Exploration of *Myrtus communis* Extract as a Novel Anti-Bacterial Agent in Uncovering Pathogenic Bacteria in Hospital Bioprocessing Units

Dhay J. Edan [1], Raghad Shubbar Jaafar*[2], Munther Abduljaleel Muhammad-Ali,[1] Fadhil Neamah Al-Kanany[2]

1) Ecology Department, Science College, University of Basrah, Basrah, Iraq.
2) Biological Development Department, Marine Science Center, University of Basrah, Basrah, Iraq.

**Corresponding author:* raghad.jaafar@uobasrah.edu.iq

ABSTRACT

The waste of biological treatment plants in hospitals includes many pathological microbes, the rollout of which leads to problems for both the environment and the general health of organisms This study seeks to use environmentally friendly methods to tackle microbial pollution from hospital biologic treatment facilities in Basrah Govenoraten, and to use Elias' (*Myrtus communis*) paper extract as an antibacterial agent. Disc diffusion method has been used to determine a different concentration efficiency of Elias plant leaf extract as an antibacterial agent. The study's findings revealed the efficacy of alcoholic and aqueous extracts of *Myrtus communis* at various concentrations (12.5, 25, and 50 mg/mL) in eradicating pathogenic bacteria isolated from these treatment facilities. The bacteria included *Staphylococcus lentus and Staphylococcus xylosus*, as well as *Sphingomonas paucimobilis* , *Escherichia coli Klebsiella oxytoca* and *Serratia ficaria*, all of which have Gram-positive and Gram-negative bacterial profiles. The diameter of the inhibition zones was used to measure the effectiveness of the extracts, which ranged from 5 to 11 mm. The efficacy of plant extracts against each type of bacteria was greatly influenced by the type of extract used. Inhibition zones in the water extract were observed to be between 5 and 11 mm, with a greater activity against Gram-negative bacteria. Conversely, the alcohol extract showed inhibition zones that were 5 to 9 mm wide, with greater efficacy against Gram-positive bacteria. Based on these findings, it can be concluded that the *Myrtus communis* plant extract is an environmentally friendly method for addressing microbial pollution that originates from hospitals.

Keywords: Hospitals, Pathogenic bacteria, *Myrtus communis*, Basrah

INTRODUCTION

Hospital wastewater (HWW) is distinct from domestic wastewater as it poses hazards and contains infectious elements. Discharges from diagnostic laboratories, operating rooms, radiology departments, and infectious wards are all part of it [1]. Hospital wastewater is loaded with harmful viruses and pathogenic bacteria, along with toxic organic pollutants, radioactive elements, and pharmaceutical compounds, which include antibiotics and psychiatric drugs. The potential danger to public health caused by the presence of numerous pathogenic microorganisms in household wastewater when it is discharged into receiving water systems is highlighted by the presence of several of them [2]. Hospital wastewater (HWW) offers a favorable environment for the growth and proliferation of pathogenic microbes, encompassing bacteria, fungi, viruses, and parasites. Consequently, wastewater generated from HWW treatment plant are regarded as hotspots for infectious microorganisms. the primarily focuses on analyzing the average quantities of microorganisms present in hospital wastewater (HWW), detecting these microorganisms holds immense importance as it allows for an evaluation of potential health risks linked to the treatment and disposal of HWW. The previous study provides specific microbial counts, encompassing total coliforms, fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa*, and *Salmonella* spp. (8.1×107 CFU·g^{-1} and 1.4×106, 3.6×105, 1.6×105, 2.2×105 and 5.5×104 CFU·g^{-1}), each of these types of microorganisms plays a pivotal role in determining the overall safety and suitability of managing HWW. By comprehending their presence and abundance, appropriate measures can be implemented to safeguard both public health and environmental well-being [3].

Ordinarily, it's imperative to manage hospital wastewater (HWW) on-site to minimize the release of pharmaceuticals, pathogens, and antibiotic resistance genes (ARGs). Nevertheless, certain locations continue to opt for the concurrent treatment of HWW alongside community wastewater. In practice, municipal wastewater treatment facilities (WWTPs) are purposefully designed to eliminate common contaminants including fats, oils, organic matter, nitrogen, and phosphorus originating from human waste [4]. Therefore, the conventional methods

employed for wastewater treatment are ineffective in eradicating pharmaceuticals, pathogens, and ARGs. This emphasizes the notion that these compounds could potentially be discharged into the environment alongside the treated wastewater when hospital wastewater (HWW) undergoes municipal wastewater treatment processes. HWW, which encompasses various hazardous materials, is frequently transported to conventional urban WWTPs. Regrettably, there could be deficiencies in appropriately treating the HWW within these WWTPs [5].

