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ASSESSMENT OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ETHANOLIC FLOWER EXTRACTS FROM *ROSA DAMASCENE* AGAINST PATHOGENIC MICRO-ORGANISMS

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Abstract: This study investigates the antimicrobial potential of *Rosa damascena* flower extract, with a focus on its antibacterial and antifungal properties. The primary objective was to assess the inhibitory activity of the ethanolic extract against a spectrum of bacterial and fungal pathogens. Using the agar disc diffusion method, the extract was evaluated at a concentration of 100 µg/ml against two Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), one Gram-negative bacterium (*Escherichia coli*), and four fungal strains (*Aspergillus fumigatus AF293*, *Aspergillus niger ATCC 16404*, *Candida albicans SC5314*, and *Monascus purpureus ATCC 1008*). The zones of inhibition produced by the extract were compared to those of standard antibiotics: *ciprofloxacin* for antibacterial activity and *fluconazole* for antifungal activity. The findings revealed significant antibacterial effects, particularly against Gram-positive bacteria, with clear zones of inhibition, suggesting that *Rosa damascena* harbors a diverse array of bioactive secondary metabolites, the extract demonstrated notable antifungal activity, with inhibition observed across several fungal strains. These results underscore the extract's promising antimicrobial potential, highlighting its efficacy as a source of bioactive compounds, the study suggests that *Rosa damascena* could serve as a valuable resource for the development of novel antimicrobial agents, particularly in light of increasing resistance to conventional antibiotics. Further investigation into the specific mechanisms of action, toxicity, and the isolation of active compounds is warranted to advance its potential as a therapeutic agent in the pharmaceutical industry. The synergistic effects with other natural compounds could enhance its therapeutic efficacy and expand its potential applications in clinical settings.

Keywords: *Rosa damascene,* Ethanolic Extract, Antimicrobial activity, Minimum Inhibitory Concentration (MIC)

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1. Introduction

Rosa damascena (Damask rose) is an important species of the flowering plant genus because of the fragrant smell and uses in medicine and cosmetics as well as in preparation of fragrances (Majedi et al., 2024). It has been cultivated for thousands of years beginning from the Middle East as mentioned by (Bari et al., 2024) It has a unique composition that consists of more than two hundred phytochemicals that include essential oils, flavonoids, and a range of phenolic compounds these components provides the extraordinary fragrance and some other therapeutic value associated with this species. *Rosa damascena* has also been employed in traditional healthcare for its capacity to treat a broad spectrum of ailments due to its impacts on inflammation, oxidation, and antimicrobial profile (Niazi et al., 2023; Wang, 2024).

There has been a rising interest in research related to *Rosa damascena* due to its highly potent antimicrobial activity and its efficiency against different pathogenic microorganisms (Poonia et al., 2024) However, in recent years, the use of plant derived antimicrobial has become increasingly important because of the emergence of antibiotic resistance bacteria, which has led to further research into the agents. *Rosa damascena* is among the few plants which contain a diverse profile of bioactive (DpBC) compounds, and as such has been identified as a potential candidate in this regard (Antoniadou et al., 2024).

Again, antibiotic resistant remains a significant threat to public health for it is gradually making infections that were hitherto easily treatable with standard antibiotics to become more complex to control (Brüssow, 2024). The overly use of antibiotics both in hospitals and in farming has led to the emergence of resistant strains, and therefore, there is growing call for natural antimicrobial compounds that should be used alongside standard

antibiotics or can perhaps be used as a replacement In this regard, plant secondary metabolites have received extensive attention for their antimicrobial activity, and, some investigations have revealed that *Rosa damascena* flower ethanolic extracts exhibited remarkable antibacterial and antifungal activities, These observations collectively indicate that the *Rosa damascena* extracts can be an effective option to control of pathogenic microbes (Antoniadou et al., 2024; Dini et al., 2024).

Furthermore; Plants, including *Rosa damascena*, produce a wide range of secondary metabolites organic compounds that, while not essential for the basic growth and development of the plant, play critical roles in defense mechanisms and reproductive processes, these secondary metabolites are structurally diverse and hold great promise for pharmaceutical applications due to their bioactive properties (Laftouhi et al., 2024; Rahimi et al., 2024). Historically, natural products derived from plants have been instrumental in the development of various medicines, improving public health and contributing to the creation of cost-effective treatments worldwide (Azizi et al., 2023; Chaughule and Barve, 2024) many of these medicines, particularly those used to treat microbial infections, have their origins in the secondary metabolites produced by plants (Niazi and Monib, 2024).

The antimicrobial and antiviral properties of secondary metabolites are well documented, with specific compounds like alkaloids, phenolics, polyphenols, flavonoids, quinones, tannins, coumarins, terpenes, lectins, polypeptides, and saponins showing particular promise (Saini et al., 2024), each of these groups exhibits unique biological activities that can be harnessed for medicinal purposes. For instance, polyphenols have recently been identified to possess antimicrobial effects and have widely applied in the food processing sectors owing to their ability to minimize the usage of chemical preservatives (Kakar et al., 2024; Islam et al., 2025). In the same way, the pharmaceutical industry is also considering polyphenols and other secondary metabolites as possible treatments for infections that result from antibiotic-resistant bacteria (Niazi, 2024). Specifically, the flowers possess important antimicrobial activity, especially *Rosa damascena* which has been used worldwide as an ethnomedical remedy for various ailments and conditions (Oargă et al., 2024). Studies done in the current years have been directed towards the isolation of bioactive compounds from the plant flowers; notably, the attention has been given to their antimicrobial activity (Bhadange et al., 2024). Among these extracts, the ethanolic ones have been reported to be the most active against various pathogenic bacteria and fungi; these results support the traditional uses of *Rosa damascena* and its possible use as a source of new antimicrobial compounds (Abdel-Nasser et al., 2024; Abdel-Malek et al., 2024). Study on *Rosa damascena* determine the antibacterial and antifungal properties of ethanolic flower extracts against important and relevant bacterial and fungal species (Fayaz et al., 2024). The antibacterial potential of *Rosa damascena* as a natural agent is examined using microbiological techniques, such as agar disc diffusion and determination of the extracts' minimum inhibitory concentrations (MIC). (El-Shouny et al., 2016; Trendafilova et al., 2023). Disc diffusion (DD) is one of the methods used in assessing the antibacterial property of plant extracts by measuring the zone of inhibition, which is an aspect of the extract's ability to inhibit the growth of microbes (Golus et al., 2016; Sarwari et al., 2024). The MIC (Minimum Inhibitory Concentration) on the other hand offers a more quantitative approach in determining the lowest concentration of the extract that would help inhibit the growth of a given microorganism (Hafidh et al., 2011).

Consequently, ought the properties of *Rosa damascena* extracts against microbes as stated be proved, the world could envision novel therapies for eradicating microbial infections (Akram et al., 2020; Gupta et al., 2024; Trivedi et al., 2025). The *Rosa damascena* extracts could replace synthetic antibiotics, particularly for microbial infections that are resistant to conventional treatment (Lee et al., 2023). The information about bioactive compounds in *Rosa damascena* may prompt more research. Into other plant species, taking the range of such natural solutions to the global challenge of resistance to antibiotics (Minteguiaga et al., 2023).

Rosa damascena is not only appreciated for its olfactory and ornamental properties, but for its healthy phytochemical content that takes promising potential for the therapeutic usage of the species (Simin et al., 2024), especially in the modern world, which faces the problem of antibiotic resistance (Farooq et al., 2020). The extensive use this plant in the traditional medicine is gradually being supplemented by scientific confirmation and its potential as a source of natural, effective remedies for microbial infections increases while the research on the antimicrobial activity of *Rosa damascena* goes on and while microbial resistance poses one of the major challenges to modern medicine, (Reisi-Vanani et al., 2024). This study aims at assessing the antimicrobial activity of ethanolic *Rosa damascena* flowers extracts against a number of pathogenic microorganisms, mainly bacteria and fungi. Using agar disc diffusion method, the inhibitory ability of the extract against these microorganisms; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Aspergillus niger* and *Candida albicans* were determined. The diameter of the inhibition zones was carefully reported and compared with normal antibiotics in order to explain the mild anticancer property in the plant. These microorganisms can cause various diseases and damage to *Rosa damascena* plants, they not only reduce the quality and yield of this valuable plant but also pose a threat to its cultivation in agricultural and horticultural settings, below is an overview of how these microorganisms can impact *Rosa damascena*.

Bacillus subtilis

While *Bacillus subtilis* is often regarded as a beneficial bacterium for plants due to its role in biocontrol and plant growth promotion, some strains may become opportunistic pathogens under specific conditions, these pathogenic strains can cause soft rot or necrosis in plant tissues by producing enzymes that degrade plant cell walls. Such infections can weaken *Rosa damascena* plants, reducing their vitality and flower production (El-Saadony et al., 2022; Niazi, 2024).

