Trop J Nat Prod Res, March 2024; 8(3):6508-6515 **ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

# Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

# Exploring the Disinfectant Potential of Plant Extracts against Bacterial Strains

Samira Jaouhar[1,2,3]*, Ikrame Zeouk[4], Samiha Dahou[5], Khadija Bekhti[2]

[1]*Higher Institut of Nursing and Health Professions of Fez-Meknes. Regional Directorate of Health Fes-Meknes, El Ghassani Hospital, Dhar El Mehraz, 30000 Fes, Morocco.*
[2]*Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Techniques, Laboratory of Microbial Biotechnology & Bioactive Molecules, PB 2202, Fez, Morocco.*
[3]*Hassan First University of Settat, Higher Institute of Health Sciences; Laboratory of Health Sciences and Technologies; Casablanca Road km 3,5 PB 555 Settat; 26000, Morocco.*
[4]*Laboratory of Pharmacology-Toxicology, Faculty of Medicine, Pharmacy and Dentistry, Sidi Mohamed Ben Abdellah University, PB 2202, Fez, Morocco.*
[5]*Sidi Mohamed Ben Abdellah University, Faculty of Medicine and Pharmacy, Laboratory of human pathology, biomedicine, and environment, Fez, Morocco*

ARTICLE INFO

*Article history:*
Received 21 September 2023
Revised 07 March 2024
Accepted 12 March 2024
Published online 01 April 2024



ABSTRACT

The massive and excessive use of disinfectants has harmful effects on ecosystems and human health. This study aimed to evaluate the potential effect of the methanol extracts of *Peganum harmala*, *Pistacia lentiscus*, *Rubia tinctorum*, and *Nardostachys grandiflora*. Antimicrobial activity was tested against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853, the disinfectant potential was determined using the dilution-neutralization method (NF EN1040/T72-152, 2006). A phytochemical assay was carried out on plant extracts employing the Folin-Ciocalteu method for the quantification of polyphenols, and the aluminum chloride method ($AlCl_3$) was used to determine the flavonoid content. The results revealed the antimicrobial activity of *Peganum harmala* against all the strains, with a minimum inhibitory concentration (MIC) between 1 and 4 mg/mL, followed by *Pistacia lentiscus* and *Rubia tinctorum*, with MICs between 2 and 16 and 4 and 16 mg/ml, respectively. However, only *Peganum harmala* showed significant disinfectant activity, with microbial reduction ranging from 4.66 $\log_{10}$ CFU/mL to 3.19 $\log_{10}$ CFU/mL after 5 minutes of contact. The phytochemical assay revealed a flavonoid content of 79 ± 2.5 µg eq Que/mg E and a phenol content of 72 ± 0.88 µg eq AG/mg E in *Peganum harmala*. *Peganum harmala* has significant potential as a natural disinfectant. Further research should focus on the development of eco-friendly and cost-effective disinfection methods. This would help mitigate the negative impacts of chemical disinfectants on ecosystems and human health.

***Keywords*:** Antimicrobial activity; Disinfectant; Dilution-neutralization methods; *Peganum harmala*; Phenolic compounds.

## Introduction

Some procedures, such as hand hygiene and surface disinfection, are essential for preventing healthcare-associated infections (HAIs) and minimizing their spread.[1,2] These infections can be caused by various pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, significantly impacting human health.[3,4] *Staphylococcus aureus* (*S. aureus*) is a common bacterium that can cause a range of infections, from minor skin and soft tissue to severe bloodstream infections.[5,6] It is a leading cause of HAIs and is known for its ability to develop resistance to multiple antibiotics.[7] Additionally, *S. aureus* can produce toxins and virulence factors that contribute to tissue damage and disease severity.[8] *Escherichia coli* (*E. coli*) remains a normal resident of the human intestinal tract, but certain strains can cause infections, especially in the urinary tract and bloodstream nosocomial.[9,10] Some pathogenic strains of Escherichia coli produce toxins, such as Shiga toxins, which can lead to severe complications like hemolytic uremic syndrome.[11]