The majority of technologies used to treat hospital wastewater (HWW) have been developed with the primary goal of eradicating microorganisms and indicators of pathogens, such as fecal bacteria, *E. coli*, and total coliforms[6]. At present, numerous research efforts are underway to explore diverse approaches for treating wastewater originating from hospital establishments. These approaches frequently involve various combinations of chemical and biological degradation processes in unique configurations [7]. The conventional chemical treatment like coagulation [8]. The predominant biological process like nitrification [9]. Combining enzymes with a root treatment plant or using activated sludge via vermifiltration alone or in combination is a viable option, the use of wood-destroying fungi or different modifications of nanomaterials, sorbents, and associated photocatalysis is speculative [9].

Within the sphere of environmental conservation and sustainable solutions, the assessment of the efficiency of plant extracts in enhancing the biological properties of hospital wastewater has garnered significant attention. Hospitals, being indispensable to healthcare, generate wastewater that often carries a load of contaminants, encompassing pathogenic bacteria. In response to this concern, researchers have directed their attention to the potential of natural remedies, specifically extracts derived from natural plants, for these reasons:- they represent cost effective, have a short processing time, and a limit environmental impact [10]. The use of plant extracts and products derived from them is a valuable resource for treating a range of medical conditions caused by bacterial and fungal pathogens [11]. Different studies dealing with the used the extracted from different parts of plants to treat wide range from pathogenic microorganisms [12]. Indicated the ability of *Myrtus. communis* leaf extract to treat Gram-positive bacterial infections with significant inhibition-zone size (9–25mm) [13]. *Myrtus munis* and *Marrubium vulgare* leaves extracts have antibacterial properties against certain periodontal pathogens. *Aggregatibacter actinomycetemcomitans* and *Eikenella corrodens* was demonstrated and two reference strains of *A. actinomycetemcomitans* [14] using of Piper betle Linn's ethanol extract against gram-positive and gram-negative bacteria, showed the highest antibacterial activity. The minimum inhibitory concentration (MIC) and the minimum bacteria concentration (MBC) of Piper betle Linn ethanol extract against *Salmonella typhimurium* were similar (1562.50mg/L); The highest concentration showed MIC and MBC against *Pseudomonas aeruginosa* 6250mg/L and 12500mg/L respectively [15]. indicated the antibacterial activities of various extracts of *Pinus halepensis* against the *Klebsella pneumar Esherchiia coli Bacilus cereus.* All of these funding of the previous studies open the door to further research, optimization, and potential application of plant-based treatments in hospital wastewater management. The primary objective of the present research is to employ eco-friendly techniques for the treatment of hospital wastewater. This entails using an extract derived from the *Myrtus communis* plant as a bacterial antagonist against pathogenic bacteria that have been isolated from specific hospital biological treatment facilities under examination.

MATERIALS AND METHODS

Sample collection

Samples of wastewater were gathered from the biological treatment plant of the specified hospitals, during the period (December, 2021 and February, 2022), and their exact locations are marked in the table.1. Glass containers with a 500ml volume were employed for collecting the samples. Prior to collection, these containers were sterilized using an autoclave. Subsequently, the samples were kept cool during transportation to the laboratory.

Table 1: Field work location

Stations	Latitude	Longitude
Al-Mawaddah	30º 30′ 1.41″ N	47º 48′ 3.84″ E
Ibn Al-Bitar	30º 29′ 37.56″ N	47º 47′ 39.68″ E
Basrah oil company	30º 3′ 31.75″ N	47º 48′ 30.92″ E
Al-Sadr Teaching	30º 30′ 21.43″ N	47º 51′ 1.51″ E

Collection of Myrtus communis plant

Samples of plants were obtained from household gardens in Basrah Province as a means of confirming their domestic origin and ruling out external imports. The study exclusively utilized plant leaves. After the collection process, the leaves underwent washing to eliminate dust particles. Subsequently, they were left to air dry in a shaded location for a period of one week. Once completely dried, an electric grinder was employed to finely grind the leaves into a powdered form.