Staphylococcus aureus

Staphylococcus aureus is primarily known as a human pathogen, but its presence on *Rosa damascena* may result from contamination during cultivation, harvest, or storage. While it does not directly cause diseases in the plant, it can form biofilms on plant surfaces, potentially facilitating the growth of other pathogens and reducing the plant's market value, especially in rose-based products like essential oils (Verešová et al., 2024).

Escherichia coli

Escherichia coli contamination in *Rosa damascena* is typically linked to irrigation with contaminated water or improper handling during harvesting and processing. Although it does not directly cause plant diseases, *E. coli* poses a significant threat to the safety of rose-based products, such as oils and extracts that are intended for human use, contaminated products can lead to gastrointestinal infections in humans, making strict hygiene essential in *Rosa damascena* cultivation (Samad and Saeed, 2024).

Aspergillus niger

Aspergillus niger is a common fungal pathogen that can infect *Rosa damascena* plants, especially under conditions of high humidity and poor air circulation. It causes black mold on plant surfaces, particularly on flowers and stems, this fungal infection can lead to reduced flower quality, discoloration, and economic losses. *A. niger* produces mycotoxins that can contaminate rose products, rendering them unsafe for human use (Bi et al., 2024; Wadhwa et al., 2024).

Candida albicans

Candida albicans is primarily a human pathogen and is not a natural plant pathogen, its presence on *Rosa damascena* may result from contamination during processing or handling, especially in moist environments. Though it does not cause direct harm to the plant, it can compromise the microbiological safety of rose-based products and contribute to spoilage during storage (Ghavam, 2024).

Microbial contamination and infection pose a significant challenge in *Rosa damascena* cultivation and processing. While some microorganisms like *Aspergillus niger* directly damage the plant, others such as *Escherichia coli* and *Candida albicans* mainly affect product safety. Implementing proper agricultural practices, hygienic handling, and effective microbial control measures are essential to ensure the quality and safety of *Rosa damascena* products (Ignatov et al., 2024).

2. Materials and Methods

2.1. Preparation of Ethanolic Extract

2.1.1. Collection of plant materials

Volatile oils and bioactive compounds used in this study were extracted from fresh *Rosa damascena* flowers that were harvested during February at their optimal bloom period, at a trusted source: The flowers were harvested early in the morning to ensure they were at their optimal blooming stage, which is widely believed to be the time when the plant has the most potent concentration of volatile oils and other valuable phytochemicals.

2.1.2. Rinsing of Samples

The flowers were collected as soon as possible and were brought to the laboratory to prevent exposure to factors that might affect the degradation of the compounds, before extraction or microbial testing, the *Rosa damascena* flowers that are newly harvested were washed under clean distilled water to eliminate dust, soil or any contaminants that may bear on the extraction process. Special attention was paid unto the flowers during washing to avoid mechanical abrasion that might physically injure the petals and cause leaching of volatile compounds (Monib et al, 2024).

2.1.3. Drying Process

Following the rinse step, the flowers were well arranged to form a single layer on clean drying trays and the drying of the flowers was done under shade since direct sunlight may affect the quality of the phenolics and essential oils. These flower samples were kept in a cool, dry, well-ventilated room with regulated airflow and temperature to minimize degradation of the compounds, the trays used to dry the flowers were kept away from contaminants or any source of moisture to prevent mold/fungal growth during the drying period, the temperature of the room was maintained at a range of 25 to 28°C (Room temperature) because this allowed flowers to dry naturally without exposure to artificial heat and affects its chemical composition.

2.1.4. Duration of Drying

The flowers were allowed to dry for a total period of 12 days, this extended drying time ensured complete removal of moisture content while maintaining the integrity of the plant's bioactive compounds, care was taken to prevent over drying, which could result in the loss of volatile oils and other thermo labile constituents, at the end of the drying period, the flowers were visually inspected to ensure they were completely dry, brittle to the touch, and free from any residual moisture, any flowers that did not meet these criteria were removed from the sample batch to avoid compromising the quality of the subsequent extract (Zhao et al., 2019).

2.1.5. Powder Preparation

Once thoroughly dried, the *Rosa damascena* flowers were manually chopped into small pieces using clean, sterilized scissors, this step facilitated easier grinding and ensured uniform particle size in the final powder form, the chopped flower material was then ground into a fine powder using a mechanical grinder. The grinding

was performed in small batches to avoid overheating of the material, as excessive heat during grinding can lead to the degradation of thermo labile compounds, particularly essential oils and certain phenolics, the grinding process was continued until a homogeneous fine powder was obtained, with particle sizes small enough to maximize surface area for efficient solvent extraction during the subsequent steps (Fathima and Murthy, 2019).

2.1.6. Storage of Powdered Material

The ground flower powder was immediately transferred into sterile, air-tight glass containers to protect it from environmental factors such as moisture, light, and oxygen, these containers were clearly labeled with the date of preparation and stored in a cool, dry, and dark location to further preserve the integrity of the bioactive compounds, the storage temperature was maintained at approximately 4°C to slow down any potential oxidation or degradation processes, the powdered flower material was stored in this condition until further use in the extraction process (Siriamornpun et al., 2012).

2.2. Preparation of plant extract Using Soxhlet Extraction

A 25-gram sample of powdered flowers was combined with 250-ml of ethanol and extracted using a Soxhlet extractor for 72 hours, keeping the temperature below the boiling point of the solvent, the extracts were then incubated at room temperature for 48 hours before being used for further analysis (López-Bascón and De Castro, 2020).

2.2.1. Weighing the Plant Material

A precisely measured 25-gram sample of the previously prepared *Rosa damascena* flower powder (as described in the previous section) was used for the extraction process, the powdered plant material was carefully weighed using an analytical balance to ensure accuracy in the extraction protocol, the powder was stored in a clean, sterile container to avoid contamination until the extraction process was initiated (Woldemichael, 2022).

2.2.2. Preparation of the Solvent

Ethanol (analytical grade, 95%) was chosen as the extraction solvent due to its efficiency in extracting a wide range of polar and non-polar compounds, particularly bioactive components such as phenolics, flavonoids, and essential oils present in the *Rosa damascena* flowers. A volume of 250 mL of ethanol was measured accurately using a graduated cylinder, the solvent was kept in a tightly sealed container to minimize evaporation or contamination before use (Anokwuru et al., 2011).

2.2.3. Soxhlet Extraction Procedure

The extraction of the plant material was performed using a Soxhlet extractor, a widely accepted and efficient method for continuous extraction of plant constituents, the 25-gram sample of powdered *Rosa damascena* flowers was carefully packed into a cellulose extraction thimble, the thimble was placed inside the main chamber of the Soxhlet apparatus (Qader et al., 2022).

2.2.4. Assembly of the Soxhlet Apparatus

The Soxhlet apparatus was assembled, with the extraction thimble placed in the extractor body and the condenser set up at the top of the apparatus, around-bottom flask containing 250 mL of ethanol was attached to the lower part of the apparatus, the flask served as the solvent reservoir and was placed on a heating mantle or water bath (Rajesh et al., 2023).

2.2.5. Extraction Conditions

The Soxhlet extraction was allowed to proceed for a continuous duration of 72 hours to ensure thorough extraction of the bioactive components from the *Rosa damascena* flower powder, throughout the extraction, the temperature was carefully monitored and kept below the boiling point of ethanol (78.37°C) to prevent degradation of heat-sensitive compounds, the heating mantle was adjusted to maintain a gentle reflux of ethanol, allowing the solvent to continuously dissolve and extract the compounds from the plant material, which were then deposited back into the boiling flask upon condensation, during the extraction process, the solvent became increasingly colored as it absorbed the flower's bioactive constituents (Carvalho, 2016).

2.3. Post-Extraction Processing

2.3.1. Filtration of Extract

After 72 hours, the extraction process was stopped, and the Soxhlet apparatus was dismantled. The ethanol extract, now containing dissolved plant compounds, was carefully removed from the round-bottom flask, to eliminate any remaining plant particles or debris, the liquid extract was passed through Whatman No. 1 filter paper into a clean container.

2.3.2. Incubation

The filtered ethanolic extract was incubated at room temperature for 48 hours in a sterile, sealed container, this step allowed the extract to stabilize and helped ensure uniform dissolution of any partially soluble compounds within the solvent (Martín et al., 2022).

2.4. Evaporation of Solvent Using Rotary Evaporator

2.4.1. Evaporation and rotary setup

The solvent is evaporated using a rotary evaporator to obtain a concentrated ethanolic extract, to obtain a concentrated ethanolic extract, the ethanol solvent needed to be removed from the extract, this was accomplished using a rotary evaporator (rotavap), a laboratory device commonly used for efficient and gentle evaporation of solvents under reduced pressure, the filtered extract was transferred to the round-bottom flask of the rotary evaporator, the apparatus was assembled by attaching the flask to the rotavap's rotating arm, and the setup was placed in a warm water bath to facilitate gentle heating (Cheng, 2003).