*Corresponding author. E mail: jaouharsam@gmail.com
Tel: +21 2688157013

**Citation:** Jaouhar S, Zeouk I, Dahou S, Bekhti K. Exploring the Disinfectant Potential of Plant Extracts against Bacterial Strains. Trop J Nat Prod Res. 2024; 8(3):6508-6515. https://doi.org/10.26538/tjnpr/v8i3.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Antibiotic-resistant strains of *E. coli*, including extended-spectrum beta-lactamase (ESBL)- producing, pose a significant public health threat.[12] *Pseudomonas aeruginosa (P. aeruginosa*) is an opportunistic pathogen that can cause infections in individuals with compromised immune systems or underlying health conditions. It is frequently associated with HAIs, particularly in intensive-care units.[13] *P. aeruginosa* has an inherent resistance to many antibiotics and can form biofilms, which contribute to its persistence and resistance to disinfection.[14]

In addition to the direct effects of these bacteria on human health, their ability to develop resistance to disinfectants is a growing concern.[15,16] The abuse and misuse of disinfectants and a lack of understanding of biosafety principles have contributed to the emergence of bacterial resistance.[15,17] With the emergence of the coronavirus disease 2019 (COVID-19), crucial actions to reduce the risk of viral transmission include wearing face masks, hand hygiene, and surface disinfection.[18,19] However, the excessive and widespread use of disinfectants has produced adverse effects on the environment and users, including asthma and allergic reactions.[20,21] Given the increasing resistance to disinfectants and their detrimental impact on the environment and human health, the search for eco-friendly disinfectants has become crucial. Plants can offer a sustainable solution against microbial resistance.[22,23]

Natural product-based drugs account for 75% of medications, with herbal medicines being more commonly used and many plants exhibiting antimicrobial activity.[24,25] Plants are commonly used in medicine due to their bioactive compounds, such as terpenoids, anthraquinones, alkaloids, flavonoids, and phenolic compounds.[26,27]

These compounds offer interesting solutions against various pathogens (*S. aureus*, *E. coli*, *P. aeruginosa* ...).[27,28] They can damage both the cell wall and the cell membrane, leading to leakage, lysis, and cellular damage. [29] To the best of our knowledge, the investigations of the disinfectant effects of plant extracts are limited and need further exploration. In this context, this study aims to evaluate the antibacterial and disinfectant effects of certain plant extracts against bacteria frequently associated with nosocomial infections.

## Materials and Methods

*Plant Collection and Identification*

In this study, interviews were conducted in 2019 with herbalists in the city of Fez (34°01'59.27"N -5°00'1.01"W), Morocco, to identify potential plants that could be used as disinfectants. Based on the recommendations provided by the herbalists, the selected plants for investigation were *Peganum harmala*, *Pistacia lentiscus*, *Rubia tinctorum*, and *Nardostachys grandiflora* (Table 1). The plants were collected from the Fez-Meknes region in April-June 2021. The voucher specimen of each plant was provided under ID numbers (Table 1).

*Plant Extraction*

The extraction procedure was based on the previously described maceration technique. [23,31] Subsequently, 20 g of the powder from each plant was macerated for 6 hours at 500 rpm with 200 mL of methanol.[32,33] Whatman filter No. 1 was used to filter the resultant mixture, and it was afterward evaporated in a vacuum. The resultant extracts were kept chilled at 4 °C in a refrigerator.

*Antibacterial Testing*

*Selection of bacteria*

Based on the prevalence of bacteria often implicated in community and nosocomial infections,[34,35] the antimicrobial activity of the different prepared extracts was tested against 3 strains namely: *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853.