Extraction of plant

The plant extract was obtained as follows: A weight of 15g of plant leaf powder was taken and placed in a thimble, which was then positioned in a Soxhlet apparatus to initiate the extraction process. Two types of solvents were used (distilled water and ethanol). The extraction process was initiated by adding 150ml of solvent size to the Soxhlet device and heating it to boiling point. This process continued for 48hrs. The extraction was considered complete when the sample's color changed from green to light yellow, indicating the end of the extraction process. A rotary evaporator was used to evaporate the obtained sample extract, resulting in a concentrated extract of 20 ml. Afterward, this extract was kept at ٢٥°C for two days to undergo the drying process. The equation is used to calculate yield 1: $Y\%=(WeWi)*100$. Where: Y% = represent the yield, We = the dry weight of matter, Wi = the weight of powder at the start of extraction process, which is fixed value equal to 15g [16].

Gas chromatography–mass spectrometry (GC–MS) analysis of extracted plant sample

The gathered samples underwent analysis using GC-MS, followed by subsequent testing at the Nahr Bin Umar laboratories. The GC-MS analysis was executed at the Basrah Oil Company Laboratory, utilizing an Agilent Technologies 7890B GC system in conjunction with an Agilent Technologies 5977A Mass Selective Detector (MSD) that featured an Electron Impact (EI) signal detector. The stationary phase employed was HP-5ms, consisting of 5% phenyl and 95% methyl siloxane, with dimensions measuring 30 meters in length, 250 micrometers in diameter, and 0.25 millimeters in thickness. The temperature program for the analysis commenced with an initial setting of 40°C, held steady for 4 minutes, followed by a gradual linear increase of 10°C per minute, ultimately reaching 300°C, and maintained for a duration of 20 minutes. Helium was utilized as the carrier gas at a flow rate of 1 mL/min, and a purge flow of 3 mL/min was also integrated. The sample injection procedure employed a split mode, where the injection temperature was established at 290°C, and a sample volume of 0.5 microliters was injected.

The mass spectrometer settings included maintaining an ion source temperature of 230°C, and the data collection encompassed a mass range spanning from 44 to 650 m/z. To corroborate and confirm the identification of compounds, the collected data underwent analysis against the Nation's Standard Reference Data (NIST) 2020 and 2014 databases.

Bioindicators Bacteria (Total Coliform and Fecal Coliform)

The membrane filtration method (SM and 9222G 9222D) was employed to effectively isolate, identify, and numbering bacterial biomarkers present in samples taken from the hospital's wastewater. Briefly decimal dilution was carried out on the samples (10^{-1} – 10^{-5}).0.1 ml from the last dilution were subsequently plated onto both selective media, (Endo Agar and MFCAgar), which made in accordance with the manufacturer's specifications (Hi media).

The total quantity of bacteria

To assess the total bacterial count in wastewater samples, using sterile distal water a series of decimal dilutions were prepared (10^{-1}–10^{-5}). Then from the final dilution, 0.1 mL was taken and plated onto a total plate count agar medium (Hi media), made in accordance with the manufacturer's specifications. For an overnight period, the plates were incubated at 37 degrees Celsius [16].

Isolation and identification of pathogenic bacteria

The isolation and purification of bacteria from hospital wastewater samples were carried out as follows: After performing a series of decimal dilutions on the samples using sterilized distilled water, from the final dilution 0.1mL (10^{-5}) was taken, and spread onto the surface of nutrient agar medium preparing by following the manufacturer's instructions (Hi media). The plates were then incubated in an incubator at a temperature of 30 degrees Celsius for 24 hours. To purify the colonies, the process of isolating and plating colonies was repeated on new agar media until a pure culture was obtained, characterized by colonies that were consistent in terms of color, shape, and texture. For diagnostic purposes, morphological, microscopic and biochemical tests (Gram stain) were conducted on the isolated colonies. Sample then sent to tested by Vitek II to confirm the diagnosis,

Antibacterial activity test

Diverse concentrations of extracts derived from *Myrtus communis* (aquace and ethanolic) were formulated (12.5, 25, and 50mg/mL) utilizing Dimethyl sulfoxide (DMSO) as the solvent agent. The disc diffusion technique was used to validate the antibacterial efficacy of these extracts from plants. Mueller Hinton agar (Himedia) plates were used to culture the isolated bacterial strains. The disks that were impregnated with varying concentrations of the plant extract suspension were placed on the agar surface and incubated for a duration of 24 hours at 37°C [14].