2.4.2. Temperature Control

The water bath temperature was set to approximately 40°C, which is below the boiling point of ethanol (78.37°C) to prevent degradation of thermolabile bioactive compounds (TBC) in the extract, this low-temperature evaporation process also minimized the risk

of altering the chemical composition of the plant compounds (Amirullah et al., 2021).

2.4.3. Vacuum Application

A vacuum was applied to the rotary evaporator system, lowering the atmospheric pressure inside the apparatus and allowing ethanol to evaporate at a lower temperature, the flask was continuously rotated to increase the surface area of the liquid extract, thereby enhancing the rate of evaporation.

2.4.4. Solvent Removal

Over time, the ethanol vapor was condensed by the condenser and collected in a separate receiving flask, the process continued until most of the ethanol was removed, leaving a concentrated residue of the ethanolic extract in the round-bottom flask, the evaporation process was carefully monitored, ensuring that no excessive heating or prolonged exposure to the rotavap occurred, which could damage the bioactive compounds (Hrubesh et al., 2021).

2.4.5. Collection of Concentrated Extract (CCE)

Once the majority of the ethanol had been removed, the rotary evaporator was stopped, and the concentrated extract was collected. The extract, now in a thick, viscous form, was transferred into a sterile, air-tight glass container using a sterile spatula, the concentrated ethanolic extract was stored at 4°C in a dark, cool environment until further use in subsequent antimicrobial and antifungal activity assays. The final concentrated extract was weighed, and the extraction yield was calculated as a percentage of the initial 25-gram plant sample, the extract was labeled with the date of extraction, concentration, and batch number for proper tracking during subsequent analysis, this detailed preparation method ensured the careful extraction of bioactive compounds from *Rosa damascena* flowers, maintaining their integrity and maximizing their potential efficacy in antibacterial and antifungal activity testing (Acharya and Hare, 2022).

2.5. Statistical Analysis

The study likely utilized a combination of descriptive and inferential statistical methods to evaluate the effectiveness of *Rosa damascena* ethanolic extract against bacterial and fungal strains (Zhang et al., 2024).

2.5.1. Measurement of Zone of Inhibition

The diameters of the zones of inhibition around the discs were measured and recorded.

Descriptive Statistics;

Mean: The average diameter of inhibition zones (calculated from two perpendicular measurements for each plate) was reported for each treatment group (extract, control antibiotics like *Ciprofloxacin*, and antifungals like *Fluconazole*).

Standard Deviation (SD): Likely calculated to quantify the variability of the inhibition zones across replicate tests.

Range: Minimum and maximum zone diameters may have been reported for completeness (Mailu et al., 2021).

2.5.2. Comparative Analysis of Inhibition Zones

To compare the antimicrobial efficacy of *Rosa damascena* extracts with standard drugs (*Ciprofloxacin* and *Fluconazole*):

One-Way Analysis of Variance (ANOVA): If multiple concentrations of the extract were tested, ANOVA could be used to determine if there were statistically significant differences in the inhibition zones among the treatments.

Post-hoc Tests (Tukey's Test): If ANOVA identified significant differences, post-hoc tests would specify which pairs of treatments were significantly different.

t-Test: A paired or independent t-test may have been used to compare the inhibition zones of the extract with standard drugs *Ciprofloxacin* and *Fluconazole* (Seidel et al., 2008).

2.5.3. Minimum Inhibitory Concentration (MIC)

The MIC determination involved a serial dilution technique to find the lowest concentration of the extract that prevented visible microbial growth (Mazzola et al., 2009).

Regression Analysis: A regression model (linear) have been used to analyze the relationship between extract concentration and microbial growth (measured by optical density at 600 nm or visual turbidity).

MIC Value Analysis: The MIC values were reported for each tested microorganism.

Descriptive comparisons of MIC values were made against standard antimicrobial agents (*Fluconazole* and *Ciprofloxacin*) to assess relative potency.

2.5.4. Data Visualization

Bar Charts or Boxplots: Graphical representation of inhibition zones or MIC values for each strain, comparing extract efficacy with standard drugs.

Line Graphs: Used to depict the concentration-dependent effects of the extract on microbial growth during MIC determination (Colclough et al., 2019).

Software and Tools: The statistical analyses have been performed using software such as SPSS, GraphPad, and Excel.

Spectrophotometric data analysis for optical density at 600 nm during MIC determination was likely supported by statistical tools for precision (Tavoosi et al., 2024).

3. Results and Discussion

3.1. Microorganisms tested

3.1.1. Preparation of Inoculum

3.1.1.1. Source of Microbial Cultures

The bacterial and fungal strains were obtained from laboratory-preserved slant cultures stored at 4°C. These slants contain actively growing microbial cells maintained under nutrient-limiting conditions to preserve their viability.

3.1.1.2. Preparation of Inoculum

A loopful of bacterial or fungal culture was aseptically transferred from the slant into a 10 mL test tube containing fresh nutrient broth for bacterial strains and Sabouraud Dextrose Broth for fungal strains, the broth cultures were incubated at 37°C for 24 hours for bacterial strains and 28°C for fungal strains, respectively, to allow optimal growth. After incubation, the bacterial

and fungal suspensions were standardized to achieve the required turbidity using McFarland standards (usually 0.5 McFarland standard equivalent to (1.5 \times 10^8\) CFU/mL), to standardize the inoculum, sterile saline solution or broth was added to dilute the microbial suspensions until the turbidity matched the McFarland standard visually by comparison against a white background (Andrews, 2001; Budiman, 2016).

3.1.1.3. Sterile Swabs and sterilization

Sterile cotton swabs were prepared by winding clean, dry cotton wool onto wooden or plastic applicators. These swabs were packaged in either culture tubes or sterile paper wrappers. For plastic swabs, autoclaving was used (121°C, 15 psi for 15-20 minutes), while wooden swabs could also be sterilized by dry heat (160-170°C for 2 hours) in a suitable oven, sterilized swabs were stored in sterile containers until further use to ensure that they remained uncontaminated. Sterile forceps were essential for placing discs onto the inoculated agar plates. Forceps were sterilized using the flame-sterilization technique: The forceps were dipped in 70% ethanol. After dipping, the ethanol was ignited to burn off and sterilize the forceps, this method ensured that the forceps were free from any microbial contaminants before use (Catalfomo and Schultz, 1966).

3.1.1.4. Inoculation of Agar Plates

Sterile Muller-Hinton agar plates (for bacterial testing) and Sabouraud Dextrose agar plates (for fungal testing) were used as the medium for microbial growth. Each plate was labeled with the corresponding microorganism to be tested (Chroho et al., 2022).

3.1.1.5. Application of Inoculum

A sterile cotton swab was dipped into the standardized inoculum and excess inoculum was removed by gently pressing the swab against the side of the culture tube to eliminate drips and ensure uniform application, the agar plates were inoculated using the streaking method, the swab was streaked across the entire surface of the agar plate three times, each time rotating the plate by approximately 60° to ensure even distribution of the inoculum. After streaking, the swab was passed along the edge of the plate to cover the entire surface, the inoculated plates were allowed to sit undisturbed with the lid closed for approximately (10-15 minutes) at room temperature to allow the inoculum to absorb into the agar surface before the application of discs (Curtis et al., 2004).

3.1.1.6. Disc Preparation and Application

Filter paper discs (6 mm in diameter) were sterilized by autoclaving, each sterile disc was then soaked overnight in a solution containing (100 µg of the ethanolic extract of *Rosa damascene,* each inoculated agar plate was divided into two sections:

In one section, a sterile disc soaked in (100 µg of the ethanolic extract was placed. In the second section, a standard antibiotic disc (containing 10 µg of *Ciprofloxacin* as a positive control for antibacterial tests) was placed using sterile forceps, for antifungal tests, a similar procedure was followed, using *Fluconazole* as the standard antifungal disc.

3.1.1.7. Pre-Incubation Diffusion

Once the discs were placed on the agar plates, the plates were kept at 4°C or room temperature for 1 hour to allow for diffusion of the extracts and antibiotics from the discs into the agar, this pre-incubation step ensured that the compounds from the discs began to interact with the microbial cells before active growth occurred. After the pre-diffusion step, the plates were transferred to an incubator set to 37°C for 24 hours for bacterial strains and 28°C for 48-72 hours for fungal strains, the closed plates were incubated in an inverted position (agar side up) to prevent condensation on the lid, which could interfere with microbial growth. After incubation, the zones of inhibition (clear areas where microbial growth was prevented around the discs) were measured. A ruler, divider, or Vernier caliper was used to measure the diameter of the inhibition zone in millimeters. For accuracy, two perpendicular measurements of the zone diameter were taken, and the average was recorded, the average diameters of the inhibition zones were recorded for both the ethanolic extract and the standard control disc (Deattu et al., 2012).