*Inoculum Preparation*

The microbial inocula was acquired by directly suspending fresh colonies. Specifically, 1 to 2 colonies were suspended in a sterile saline solution (0.9% NaCl) and then compared to a 0.5 McFarland standard to attain a $10^8$ CFU/mL concentration.[32]

*Agar Well Diffusion Method*

Spreading 1 mL of the bacterial inoculum over the Petri dish's agar surface served as the inoculation. Using a sterile tip, an aseptic hole was made, measuring 6 mm in diameter. 80 to 100 µL of each extract solution (50 mg/mL extract + 2% dimethyl sulfoxide (DMSO)/distilled water) was added to each well.[32] The agar plates were then kept in an incubator for 24 hours at 37°C. Imipenem (10 µg/disk) was utilized as the positive control, while distilled water (2% DMSO + 98% water) was used as the negative control. The means were computed after the diameter of the inhibition zones surrounding the wells was measured.

*Determination of the Minimum Inhibitory Concentration (MIC)*

The macrodilution method in a solid medium (before solidification) was employed.[36] Different concentrations of each extract were prepared in 2% DMSO. Subsequently, 1 mL of each dilution was added to a tube containing 9 mL of sterile Luria Bertani (LB) medium to produce a series of concentrations ranging from 16 to 0.5 mg/mL.[33] The mixture was thoroughly mixed and aseptically distributed into Petri dishes. Once the medium solidified, 5 µL spots of the bacterial suspension, adjusted to $10^6$ CFU/mL, were aseptically deposited on the agar surface. A growth control without plant extracts was also prepared. The dishes were then incubated for 24 hours at 37°C.

*Disinfectant Activity of Plant Extracts*

This test aims to determine the reduction in the bacterial count after contact times of 5 minutes and 30 minutes (NF EN 1040/T72-152, 2006) at a temperature of 20°C ± 1°C.

The disinfectant effect of the extracts was tested against four strains according to NF EN 1040/T72-152 (2006) (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *Enterococcus* sp.) using the dilution-neutralization method. After conducting a series of tests to optimize a concentration that can achieve a bacterial reduction close to 5 $\log_{10}$, we selected two concentrations: 76 mg/mL (C1) and 38 mg/mL (C2).

Following the preparation of the bacterial inocula as described above, 0.1 mL of the inoculum was serially diluted in a saline solution up to a dilution factor of $10^{-7}$. Then, 100 µL of the bacterial solution was plated on PCA (Plate Count Agar) agar. After 24 hours of incubation at 37°C, the colonies were counted, and the viable bacterial concentration was expressed as CFU/mL (growth control).

For the disinfectant test, the dilution-neutralization method was employed following the protocol described by St-Pierre et *al*.[37] This method is specifically designed for assessing disinfectants used on surfaces in contact with food and medical devices.[38]

Firstly, the antibacterial activity of the neutralizer needed to be evaluated. In a tube, 100 µL of the bacterial inoculum was combined with 900 µL of the neutralizer solution, consisting of egg or soy lecithin (3 g/L), Tween 80 (30 g/L), L-Histidine (1 g/L), and sodium thiosulfate (5 g/L).[38,39] After a 5-minute contact time, a serial dilution was performed in sterile Eppendorf tubes containing a saline solution. Spreading inoculation (100 µL) on PCA agar was carried out, and after 24 hours of incubation at 37°C, the surviving microorganisms were counted.

Secondly, to assess the effectiveness of the dilution-neutralizer method, 100 µL of the extract (dissolved in 2% DMSO) was added to 900 µL of the neutralizer. After a 5-minute contact time, 900 µL was taken and mixed with 100 µL of a bacterial suspension. This solution was then serially diluted in Eppendorf tubes containing physiological water, and 100 µL of the diluted bacterial solution was inoculated on PCA agar. A colony count was performed after 24 hours of incubation at 37°C.

For each extract, 100 µL of the bacterial suspension was transferred to a sterile Eppendorf tube containing 900 µL of the extract (C1 and C2). After a designated contact time (5 and 30 minutes), 100 µL of each tube was transferred to 900 µL of the neutralizing solution. After a series of physiological water dilutions, PCA Petri dishes were inoculated with 100 µL of the bacterial suspension and incubated at 37°C for 24 hours. The agar colonies were counted and compared to the initial colonies to determine the logarithmic reduction. A commercial disinfectant (Hexanios G + R) was tested as a positive control at a concentration of 0.5%. The logarithmic reduction of bacteria was calculated using the following formula:

X Log10 = -Log10(n/N)

X Log10: Log reduction

n: Number of viable bacteria per mL after contact with the disinfectant

N: Initial number of bacteria per mL

*Phytochemical Assay*

*Total Phenolic Quantification*

The total polyphenol content of the prepared extracts was determined using the Folin-Ciocalteu method.[40] Briefly, 200 µL of each extract (2 mg/mL in methanol) were mixed with 1.5 mL of Folin-Ciocalteu reagent (10%). The mixture was carefully agitated and allowed to react for 5 minutes in the dark. Subsequently, 1.5 mL of 5% sodium carbonate ($Na_2CO_3$) solution was added. After incubating the mixture in the dark at room temperature for 2 hours, the optical density (OD) values were measured at 750 nm using a spectrophotometer (BioTek L800). Negative control was prepared under similar conditions using methanol alone.

**Table 1:** Studied plants

| Plants (*Voucher*) | Family | Used parts |
|---|---|---|
| *Peganum harmala* BLMUP350 | Zygophyllaceae | Seeds |
| *Pistacia lentiscus* BLMUP351 | Anacardiaceae | Leaves |
| *Rubia tinctorum* BLMUP352 | Rubiaceae | Roots |
| *Nardostachys grandiflora* BLMUP353 | Valerianaceae | Whole Plant |

A standard range was established using gallic acid with concentrations ranging from 25 µg/mL to 300 µg/mL, following the same experimental conditions to determine the concentration of phenolic compounds in the extracts. The results were expressed as micrograms of gallic acid equivalent per milligram of dry extract (eq GE/mg of E).

*Total Flavonoid Quantification*

The quantification of flavonoids was performed following the protocol described by Bahorun *et al*.[41] Briefly, 0.5 mL of each extract (2 mg/mL) was mixed with 0.1 mL of aluminum chloride (AlCl3) solution (10%), 0.1 mL of potassium acetate (1 M), and 4.3 mL of distilled water. The mixture was thoroughly agitated and incubated for 30 minutes at room temperature. The OD values were measured at 415 nm using a spectrophotometer. The concentration of flavonoids was determined using a calibration range established with quercetin (25 to 300 µg/mL) under the same experimental conditions. The results were expressed as micrograms of quercetin equivalent per milligram of dry weight extract (eq Que/mg of E).

*Data Analysis*

The results were presented as mean values ± standard deviation (SD), and statistical analyses were performed using ANOVA with IBM SPSS Statistics 21. Differences with a p-value less than 0.05 were considered statistically significant.

## Results and Discussion

The present study aims to assess the antibacterial and disinfectant effects of *P. harmala*, *P. lentiscus*, *R. tinctorum*, and *N. grandiflora*. Among the four plants, the extract of *P. harmala* exhibited the highest activity, with zone diameters of 23.5 mm ± 0.5 for *S. aureus*, 21.5 ± 0.5 mm for *E. coli*, and 26 ± 0 mm for *P. aeruginosa* (Table 2). These findings are consistent with the results reported by Arshad et *al*. and Senhaji et *al*.[42,43] *R. tinctorum* also demonstrated significant activity, with zone diameters of 15.5 ± 0.5 mm against *P. aeruginosa*, 14 ± 1.0 mm for *S. aureus*, and 13.5 ± 0.5 mm for *E. coli* (Table 2). Similar results were reported by Ghafari et *al*.[44] *P. lentiscus* exhibited moderate inhibitory effects against all tested strains, which aligns with the result of Djebari et *al*.[45]

The MIC of *P. harmala* ranged from 1 to 4 mg/mL (Table 3), which is relatively higher compared to the findings of Edziri et *al*. and Senhaji et *al*.[46,47]In our study, the MIC of *P. lentiscus* ranged from 2 to 16 mg/mL, which is consistent with the results reported by Benhammou et *al*. and Djebari *et al.*[48,45] Furthermore, the MIC of *R. tinctorum* ranged from 4 to 16 mg/mL, which agrees with findings from various investigations.[49,50]