RESULTS AND DISSCUSSION

Results of GC mass analysis

Table 1,2 illustrates the chemical characterization achieved through Gas Chromatography-Mass Spectrometry (GC-MS) for the aqueous and alcoholic extract of *Myrtus communis.* The tables highlights the

detection of 9 distinct chemical compounds, which were identified through analytical assessment. The identification of these essential compounds was carried out by utilizing their retention time, molecular formula, compound name, and mass spectrum. As can be detected from the tables that the type of extraction plays important role in the type of the identified chemical compound. The high number of detection compound contribute to the effectiveness of plant extract as antibacterial agent, however the detected compound in the present study may differ from compound identify during previous study, and this may illustrated by many causes; This may be related to various factors in the environmental like geography, temperature, length of day, nutrients, precipitation, soil type, as well as to the plants population genetic dynamics [12]. The table data indicates that the compound with the highest proportion was pyrogallol, accounting for 39.3% in comparison to other compounds. This substantial presence could be linked to the potent antibacterial properties exhibited by the Elias-derived extract against pathogenic bacteria [17]. Numerous research studies have documented the antibacterial effects of compounds derived from pyrogallol. In a specific study, pyrogallol demonstrated the second most potent inhibition of growth among 48 polyphenols against periodontopathic bacteria, surpassed only by curcumin. This effect was observed at each of the biofilm and planktonic bacterial growth forms [18]. In a different research investigation, incorporating pyrogallol into antibacterial films created using a blend of sodium alginate and carboxymethyl cellulose was discovered to be further effective in countering *E. coli* and *S. aureus* [19].

Table 2: The chemical structures to the diagnosed compounds that identified by GC- Mass for water extracted Myrtus *communis*.

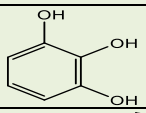
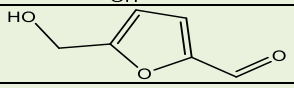
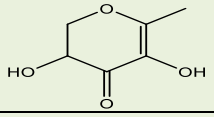
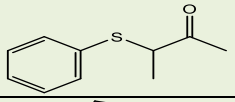
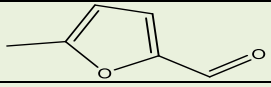
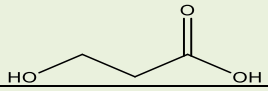
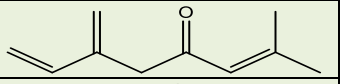
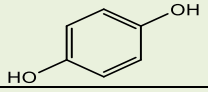
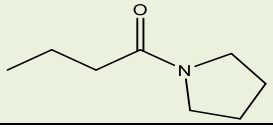
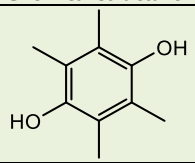
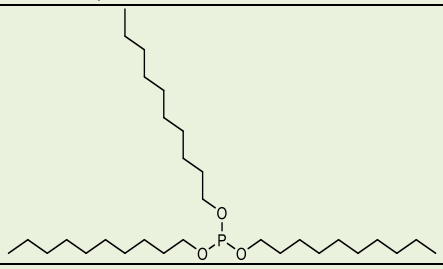
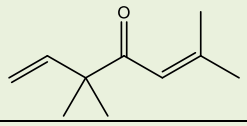
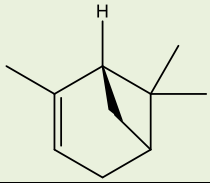
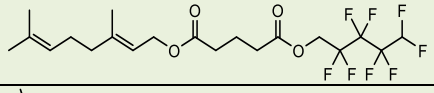
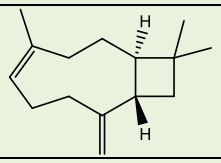
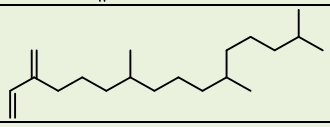
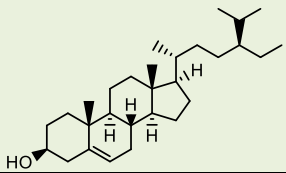
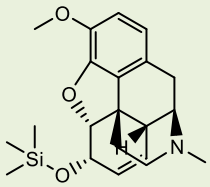
Peak	RT	Area %	Library/ID	Chemical formula	Chemical structure
38	15.9316	39.1388	1,2,3-Benzenetriol	$C_6H_6O_3$	
31	13.8223	19.3397	5-Hydroxymethylfurfural	$C_6H_6O_3$	
25	12.4284	4.8714	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	
41	17.7828	3.6382	3-phenylthio-2-Butanone	$C_{10}H_{12}OS$	
9	9.0652	3.3683	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	
45	18.9924	3.2829	Propanoic acid, 3-hydroxy-	$C_3H_6O_3$	
53	20.7698	2.766	2-Methyl-6-methyleneocta-2,7-dien-4-one	$C_{10}H_{14}O$	
32	14.5451	2.2533	Hydroquinone	$C_6H_6O_2$	
46	19.8406	2.0796	Pyrrolidine, 1-(1-oxobutyl)-	$C_8H_{15}ON$	