3.1.2. Assessment of Antifungal and Antibacterial Activates of Rosa Damascene Ethanolic Extracts

3.1.2.1. Source of Microbial Cultures

Fungal strains were obtained from preserved laboratory slant cultures, stored at 4°C to ensure long-term viability, these slants contained actively growing fungal cells, maintained in nutrient-limiting conditions.

3.1.2.2. Preparation of Inoculum

In this experiment, the efficacy of the *Rosa damascena* ethanolic extract on fungal strains was determined by conducting in vitro antifungal susceptibility testing on five different fungal strains (*Candida albicans* SC5314, *Aspergillus niger* ATCC 16404 and *Trichophyton* ATCC 9533). A loopful of the preserved fungal slant culture was streaked or inoculated into a test tube containing a fresh nutrient enriched *Sabouraud Dextrose Broth (SDB)*, suitable for fungal growth (Francis et al., 2024).

3.1.2.4. Incubation and Standardization

The broth culture was further grown at 25-30°C for 24-48 hs so that the fungi develop well enough for identification. After reaching log phase *(Optimal Growth Phase)*, the relative density of the suspension was measured visually and the fungal inoculum was standardized to the 0.5 McFarland turbidity, which is equivalent to approximately (1\times 10^8\) colony-forming units per millilitre). If turbidity of the sample was too high in there, the suspension was diluted by adding sterile saline solution or fresh broth. On the other hand, if the turbidity was very low, more incubation was kept in the water to allow the bacteria to produce turbidity (Gavra et al., 2022).

3.1.2.5. Assessment of Antibacterial Activity of *Rosa damascena* Extracts

In this initial description of the antibacterial potential of *Rosa damascena* ethanolic extracts, inhibition zones were determined around both the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. Preparation of Sterile Swabs: Sterile cotton wool swabs were prepared by tightly winding clean cotton onto sterile wooden or plastic applicators. For plastic swabs, the swabs were sterilized using autoclaving (121°C, 15 psi, for 20 minutes). For wooden swabs, dry heat sterilization was used (160°C for 2 hours). The wooden swabs were placed in sterile culture tubes or sterile paper wrapping to maintain their sterility, the swabs were stored in sterile containers until use to avoid contamination, and the forceps used for handling the discs were sterilized by immersing them in 70% ethanol, followed by immediate flaming to burn off the ethanol. This method ensured aseptic conditions during disc placement Figure 1 (Moussaoui and Alaoui, 2016; Hassand et al., 2024).

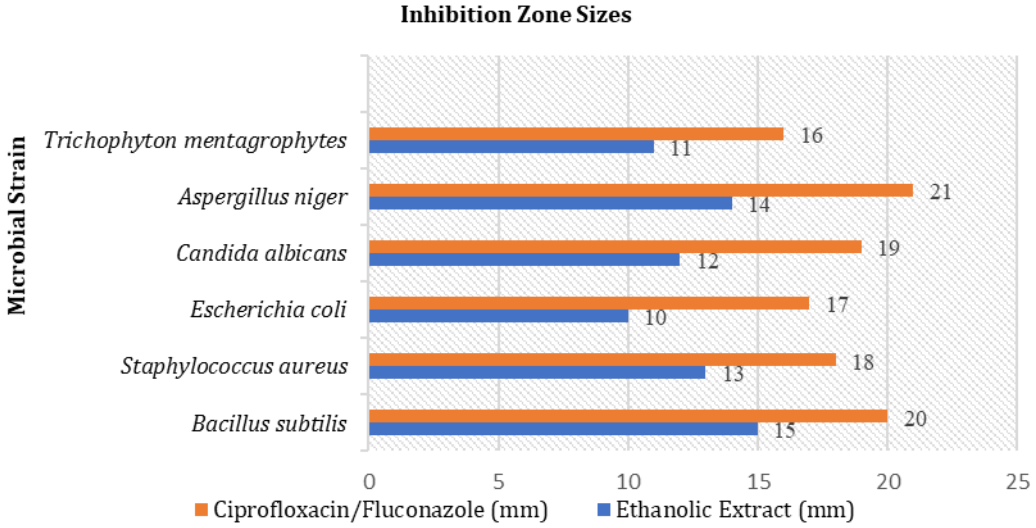

Figure 1. It illustrates the antifungal and antibacterial activity results using a bar chart. Each bar represents the diameter of inhibition zones for different microorganisms, comparing the *ethanolic* extract to the standard control disc (antibiotic and antifungal agent) across various bacterial and fungal strains, enabling a direct comparison of effectiveness.

3.1.2.6. Selection of Fungal Strains for Antifungal Activity Testing

The following fungal strains were selected for the antifungal evaluation: *Candida albicans (SC5314)* - a common yeast responsible for candidiasis, *Aspergillus niger* (*ATCC 16404)* - a filamentous fungus that causes aspergillosis, and *Trichophyton mentagrophytes (ATCC 9533*) - responsible for *dermatophytosis*, these fungi were chosen for their clinical relevance and common pathogenicity.

3.1.2.7. Inoculating the Plates, Agar Medium Preparation and Drying the Inoculated Plates

Sterile Sabouraud Dextrose Agar (SDA) plates were prepared and allowed to cool. SDA provides a nutrient-rich environment conducive to fungal growth, a sterile cotton swab was dipped into the standardized fungal inoculum. Excess inoculum was removed by pressing and rotating the swab against the inner side of the culture tube above the liquid level, the swab was streaked across the surface of the SDA plates in a systematic manner, the streaking was done in three directions, rotating the plate by 60 degrees after each streak, ensuring even distribution of the inoculum, the swab was passed around the edge of the plate for complete coverage of the agar surface, the inoculated plates were left at room temperature for 10-15 minutes with their lids closed, allowing the fungal inoculum to absorb into the agar surface Figure 2 (Saghafi et al., 2021).

3.1.2.8. Procedure for Antifungal and Antibacterial Testing

The standardized fungal and bacterial inoculums were aseptically introduced onto the prepared agar plates using the method described above, ensuring even distribution across the surface, each Petri dish was divided into two sections: In one section, a sterile paper disc (6 mm in diameter) soaked in 100 mg of the *Rosa damascena* ethanolic extract was placed. The discs had been prepared by soaking overnight in the extract solution to enhance potency Figure 3 (Shi et al., 2024).

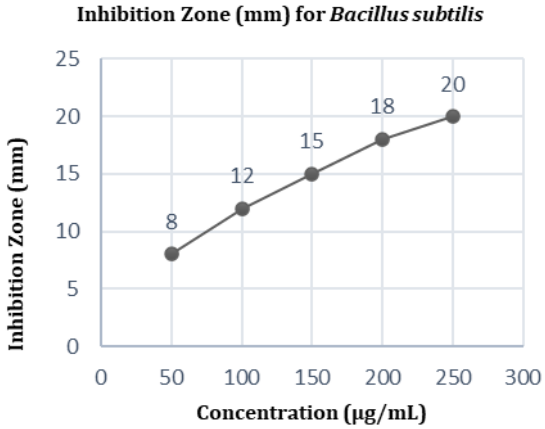

Figure 2. The graphical representation illustrates the relationship between the concentration of the extract (µg/mL) and the inhibition zone (mm) for *Bacillus subtilis*. The X-axis represents the concentration of the extract, while the Y-axis shows the corresponding inhibition zone in millimeters. The trend indicates that as the concentration of the extract increases, the inhibition zone also expands, suggesting a dose-dependent antibacterial effect.

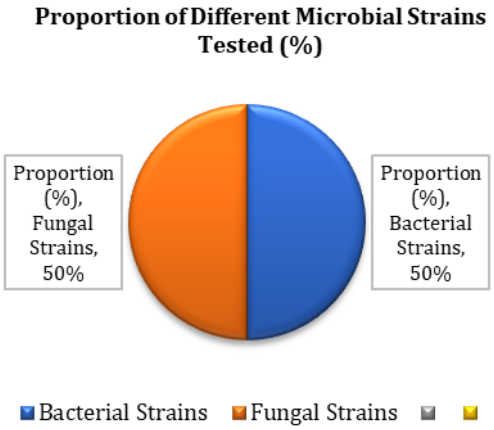

Figure 3. It illustrates the distribution of microbial strains tested for antifungal and antibacterial activity through a pie chart. The chart displays the proportions of strains such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*, providing a clear visual of the microbial diversity in the experimental sample.