The phytochemical assay revealed that the flavonoid content in *P. harmala* was 79 ± 2.5 µg eq Que/mg E and 72 ± 0.88 µg eq AG/mg E for phenols (Table 4). Previous studies have reported similar findings.[51,44,47] The antibacterial activity of *P. harmala* has been attributed to the presence of harmane, an alkaloid that exerts its antibacterial effects through DNA intercalation.[52,53] Several studies have confirmed the high content of phenols, tannins, and flavonoids in *P. lentiscus* leaf extract.[54,55] In the case of *R. tinctorum*, the total phenol and flavonoid contents were approximately 25.16 ± 0.09 µg eq GA/mg E and 21.63 ± 1.25 µg eq Que/mg E, respectively (Table 4). Essaidi et *al*. reported similar results with contents of 38.84 ± 0.6 µg eq GA/mg E and 13.41 ± 0.34 µg eq Que/mg E.[50] Previous research has indicated that the flavonoids in *R. tinctorum* constitute approximately 1.2% of its dry mass.[56] Anthraquinones are the primary components of *R. tinctorum*, and their antimicrobial mechanism has been demonstrated.[31] Moreover, it has been demonstrated that flavonoids possess the ability to hinder several bacterial virulence factors, encompassing quorum-sensing signal receptors, enzymes, and toxins.[57] The antibacterial efficacy of diverse flavonoids can also be attributed to additional mechanisms, such as impeding bacterial energy metabolism, nucleic acid synthesis, and cytoplasmic membrane function.[57]

**Table 2:** Antibacterial activity of plant extracts

| Plant extracts | Bacterial strains | | |
|---|---|---|---|
| | ***S. aureus* ATCC 29213** | ***E. coli* ATCC 25922** | ***P. aeruginosa* ATCC 27853** |
| *Peganum harmala* | 23.5 ± 0.5 | 21.5 ± 0.5 | 26 ± 0.0 |
| *Rubia tinctorum* | 14 ± 1.0 | 13.5 ± 0.5 | 15.5 ± 0.5 |
| *Pistacia lentiscus* | 11.5 ± 0.5 | 12.5 ± 0.5 | 12.5± 0.5 |
| *Nardostachys grandiflora* | _ | 10 ± 0.0 | _ |
| Imipenem (10 µg/disk) | 40 | 28 | 30 |

**Table 3:** The minimum inhibitory concentrations of the active extracts (mg/mL)

| Plant extracts | Bacterial strains | | |
|---|---|---|---|
| | ***S. aureus* ATCC 29213** | ***E. coli* ATCC 25922** | ***P. aeruginosa* ATCC 27853** |
| *P. harmala* | 1 | 4 | 1 |
| *R. tinctorum* | 4 | >16.00 | 4 |
| *P. lentiscus* | 2 | >16.00 | 2 |

**Table 4:** Total phenolic and flavonoid content of the methanolic extracts

| Plant extracts | Total flavonoid contents (µg equivalent of quercetin/mg of extract) | Total phenolic contents (µg equivalent of gallic acid/mg of extract) |
|---|---|---|
| *P. harmala* | 79 ± 2.5 | 72 ± 0.88 |
| *R. tinctorum* | 21.63 ± 1.25 | 25.16 ± 0.09 |
| *P. lentiscus* | 40.44 ± 1.38 | 187.34 ± 0.36 |
| *N. grandiflora* | 176.44 ± 2.65 | 17.91 ± 0.09 |

*P. harmala*, *P. lentiscus*, and *R. tinctorum* exhibited activity against the tested strains in vitro after 24 hours of incubation. However, an effective disinfectant should demonstrate a rapid action spectrum with a contact time of 5 minutes (other durations are possible) and achieve a microbial reduction of 5 $\log_{10}$, corresponding to the elimination of 99.999% of bacterial strains (*S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922,).