Table 3. The chemical structures to the diagnosed compounds that identified by GC- Mass for Alcohol extracted *Myrtus communis*

PK	RT	Area %	Library/ID	Chemical formula	Chemical structure
22	17.6501	14.8852	Durohydroquinone	$C_{10}H_{14}O_2$	
28	19.8848	5.5967	Phosphorous acid, tris(decyl) ester	$C_{30}H_{63}O_3P$	
34	20.8141	5.5194	1,5-Heptadien-4-one, 3,3,6-trimethyl-	$C_{10}H_{16}O$	
44	25.8293	4.6565	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	
45	26.0948	3.8996	Glutaric acid, 2,2,3,3,4,4,5,5-octafluoropentyl geranyl ester	$C_{20}H_{26}F_8O_4$	
15	16.3447	3.8387	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	
35	21.0649	3.2762	Neophytadiene	$C_{20}H_{38}$	
60	33.0571	2.982	beta-Sitosterol	$C_{29}H_{50}O$	
57	30.4389	2.974	Codeine, TMS derivative	$C_{21}H_{29}NO_3Si$	

The total count of the bacteria

Table 4 contain the overall count of isolated bacteria in addition to the total number of the bioindicator bacteria (total Coliform and fecal Coliform) (CFU/ml) in the collected sample from the studied hospital. Results indicated that the most un effective biological treatment plant was in the Al-Sadr Teaching, where the number of bacteria was high (3, 26, 13 CFU/ml), for the bioindicator bacteria and the total plate count respectively. Bioindicator bacteria, such as *Escherichia coli* and *Enterococcus* spp., are widely acknowledged indicators of fecal pollution in aquatic environments. Detecting their presence in hospital wastewater can signal the emission of pathogens into the surroundings, carrying risks for both aquatic ecosystems and public health[20]. The counts observed in this study could pose a risk to the surrounding environment if the water discharged from the hospital under investigation is released without treatment. The bacterial counts in this water exceed international standards. For instance, the US Environmental Protection Agency (US EPA) recommends that the geometric mean of E. coli and Enterococcus in freshwater intended for recreational purposes should not exceed 126 CFU/100mL and 35 CFU/100mL, respectively [21] . Similarly, the WHO recommends that E. coli should not be detected in any water (100mL) meant for human consumption [22] .

Table 4. The total count (CFU/ml) of the bacteria in the studied hospital

Hospitals	Fecal coliform (10^{-5})	Total coliform (10^{-5})	Total plate count (10^{-5})
Ibn Al-Bitar	nil	2	2
Al-Mawaddah	3	nil	nil
Al-Sadr Teaching	3	26	13
Oil	nil	nil	nil

Pathogenic Bacteria

In Table 5, the study showcased the noteworthy pathogenic bacteria identified at the hospital station. The outcomes highlighted the existence of various pathogenic bacteria from distinct phyla. *Pseudomonadota* emerged as the dominant bacterial phylum, while *Staphylococcus lentus* stood out as the most commonly occurring bacterium [23]. identified eight 8 bacterial genera. The bacterial isolates were *Klebsiella, Pseudomonas, Escherichia, Serratia, Staphylococcus, Streptococcus, Proteus and Bacillus* [24]. in his study recorded 7 different pathogenic bacteria, that included *Staphylococcus aureus*, *Escherichia coli* ,*P. aeruginosa* , *S. epidermidis* , *Enterobacter* spp, *S.pyogene* and *Shigel la*.[25], There were 64 strains isolated, and 49 of them were identified to be from different families.: Enterobacteriaceae (e.g., Escherichia coli, Klebsiella sp., Citrobacter sp.) comprised 572% of th e identified bacteria non-Enterobacteriaceae (e.g., Aeromonas sp., Pseudomonas sp.) comprised 30.6%, and Streptococcaceae (e.g., Enterococcus sp.) compri sed 12.2%.