In the second section a control disc of *Fluconazole* (20 µg) was incorporated during the antifungal susceptibility testing. In the antibacterial testing, *Ciprofloxacin* (10 µg) was used as the control antibiotic disc. Both extract and control discs were applied onto the plates with the aid of the sterile forceps to prevent contamination. After the discs were placed on the inoculated agar plates, the plates were either: Kept in the refrigerator at 4°C for an hour with slow diffusion or left at room temperature to permit the extract and the standard drugs to diffuse into the surrounding medium. After the diffusion period, the plates were incubated at appropriate temperatures:

For bacterial cultures, plates were incubated at 37°C for 24 hours. For fungal cultures, plates were incubated at 25-30°C for 24-48 hours. The plates were placed in an inverted position (agar side up) during incubation to prevent condensation from dripping onto the agar surface, which could interfere with microbial growth.

3.1.2.9. Observation and Measurement and Data Recording

After incubation, the plates were carefully examined for zones of inhibition (clear zones around the discs where microbial growth was prevented by the extract or control agent), the diameter of each zone of inhibition was measured in millimeters (mm) using a Vernier caliper, ruler, or divider, two perpendicular diameters were recorded for each zone, and the average diameter was calculated, the zones of inhibition were recorded for both the ethanolic extract of *Rosa damascena* and the standard antimicrobial agents (*Fluconazole* and *Ciprofloxacin*), the results were compared to assess the efficacy of the extract in inhibiting the growth of the selected bacterial and fungal strains.

3.1.2.10. Minimum Inhibitory Concentration (MIC) Determination for *Rosa damascena* Ethanolic Extract

The Minimum Inhibitory Concentration (MIC) is a critical measure used to determine the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after incubation. This method involves precise preparation of both the test antimicrobial extract and the microbial inoculum, followed by a series of dilutions to assess the effectiveness of the extract at varying concentrations.

3.1.3. Preparation of Test Drug (Extract) for MIC Testing

3.1.3.1. Stock Solution Preparation

The ethanolic extract of *Rosa damascena* was first prepared by dissolving the dried extract in Dimethyl Sulfoxide (DMSO) or sterile distilled water, this served as the stock solution, the concentration of the stock solution was carefully adjusted to be 1,000 µg/mL by dissolving a precise amount of the extract in the solvent. For instance, to prepare a 1,000 µg/mL stock solution, 100 mg of extract was dissolved in 100 mL of solvent, to establish a range of concentrations for MIC determination, serial two-fold dilutions of the stock solution were performed using Muller-Hinton Broth (MHB) as the diluent, a series of 10 to 15 dilutions were prepared to cover a concentration range from 100 µg/mL to 1.56 µg/mL, this range ensures a broad spectrum to evaluate the antimicrobial potential of the extract.

3.1.3.2. Stepwise Serial Dilution

One milliliter of Muller-Hinton Broth was introduced into each of 10 sterile test tubes with numbers labelled. Tube 1 contained 1 mL from the 1,000 µg/mL stock solution diluted to yield 100 µg/mL in the final solution, 1 mL of the solution in tube 1 was then transferred to tube 2 resulting in another dilution to 50 µg/mL; the process

continued to the final dilution of 1.56 µg/mL in tube 10; in each step capping and shaking the tubes (Shohayeb et al., 2014).

3.1.3.3. Preparation of Inoculum for MIC Testing

From the study the target bacterial strains were *Escherichia coli (K-12), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (PAO1)* and *Bacillus subtilis (168).* Inoculation of each bacterial strain was done in Muller-Hinton Agar (MHA) plates and incubated at 37°C for 24 hours to obtain fresh, pure colonies of the bacteria. In the overnight culture, well isolated colony was picked up and enriched in Muller-Hinton Broth (MHB) at 37°C for 18-24h. After incubation, the bacterial suspension was standardized to match 0.5 McFarland standard, which corresponds to a bacterial density of approximately 1-2 × 10^8 CFU/mL, to achieve this, the overnight culture was diluted using sterile Muller-Hinton Broth to create a 1:1% dilution or 10 ^ -2 molar concentration. This dilution makes the bacterial inoculum in the right concentration for the MIC assay as stated by (Talib and Mahasneh, 2010).

3.1.3.4. MIC Test Setup

Eight sterile test tubes were labeled from 1 to 8. Tubes 1 to 6 were designated for the serial dilutions of the extract. Tube 7 was used for the lowest extract concentration (0.5 mL), and tube 8 served as the negative control. 1 mL of Muller-Hinton Broth was added to each of the first five tubes. Tubes 6 and 7 each received 0.1 mL of the broth.

3.1.3.5. Inoculation of Tubes with Extract: In the first tube (Tube 1), 1 mL of the stock extract solution was added and thoroughly mixed to achieve the highest concentration of the antimicrobial agent (100 µg/mL), From Tube 1, 1 mL of the solution was transferred to Tube 2, mixed thoroughly, and this process was continued for Tube 3 through Tube 6, in Tube 7, 0.5 mL of the diluted extract solution from Tube 6 was added to achieve the lowest concentration for testing, tube 8 was left as a control, containing only the Muller-Hinton broth and inoculum, but no extract. Following the serial dilutions of the test extract, 0.1 mL of the standardized inoculum (10^-2 bacterial suspension) was added to each of the test tubes (Tubes 1 through 7), Tube 8 was treated similarly by adding 0.1 mL of inoculum but served as the growth control, ensuring no antimicrobial agent was present (Waksman and Reilly, 1945).

3.1.3.6. Incubation, Measurement and Conditions

All test tubes were incubated at 37°C for 18-24 hours in a shaking incubator to ensure thorough mixing and optimal growth conditions for the microorganisms, the incubation period allowed sufficient time for the bacterial cells to interact with the different concentrations of the *Rosa damascena* extract and grow in the absence of inhibitory concentrations. After the incubation period, each tube was visually inspected for turbidity, which indicates microbial growth, clear tubes suggest inhibition, while cloudy tubes signify microbial proliferation, for more accurate determination of MIC values, spectrophotometric readings were taken using a spectrophotometer set at an optical density (OD) of 600 nm, the OD value for each tube was recorded to quantify the microbial growth, lower OD values indicate less microbial growth due to the inhibitory effects of the extract, while higher values indicate substantial growth, the MIC is defined as the lowest concentration of the extract that resulted in no visible turbidity (clear solution) or no significant increase in OD compared to the control tube (Tube 8), for example, if Tube 5 (containing 6.25 µg/mL of the extract) is the first tube that appears clear (or shows no significant increase in OD compared to the control), then the MIC of the *Rosa damascena* extract against the tested bacterial strain would be reported as 6.25 µg/mL, the MIC values for each tested microorganism are compared to standard MIC values for known antimicrobial agents (*Fluconazole* and, *Ciprofloxacin*), lower MIC values suggest that the *Rosa damascena* ethanolic extract has strong antimicrobial properties, while higher MIC values indicate lower efficacy (White, 1965).

The ethanolic flower extract of *Rosa damascena* exhibits both antibacterial and antifungal properties, with greater potency against gram-positive bacteria and certain fungal strains, this could be attributed to the presence of bioactive compounds such as flavonoids, terpenes, and phenolic acids in the extract, the antimicrobial properties of these compounds disrupt the cell walls and membranes of pathogens, leading to cell death. For the antibacterial activity screening of the ethanolic flower extract of *Rosa damascena*, bacterial strains including *Bacillus subtilis 168, Staphylococcus aureus ATCC 25923*, and *Escherichia* coli *K-12* were utilized, in the antifungal screening, the following fungi were tested: *Aspergillus niger ATCC 16404, Aspergillus fumigatus AF293, Monascus purpureus ATCC 1008,* and *Candida albicans SC5314*.

Table 1. Antibacterial and Antifungal Properties of the Ethanolic Flower Extract of *Rosa damascena*