The results obtained for *P. harmala* demonstrated a 1.61 $\log_{10}$ CFU/mL reduction (*P. aeruginosa* ATCC 27853) and a 4.98 $\log_{10}$ CFU/mL reduction (*E. coli* ATCC 25922) at 76 mg/mL after 5 minutes of exposure (Figure 1A). When the contact time was extended to 30 minutes, *P. harmala* exhibited a range of bacterial reduction, with values ranging from 1.67 CFU/mL (*P. aeruginosa* ATCC 27853) to 5.98 CFU/mL (*E. coli* ATCC 25922) (Figure 1B). However, at a concentration of 38 mg/mL, reductions in colony log counts were observed between 4.66 $\log_{10}$ CFU/mL and 3.19 $\log_{10}$ CFU/mL after 5 minutes (Figure 2C). These findings indicate that the antimicrobial potential of *P. harmala* was not significantly affected by the concentration and contact time, as no significant differences were observed in the results ($p > 0.05$). Notably, only *P. aeruginosa* ATCC 27853 had a significant impact on bacterial reduction ($p < 0.01$). For *R. tinctorum* extracts, an increased contact time demonstrated a correlation with antimicrobial efficacy ($p < 0.01$). For instance, the reduction in colony log counts increased from 2.06 log10 CFU/mL at 5 minutes to 4.34 $\log_{10}$ CFU/mL at 30 minutes for *S. aureus* ATCC 29213 (38 mg/mL) (Figure 2C, 2D). However, with a concentration of 76/mL, the bacterial reduction could not exceed 2.18 $\log_{10}$ for all bacterial strains (Figure 1A, 1B).

The results for *P. lentiscus* demonstrated a correlation between higher concentrations and increased antimicrobial efficacy. The reduction in colony log counts for *S. aureus* ATCC 29213 increased from 1.17 $\log_{10}$ CFU/mL at 38 mg/mL to 4.44 $\log_{10}$ CFU/mL at 76 mg/mL ($p < 0.05$) (Figure 2C, Figure 1A). Additionally, *P. aeruginosa* ATCC 27853 exhibited a significant impact on colony log reduction values compared to other bacteria ($p < 0.01$). Regarding the commercial disinfectant, a reduction in colony log counts was observed between 5.51 $\log_{10}$ CFU/mL and 4.01 $\log_{10}$ CFU/mL at 5 minutes (Figure 1A). With a contact time of 30 minutes, a reduction of more than 5 $\log_{10}$ was observed for all strains.

Our study revealed the potential disinfectant effect of *P. harmala* extracts. Specifically, at a concentration of 38 mg/mL, this natural extract achieved a reduction ranging from 4.66 $\log_{10}$ CFU/mL to 3.19 $\log_{10}$ CFU/mL within a 5-minute contact time. However, the bacterial reduction for *P. aeruginosa* did not exceed 3.3 $\log_{10}$ at the same contact time. The use of *P. harmala* as a disinfectant in traditional medicine has been previously documented.[58] Phytochemical analyses of the seeds have identified the presence of saponins, tannins, glycosides, alkaloids, anthraquinones, terpenoids, and steroids, which exhibit intercalation with DNA.[52,59,60]

Furthermore, *P. lentiscus* also exhibited significant disinfectant potential, particularly at a concentration of 76 mg/mL, where the reduction in $\log_{10}$ exceeded 5 for *Enterococcus* sp. For *E. coli* and *S. aureus*, the reduction ranged from 3 to 4.44 $\log_{10}$. However, *P. aeruginosa* showed lower sensitivity. The phytochemical analysis confirmed the abundance of phenolic compounds in *P. lentiscus*, suggesting that its antibacterial activity can be primarily attributed to these major constituents.[61] Benhammou et *al*. demonstrated the presence of gallic acid, flavonol glycosides, and anthocyanins in *Pistacia*. sp.[48] The antimicrobial activity of these metabolites has been demonstrated in various studies.[62]