Table 5: Bacteria that were identified and verified in the studied hospitals based on their phylum.

Phylum	Identified Bacteria	Gram stains	Shape and color
Bacillota	*Staphylococcus lentus*	+	Coccia/ white
	Staphylococcus xylosus		
Pseudomonadota	*Sphingomonas paucimobilis*	-	Rod/ yellow
	Escherichia coli	-	Rode/off-white
	klebsiella oxytoca	-	circular /Greyish white
	Serratia ficaria	-	Rode/pink

Antibacterial activity

To assess antibacterial activity of plant extracts studied against pathogenic bacteria, different

concentrations (12.5, 25, and 50mg/mL) was used, and tested against various types of isolated bacteria, including two Gram-positive strains (*Staphylococcus lentus* and *Staphylococcus xylosus*) and several Gram-negative ones (*Sphingomonas paucimobilis*, *Serratia ficaria*, *Escherichia coli*, and *Klebsiella oxytoca*). All concentrations used exhibited good antibacterial activity. The plant extracts probably function as antimicrobial agents this funding is consistent with [26] and [14], acting on bacterial cells through specific mechanisms. They can potentially disrupt the synthesis of cell walls and proteins, while, also affecting the integrity of the cytoplasmic membrane and cellular metabolism [27]. The type of extract plays vital role in the activity of the plant extracts against each type of bacteria (Table 9). The water extract recorded diameter inhibition zone range (5-11mm), with more activity against Gram negative bacteria, while in alcohol extract the diameter range(5-9mm), with high activity against positive ones. The explanation of these finding is related to the differences found in the cell wall of these two class of bacteria[28] , in his study recorded strong inhibitory effect of ethanolic extract of *Myrtus communis* against Gram-positive and acid-fast bacteria, but the growth of gram-negative bacteria was unaffected [13]. Their investigation revealed that the aquatic and methanological extracts of *M. communis* had antibacterial effects on tested strains., i.e., *A. actinomycetemcomitans* and *E. corrodens*. *M. communis*. It has been discovered that leaf extracts possess antibacterial properties against Gram-positive (*Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, and Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, and Campylobacter jejuni*).

Table 6. The antibacterial activity of leaf extracts of *Myrtus communis*

Bacteria	Water plant extract concentration (mg/mL)			Alcohol plant extract concentration (mg/mL)		
	12.5	25	50	12.5	25	50
	Inhibition zone (mm)					
Staphylococcus lentus	5	5	5	6	8	9
Staphylococcus xylosus	5	5	5	7	8	9
Serratia ficaria	8	8	10	5	5	5
Sphingomonas paucimobilis	11	10	5	5	7	7
Escherichia coli	5	5	5	7	8	9
Klebsiella oxytoca	9	9	7	5	5	5

CONCLUSIONS

The study's findings suggest that *Myrtus communis* extract shows promise as an environmentally friendly antibacterial agent for addressing microbial pollution in hospital biological treatment facilities. The extract demonstrated efficacy against various pathogenic bacteria, with different effectiveness depending on whether an alcoholic or aqueous extract was used. Further research is needed to explore the extract's mechanism of action, concentration-dependent efficacy, safety considerations, and practical application in hospital settings. Scaling up production and exploring other environmental applications are also potential areas for future investigation.

ETHICAL ISSUES

No life science threat was practiced in this research.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

AUTHORS' CONTRIBUTIONS

Author 1,2,3 Conceptualization, Methodology, Data Collection, and Writing - Original Draft. Author 1, 2,3,4 Data Analysis, Visualization, and Writing - Review & Editing.
Author Name 2, 3 Supervision, Funding Acquisition, and Project Administration. Each author has read and approved the final manuscript.

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