Zone of Inhibition (mm)									
Microorganism (strain tested)	Type	Inhibition by Extract	Mechanism of Action	*Rosa damascena* flower Extract (100µg)	Standard *ciprofloxacin* (10µg)	Observation and Measurement	MIC for *Rosa damascena* Extract (µg/mL)	Preparation and Incubation Conditions	Effectiveness
Bacteria									
Staphylococcus aureus	Gram-positive cocci	Significant	-	10 mm	18 mm	Zones of inhibition measured with Vernier caliper; average diameter calculated from two measurements	6.25	Inoculated on Muller-Hinton Agar; incubated at 37°C for 24 hours; standardized inoculum (0.5 McFarland) used for MIC testing	Significant inhibition against Gram-positive bacteria; effective at moderate concentrations
Escherichia coli	Gram-negative rod	Moderate	Outer membrane barriers	8 mm	15 mm	Clear zones around discs measured; lower susceptibility observed for Gram-negative bacteria	12.5	Prepared through serial two-fold dilutions from 1000 µg/mL to 1.56 µg/mL; incubated at 37°C with shaking for 18-24 hours	Moderate inhibition due to outer membrane barriers
Bacillus subtilis	Gram-positive rod	Highly susceptible	-	9 mm	12 mm	Zones accurately recorded and averaged; substantial inhibition at low MIC values	3.12	Stock solution prepared in DMSO; incubated at 37°C for 24 hours; bacterial suspension standardized to 1-2 × 10^8 CFU/mL	Good antibacterial action; effective at low MIC values
Fungi									
Aspergillus fumigatus	Filamentous fungus	Moderate	Increased cell permeability, oxidative damage	13 mm	22 mm	Zones measured, averaged, and compared to *fluconazol*e for assessment of antifungal efficacy	25	Serial dilutions prepared; incubated at 37°C for 18-24 hours in shaking incubator; turbidity visually inspected for MIC	Moderate susceptibility; antifungal effects at higher concentrations
Monascus purpureus	Mold	Mild	Inhibition of metabolic pathways	11 mm	20 mm	Inhibition zones recorded and compared; mild inhibition observed at standard extract concentrations	50	MIC test performed with diluted stock solution; incubated at 37°C; measurements taken at 600 nm OD for accuracy	Lower effectiveness; resistance observed, requiring higher concentrations
Candida albicans	Yeast	Significant	Disruption of cell membrane, inhibition of enzymes	11 mm	13 mm	Measurement of inhibition zones using standard techniques; consistent inhibition pattern observed	12.5	Inoculated on selective agar; incubated at 37°C; MIC readings recorded for OD consistency	Good antifungal activity; effective in inhibiting common pathogenic yeast
Aspergillus niger	Filamentous fungus	Moderate	Oxidative stress, cell wall disruption	10 mm	11 mm	Zones of inhibition observed; moderate inhibition indicating extract's limited potency against filamentous fungi	37.5	Prepared using 1:10 dilutions; inoculated and incubated with appropriate controls; OD measured post-incubation	Moderate inhibition; higher concentrations needed compared to yeast-like fungi

Table 1. Illustrate The *Rosa damascena* ethanolic flower extract generally exhibits better antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. Gram-negative bacteria tend to have lower susceptibility due to the presence of an outer membrane that acts as a barrier to many antibacterial agents. The extract shows significant inhibition against bacterial strains such as *Staphylococcus aureus ATCC 25923*, *Bacillus subtilis 168,* and *Listeria monocytogenes EGD-e*, even at lower MIC values. Resistant strains are also somewhat susceptible to the extract, which suggests its potential in combating drug-resistant bacterial infections. In terms of antifungal activity, the extract's effectiveness is detailed in Table 1, where the micro-organisms listed include, pathogenic fungal species used in the study. The inhibition by the ethanolic extract is categorized as mild, moderate, or significant, depending on the observed level of antifungal activity. The mechanism of action varies, with the extract acting through mechanisms such as increasing cell permeability, causing oxidative damage, or inhibiting metabolic pathways. The zone of inhibition (mm) is measured to

assess the antifungal potency, with larger zones indicating more effective inhibition. The extract showed moderate to significant antifungal activity against the tested strains, with varying mechanisms of action. The standard antifungal agent, *fluconazole*, generally produced larger inhibition zones, indicating its higher potency compared to the plant extract.

The study revealed that the ethanolic flower extract of *Rosa damascena* exhibited the highest antibacterial activity against *Staphylococcus aureus*, with a recorded zone of inhibition measuring 12 mm, indicating a significant capacity to impede the growth of this organism. In contrast, the antibacterial activity against the other bacterial strains, *Bacillus subtilis 168* and *Escherichia coli K-12*, was observed to be moderate, as reflected in the zone of inhibition of 10 mm for both strains during the disc diffusion assay. Notably, the extract produced a relatively similar zone of inhibition across all tested bacterial strains, suggesting consistent antibacterial properties.

This investigation clearly indicates that the antimicrobial activity of the flower extract may vary slightly depending on the bacterial strain being tested. Typically, plant extracts tend to show greater efficacy against Gram-positive bacteria compared to Gram-negative bacteria. In this context, the present study screened the antibacterial effects of *Rosa damascena* flowers, and the results demonstrated moderate activity against all tested bacterial strains when compared to the standard antibiotic, *ciprofloxacin*, this investigation confirms that the flower extract of *Rosa damascena* possesses antibacterial properties against both Gram-positive and Gram-negative bacterial strains, highlighting its potential as a natural antimicrobial agent.

3.1.3.7. Minimum Inhibitory Concentration in Ethanolic Flower Extract of *Rosa Damascena*

The minimum inhibitory concentrations (MIC) of the ethanolic flower extract of *Rosa damascena* were evaluated against three bacterias and four funguses, including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Aspergillus niger, Monascus purpureus*, and *Aspergillus fumigatus*, the results of this study are presented in the accompanying in table 3, which details the MIC values for each of the tested microbial strains.

In this investigation, the minimum concentration at which there was an absence of microbial growth was systematically determined. This critical parameter provides valuable insight into the effectiveness of the ethanolic extract in inhibiting the growth of various pathogenic organisms, by identifying the MIC for each microorganism, the study contributes to understanding the potential of *Rosa damascena* as a natural antimicrobial agent, highlighting its relevance in the development of alternative treatments for infections caused by these bacteria and fungi.

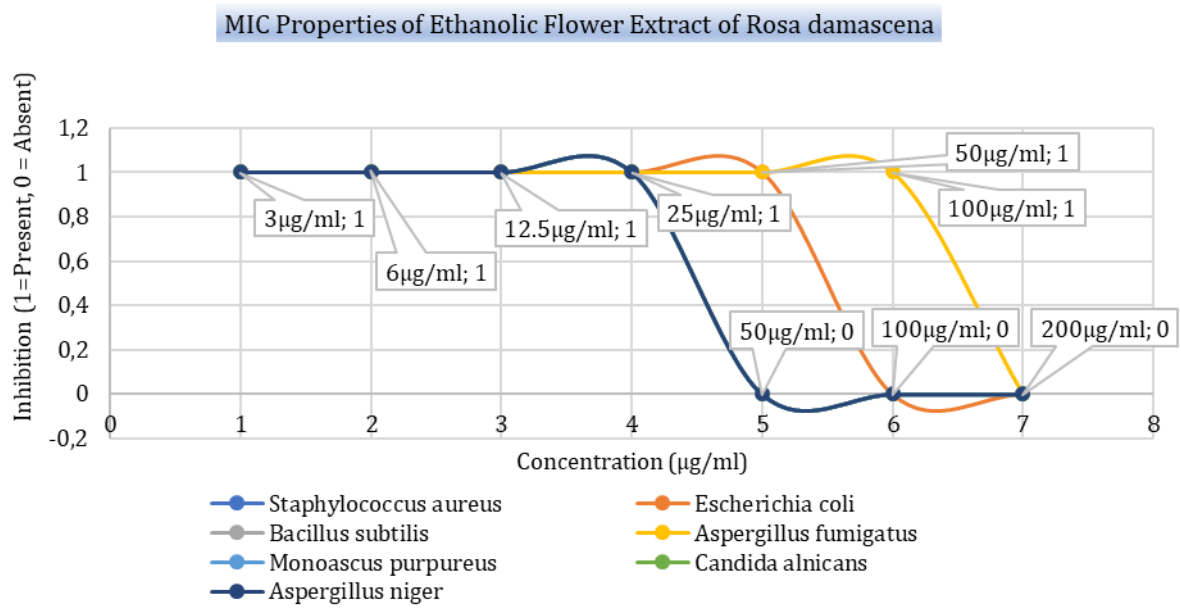

Figure 4. MIC properties of the ethanolic flower extract of *Rosa damascene* against following micro-organism (Pathogen data shown at different concentrations of the substance (in 3-200µg/ml).

Detailed Explanation of Figure 4. Pathogen: Lists the bacterial and fungal species used in the study to assess the MIC of *Rosa damascena* ethanolic extract. Concentrations (µg/ml): Ranges of concentrations tested, from 3 µg/ml to 200 µg/ml. Symbols (+/-): (+) indicates microbial growth, meaning that the concentration of the extract was not sufficient to inhibit growth at that level. (-) indicates no microbial growth, showing that the extract was effective in inhibiting the growth at that concentration. Findings: For *Staphylococcus aureus ATCC 25923* and *Bacillus subtilis 168*, inhibition started at concentrations between 25 µg/ml and 50 µg/ml. *Escherichia coli K-12* required a higher concentration (50 µg/ml) for inhibition. *Aspergillus fumigatus AF293* and

Aspergillus niger ATCC 16404 exhibited inhibition starting at 50 µg/ml. Fungal pathogens *Monascus purpureus ATCC 1008* and *Candida albicans SC5314* showed no growth at 50 µg/ml or higher. This data provides an overview of the antimicrobial efficacy of *Rosa damascena* extracts, demonstrating higher concentrations are needed to inhibit Gram-negative bacteria and certain fungi, while Gram-positive bacteria show susceptibility at lower concentrations).