The results obtained with *R. tinctorum* extract demonstrated that increasing the contact time correlated with an increase in the disinfectant effect, although it did not reach the 5 $\log_{10}$ threshold. However, surpassing the concentration of 38 mg/mL is not a viable option. The use of disinfectants at sub-lethal concentrations may lead to the development of antimicrobial resistance.[63] Nevertheless, the disinfectant efficacy of this plant can be optimized by performing acid hydrolysis extraction, as studies have demonstrated an increase in the antimicrobial activity of *R. tinctorum* through acid hydrolysis, which enhances the extraction of various metabolites.[37] The impact of *R. tinctorum* on the strains can be attributed to the presence of anthraquinones.[64]Anthraquinones isolated from plant extracts have demonstrated activity against Gram-negative bacteria, particularly *P. aeruginosa*, and Gram-positive bacteria such as *S. aureus*.[65] The antibacterial mechanisms of anthraquinones are diverse, including cell wall destabilization, alteration of metabolic pathways, and DNA intercalation.[66]

Efforts should be directed toward increasing efficacy within a shorter contact time. One possibility is to enhance antimicrobial activity through combinations with other well-known antimicrobial agents.[67] Another approach to improving antimicrobial effectiveness is the addition of surfactants to the disinfectant. The amphiphilic properties of surfactants grant them the ability to function as solubilizing agents by encapsulating antimicrobial compounds within surfactant micelles, thus aiding in their dispersion in the solution.[37] The polar segments of surfactants facilitate improved interaction with particular cellular components that influence microbial viability.[68] Bolfoni et *al*. (2014) demonstrated that the addition of surfactants like surfynol, cetrimide, and polypropylene glycol led to heightened antimicrobial efficacy in disinfectants.[69]

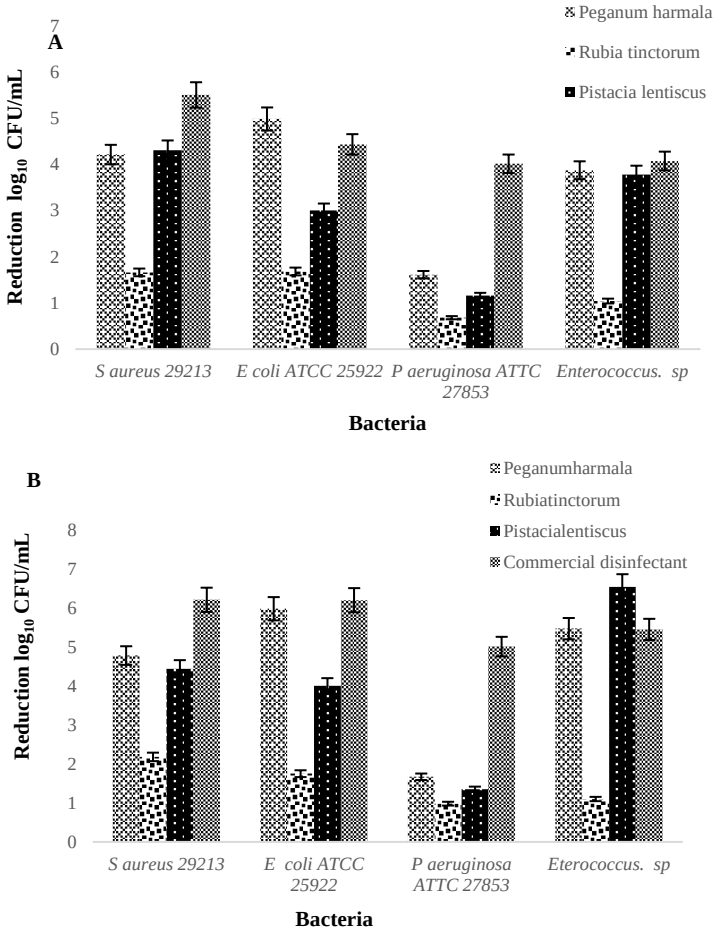


**Figure 1:** Disinfection efficiency of the extracts; Figure 1A: Reduction $\log_{10}$ of bacteria colonies (CFU/mL) according to extracts concentration at 76 mg/mL with contact 5 minutes. Figure 1B: Reduction $\log_{10}$ of bacteria colonies (CFU/mL) according to extracts concentration at 76 mg /mL with contact 30 minutes. The contact time doesn't have significant differences ($p > 0.05$). The bacterial strains had a significant impact on bacterial reduction ($p < 0.01$).

