidis*

Aqueous Extract Antibacterial Activity			
<i>S. aureus</i>		<i>S. epidermidis</i>	
Concentration of Extract [g/mL]	Inhibition zone [mm]	Concentration of Extract [g/mL]	Inhibition zone [mm]
0.00625	13	0.00625	16
0.0125	14	0.0125	17
0.025	17	0.025	19
0.05	20	0.05	22
0.1	22	0.1	27

Table-2 Antibacterial activity of ethanol extract of *R. damascena* against *S. aureus* and *S. epidermidis*

Ethanol Extract Antibacterial Activity			
<i>S. aureus</i>		<i>S. epidermidis</i>	
Concentration of Extract [g/mL]	Inhibition zone [mm]	Concentration of Extract [g/mL]	Inhibition zone [mm]
0.00625	10	0.00625	11
0.0125	15	0.0125	14
0.025	17	0.025	15
0.05	19	0.05	17
0.1	21	0.1	23

Note: Either of the extracts did not exhibit any significant antibacterial effects on the *E. coli*. Antibiotics Amikacin [AK30], Ciprofloxacin [CIP5] were used as positive control for *E. coli* and *Streptococcus* species respectively and Ampicillin [AM10] and Methicillin [ME5] were used as negative control for *E. coli* and *Streptococcus* species respectively

### Discussion

In this study, aqueous extract of *R. damascena* petals using the Soxhlet extraction method showed highest antibacterial activity against *S. epidermidis* with the inhibition zone of 27 mm at 0.1 g/mL of the extract, and an inhibition zone of 22 mm against *S. aureus* [Fig-1] using the same concentration, which even showed a larger inhibition zone than the antibiotic Ciprofloxacin (CIP10), which is an antibiotic commonly used for the treatment of *S. aureus* infections (which also had the largest inhibition zone out of the used controls in this research). While the ethanol extract showed inhibition zones of 23 mm against *S. epidermidis* and 21 mm against *S. aureus* [Fig-2] at a concentration of 0.1 g/mL of the extract. Both types of extracts did not show inhibition zones against *E. coli*, which can be due to several reasons that would require further investigation in order to find out the possible causes which can be of use for further researches.

Aqueous and ethanol extracts of *R. damascena* have been reported to inhibit the growth of *E. coli*, *S. aureus* and *S. epidermidis* [12]. Of both of the extracts, aqueous extracts showed highly significant antibacterial activity and the zone of inhibition ranged from 13-28mm, while ethanol extract showed smaller inhibition zones.

The broad-spectrum antimicrobial activity of the petals of *R. damascena* extracts use in folklore medicine. The petal extract of *R. damascena* may be used for the treatment of skin infections and for the throat infections [13]. The petals of rose varieties may be used against infection caused by pathogenic bacteria that may decrease the use of antibiotics and its ill effects [14].

This study shows that *R. damascena* petals possess chemical constituents which are responsible for its antimicrobial activity, which can be used to minimize the use of antibiotics to an extent. However, further studies are necessary for screening of the volatile and non-volatile active antimicrobial constituents of *R. damascena* petals to utilize them in pharmaceutical industries.

### Conclusion

Plant derived medicines provide a natural source of treatment with greater accuracy than chemotherapeutic agents. Medicinal plants are effective as alternative treatment for infectious diseases and alleviating the side effects associated with synthetic antimicrobials. Our scientific investigation has made it clear that *R. damascena* petal extracts are capable of inhibiting *S. aureus* [MRSA] and *S. epidermidis* [MRSE].

This application can overall decrease the use of chemical antimicrobial agents and improves the scope for the natural product-based medications to control the opportunistic bacterial complications.

**Application of research:** The un-desired side effects of the chemical formulations to manage various bacterial infections has influenced many research groups across the globe to explore various natural resources for extraction of bioactive components to be used as potential leads for pharmaceuticals. Our research strongly suggests to explore alternative medicines and formulations from natural resources like plants.

**Research Category:** Alternative medicines, Phyto-chemicals

**Abbreviations:** g: Grams, mL: Milliliters, mm: Millimeters

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Research project name or number: Research station study

**Author Contributions:** All authors equally contributed

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**Study area / Sample Collection:** Medical Laboratory Science, Komar University of Science and Technology, Sulaymania, 46001

**Cultivar / Variety / Breed name:** *Rosa damascena* Mill

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

Author agree to submit ethical clearance certificate from the concerned ethical committee or institutional biosafety committee, if the project involves field trails/experiments/exchange of specimens, human & animal materials etc.

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