The present study revealed that antimicrobial properties of ethanolic extract of *Rosa damascena* flowers are effective against a number of different bacteria. Among all the tested microorganisms, *S. aureus, C. albicans, A. niger, Monoscus purpures*, and *Aspergillus fumigates* were found to be highly sensitive to the flower extract. As illustrated in Table 3, their development was nearly suppressed at the concentration of 50µg/ml of the extract. This suggests that at a molecular level, the *Rosa damascena* extract possesses the capability to inhibit these particular bacterial and fungal strains robustly at very low concentrations.

Escherichia coli K-12 and *Bacillus subtilis 168* had the higher tolerance towards the extract, and above this concentration their growth was significantly affected, thus the minimum inhibitory concentration (MIC) of extract against these two bacterial strains was observed to be 100µg/ml. Nevertheless, it is significant to understand that *Rosa damascena* was still capable of exhibiting stronger antimicrobial effect in companionship with these microorganisms but with more concentrated extract.

In the present study, the percentage growth inhibition of the extract at the concentration of 100µg/ml was high both in case of antifungal and antibacterial activities for all the tested microorganisms. Therefore, based on these findings, it can be concluded that the extract derived from *Rosa damascena* flowers displays versatile antimicrobial activity against both bacteria and fungi at moderate concentrations. These results therefore provide credence to the application of this flower extract in the formulation of natural antimicrobial agents especially in addressing infections by both the Gram-positive and Gram-negative bacteria in addition to different species of fungal forms. Focusing more exploration on the properties of the bioactive compounds present in *Rosa damascena* could lead to the discovery of even more effective natural substitutes for stan dard antimicrobial treatments. Thus, by purifying these compounds, the authors could determine particular molecules that would be responsible for the plant's antibacterial activity and be useful in developing effective and eco-friendly methods for dealing with bacteria and mold, which would be especially important given the increase in antibiotic resistance and the need to find new forms of treatment that would not harm the environment.

Table 2. Some of the distinguishing features between MIC, antifungal and antibacterial properties of *Rosa damascena* extract are stated below

Property	MIC Properties	Antifungal Properties	Antibacterial Properties	Comparison
Concentration Range	3 - 200 µg/ml	100 µg/ml	100 µg/ml	MIC concentration for antifungal and antibacterial action varies.
Tested Microorganisms	Bacteria & Fungi	Fungi	Bacteria	Includes both Gram-positive, Gram-negative bacteria, and fungi.
Pathogens	- *Staphylococcus aureus* - *Escherichia coli* - *Bacillus subtilis* - *Aspergillus fumigatus* - *Monascus purpureus* - *Candida albicans* - *Aspergillus niger*	- *Aspergillus fumigatus* - *Monascus purpureus* - *Candida albicans* - *Aspergillus niger*	- *Staphylococcus aureus* - *Escherichia coli* - *Bacillus subtilis*	Specific pathogens tested across both antimicrobial activities.
Mechanism of Action	General inhibition of growth at varying concentrations	- Cell membrane permeability - Oxidative damage - Inhibition of metabolic pathways	Not specified for antibacterial action	Mechanisms for antifungal action are more specific.
Effectiveness by Organism	- Effective at 50 µg/ml against *Staphylococcus aureus, Bacillus subtilis, and Candida albicans* - Higher concentration (100 µg/ml) required for *Escherichia coli* and fungi like *Aspergillus fumigatus and Aspergillus niger*	- Most effective against *Candida albicans* (11 mm zone of inhibition) - Moderate effectiveness against filamentous fungi like *Aspergillus fumigatus and Aspergillus niger*	- Significant inhibition against Gram-positive *bacteria, e.g., Staphylococcus aureus* (12 mm zone of inhibition) - Moderate inhibition for *Gram-negative bacteria, e.g., Escherichia coli*	The effectiveness varies based on microorganism type and concentration used.
Zone of Inhibition	Not measured in MIC studies	Ranged from 10-13 mm for fungi	Ranged from 10-12 mm for bacteria	Zone of inhibition varies by pathogen and extract concentration.
Reference Standards	No reference drug used	Compared to *fluconazole* (20 µg/ml)	Compared to *ciprofloxacin* (10 µg/ml)	No reference drug for MIC properties; antifungal and antibacterial efficacy compared to standard drugs.

3.2. Future Perspectives and Challenges

3.2.1. Another Area of Interest is the Synthesis, Isolation and Identification of New Bioactive Compounds

Thus, the exploration of antibacterial and antifungal properties of *Rosa damascena* flower extracts ushers the world to further research in the extraction of new bioactive compounds. As such, there is infinite scope for the discovery of fresh phytochemical species that may demonstrate new modes of antibacterial activity or may have a different range and spectrum of efficacy. Further studies could include the isolation of the molecules that cause the observed effects to be able to come up with more selective treatments, more variable research could be done on how these compounds work with microbial cells to provide a broader understanding on how these can be used in developing more localized treatments.

3.2.2. Interactions with Other Antibiotics

Because of the growing resistance rate to routine antibiotics, a future research direction is to examine the potential interaction between *Rosa damascena* extracts and conventional antibiotics. Together with plant extracts, chemical antimicrobial drugs could be made more effective, used in smaller concentrations, and have fewer side effects. Future research should try to implement different combinations in clinical trials in order to see if they have the ability to decrease or even halt the further development of antibiotic resistance.

3.2.3. Three Applications: Development of Non-Pharmacological Treatments and Natural Conservants

The outcome of such studies could help to formulate plant derived antimicrobial agents and therapies compared to synthetic antibiotics, especially for resistant strains. Furthermore, more so, the extracts obtained from *Rosa damascena* can also be used as preservatives in food processing and cosmetics since they contain natural antimicrobial properties which consumers are leaning towards in the present world than using chemicals. Other researchers can aim at fine-tuning the extraction techniques and increasing the yield to suit the industrial requirements, and assessing long-term effects of these products.

3.2.4. Standardization and Quality Control have been mentioned as some of the challenges encountered in the development of learning objects

Pronostic and perspectives: Among the numerous problems that must be solved in the future, the issue of establishing an international standard for the extracts of *Rosa damascena* appears to be one of the most significant. Different growing conditions, plant cultivars, harvesting times, and extraction methods can cause differences in the bioactive compound content; therefore, guidelines for how to obtain and measure the effective compounds need to be established. Setting high quality control measures that will be accepted by the regulatory authorities will be paramount for using these extracts in therapeutic and commercial purposes.

3.2.5. Clinical Trials and Toxicological Studies

Often times, when initial studies are conducted on tissue cultures, the results could be very encouraging, but the biggest challenge comes when it has to be developed for human use. Well-controlled clinical trials and meta-analyses are required before asserting the safety, effectiveness, and adverse effects of *Rosa damascene* extracts in clinical populations. These are crucial in the determination of safe dosages, and also the examination of any health risks associated in the periodic use of the product under test. Further studies should also focus on translating the findings from the laboratory to the real-world practices.

3.2.6. Regulatory and Commercialization Hurdles

There is a challenge in the commercialization of *Rosa damascena* extracts as they function as antimicrobial agents that comes with legal constraints. It may take time and go through lengthy and complicated procedures to get approval from the regulatory body for new plant-based medicines on discoveries of naturally occurring substances, there might be issues relating to intellectual property rights. These barriers will need the collaboration of researchers, pharmaceutical companies, and governmental regulatory bodies to tackle after defining the roles of each for the simplification of the process involved all in the appraisal of new drugs without compromising the drug's quality and effectiveness.

3.2.7. Environmental and Economic Sustainability

As the demand for natural antimicrobials increases in the future, the supply of *Rosa damascena* for its production will play a critical role. Excessive fishing and farming practices also have negative impacts on the environment, for example, through soil erosion and loss of biological diversity. Due to this, it is important that subsequent attempts are aimed at producing crops in a manner that is friendly to the environment in order to feed the ever expanding population. Further, studies that will focus on the possibility of large-scale plantations and extraction of *Rosa damascena* will be critical to guarantee the products' market pricing Figure 5.

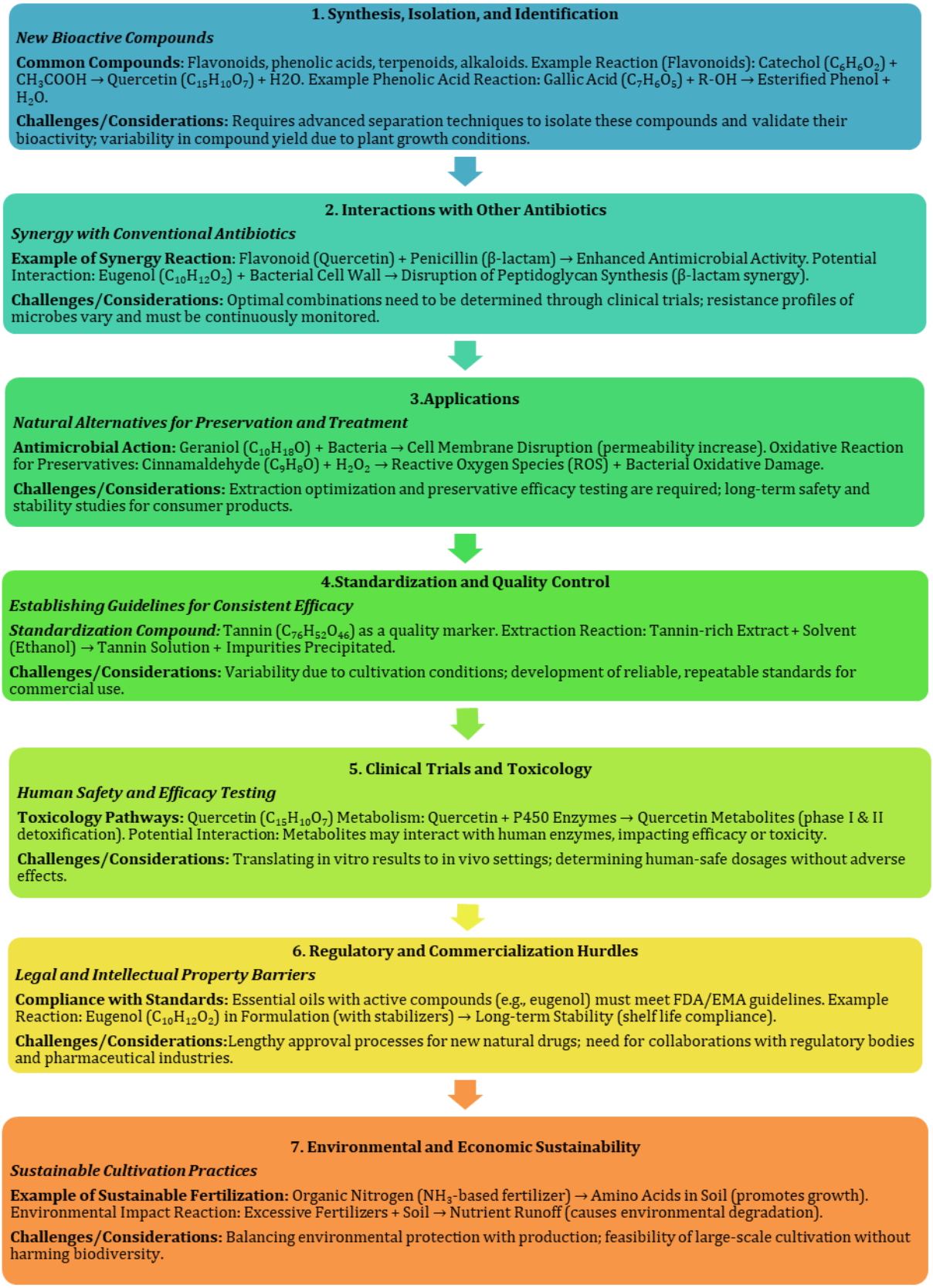

Figure 5. Future prospects for *Rosa damascena* extracts emphasize isolating potent bioactives for innovative antimicrobial applications. Synergizing these extracts with antibiotics could amplify efficacy and mitigate resistance, while their natural preservative properties show promise for food and cosmetic sectors. Rigorous standardization and clinical validations are imperative to ensure therapeutic safety, yet regulatory and commercialization obstacles necessitate strategic industry collaboration. Additionally, sustainable cultivation practices are crucial to align increased production with ecological stewardship.

4. Discussion

The findings of this study confirm the notable antimicrobial potential of the ethanolic extract from *Rosa damascena* flowers, supporting its traditional application in treating microbial infections. The extract demonstrated a broad-spectrum activity against both Gram-positive and Gram-negative bacterial strains, as well as multiple fungal species, which is especially relevant given the current global challenge of antimicrobial resistance. Observing effective inhibition zones in the disc diffusion method and obtaining minimum inhibitory concentration (MIC) values that quantify the extract's potency provide a reliable basis for recognizing *Rosa damascena as* a natural source of antimicrobial agents. This result aligns well with prior studies that have highlighted the medicinal properties of this plant, particularly in folk medicine.

The inhibitory effects of *Rosa damascena* were comparable to standard antibiotics such as *ciprofloxacin* (for antibacterial activity) and *fluconazole* (for antifungal activity). This suggests that the extract contains bioactive compounds capable of exerting effects similar to conventional antibiotics, albeit through potentially different mechanisms of action. The presence of secondary metabolites, as observed in the drastic decrease in bacterial and fungal growth, may account for these effects. Phenolic compounds, flavonoids, and other phytochemicals commonly found in rose extracts are known to disrupt microbial cell walls, inhibit protein synthesis, and impact nucleic acid functions, which could explain the antimicrobial properties observed in this study.

The extract's success in inhibiting growth across diverse microbial species emphasizes its potential as a broad-spectrum antimicrobial agent. Importantly, the extract showed effectiveness against *Staphylococcus aureus ATCC 25923* and *Escherichia coli K-12*, which are common pathogens responsible for various human infections, as well as against *Candida albicans SC5314*, a prevalent fungal pathogen. This wide range of activity could have significant implications for clinical applications, particularly in light of the rise in resistant microbial strains and the limitations of synthetic antimicrobials. Additionally, the findings raise the possibility of developing *Rosa damascena* extracts into topical antimicrobial products, especially for skin infections caused by bacterial and fungal pathogens.

The study also underscores the importance of further investigating the specific bioactive components responsible for the extract's antimicrobial properties. Identifying these compounds could lead to the isolation of new, plant-based antimicrobial agents, with potential applications in both pharmaceutical and cosmetic industries, the study has laid a foundation by demonstrating the antimicrobial efficacy of *Rosa damascena*, future research should focus on the chemical characterization of its active components, the mechanisms of antimicrobial action, and the evaluation of its safety and efficacy in vivo. The synergistic effects of *Rosa damascena* flower extracts with conventional antibiotics could enhance their effectiveness, offering a valuable addition to the fight against resistant pathogens. The observed antimicrobial activity of these extracts shows significant potential for addressing microbial infections and reducing dependence on synthetic antibiotics. Building on the promising findings of this preliminary study, comprehensive in vitro and in vivo investigations are essential to confirm the extract's therapeutic potential. This work adds to the growing body of research supporting the use of medicinal plants as sustainable and effective alternatives in antimicrobial therapy, addressing the urgent global challenge of antimicrobial resistance.

5. Conclusion

The ethanolic extract of *Rosa damascena* flowers demonstrated significant antimicrobial activity against a variety of clinically isolated bacteria and fungi, showing effectiveness comparable to standard antimicrobial drugs, this supports the traditional use of *Rosa damascena* in treating infections caused by microorganisms and highlights its broad-spectrum potential. Both Gram-positive and Gram-negative bacteria, as well as several fungal species, were inhibited by the extract, indicating its applicability as a natural antimicrobial agent. While the findings provide a solid foundation for further exploration of the plant's bioactive compounds, more in-depth research is needed to fully assess the extract's antimicrobial efficacy, ongoing studies are focused on isolating and identifying the specific components responsible for its antibacterial and antifungal properties, which could lead to the discovery of new plant-based antimicrobial agents, the study highlights the extract's potential applications in the pharmaceutical and cosmetic industries for developing natural antimicrobial products, the ethanolic extracts of *Rosa damascena* flowers are expected to exhibit inhibitory effects against the tested bacterial and fungal pathogens, indicating their potential as antimicrobial agents, the MIC and zone of inhibition data will help quantify this activity, the disc diffusion method provided clear zones of inhibition that were measurable and allowed for a comparative assessment of the efficacy of the extract against both bacterial and fungal pathogens, the MIC determination method is essential for evaluating the antimicrobial potency of natural extracts such as *Rosa damascene*, by preparing a series of dilutions and assessing the growth inhibition at different concentrations, the lowest effective concentration can be identified, providing insight into the extract's potential as a therapeutic antimicrobial agent, this process is crucial for both antibacterial and antifungal activity assessments, the assessment of the antibacterial and antifungal activities of ethanolic flower extracts from *Rosa damascena* holds great promise for addressing the growing issue of antimicrobial resistance.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.B.H.	O.A.	P.N.
C	10	10	80
D	50	50	
S			100
DCP			100
DAI			100
L	20	20	60
W	10	30	60
CR	20	20	60
SR		50	50
PM		100	
FA		100	

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declare that the research was conducted without any financial and personal relationships with other people or organization that could inappropriately influence or bias the work.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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The author, Parwiz Niazi, responsible for Conceptualization, Data curation, Formal analysis, Writing-original draft and Supervision. Obaidullah Alimyar responsible for funding acquisition, Software, Validation and Writing-review and editing. Abdul Bari Hejran responsible for Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - review and editing.

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