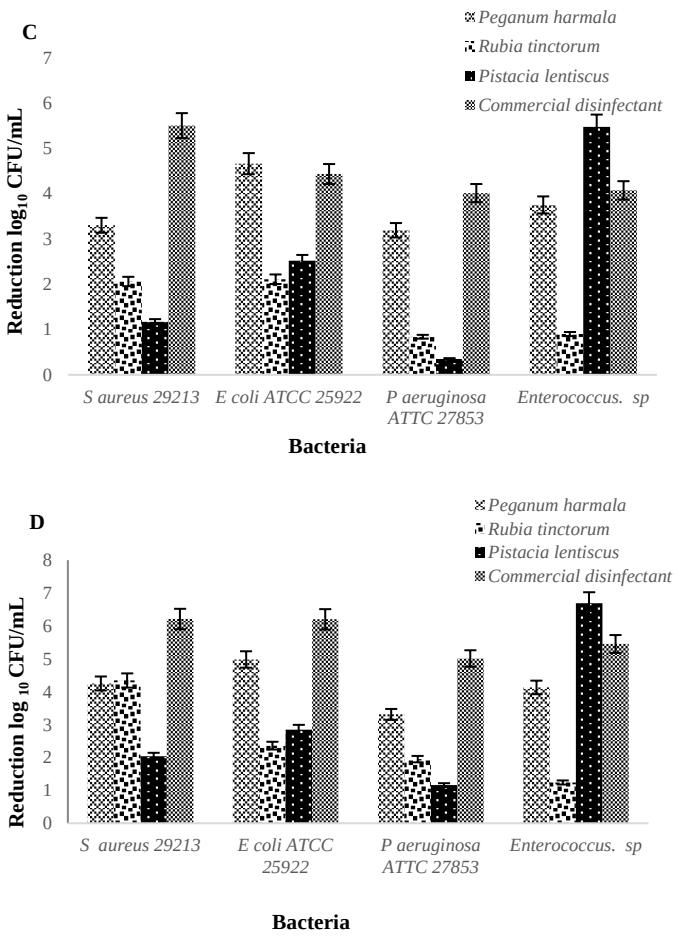


**Figure 2:** Disinfection efficiency of the extracts; Figure 2C: reduction $\log_{10}$ of bacteria colonies (CFU/mL) according to extracts concentration at 38mg/mL for 5 minutes. Figure 2D: reduction $\log_{10}$ of bacteria colonies (CFU/mL) according to extracts concentration 38mg/mL for 30 minutes. The bacterial strains had a significant impact on bacterial reduction ($p < 0.01$).

## Conclusion

The study revealed significant antibacterial activity of plants against various bacteria. P. harmala showed the highest activity, followed by R. tinctorum and P. lentiscus. In terms of disinfectant potential, *P. harmala* achieved a reduction in bacterial strains within a 5-minute contact time but fell short of the desired 5 $\log_{10}$ reduction for *P. aeruginosa. P. lentiscus* exhibited significant disinfectant potential, particularly against *Enterococcus sp*., with lower sensitivity to *P. aeruginosa*. The study suggests improving the efficacy of these plant extracts as disinfectants, considering strategies such as combining them with other antimicrobial agents or incorporating surfactants to enhance effectiveness.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## References

1. Tong C, Hu H, Chen G, Li Z, Li A, Zhang J. Disinfectant resistance in bacteria: Mechanisms, spread, and resolution strategies. Environ Res. 2021; 195: 110897. Doi: 10.1016/j.envres.2021.110897.
2. Assadian O, Harbarth S, Vos M, Knobloch J.K, Asensio A, Widmer A.F. Practical recommendations for routine cleaning and disinfection procedures in healthcare institutions: a narrative review. J Hosp Infect. 2021; 113(2021): 104-114. Doi 10.1016/j.jhin.2021.03.010.
3. Ergonul O, Tokca G, Keske S,Donmez E, Madran B, Kömür A, Gönen M, Can F. (2022). Elimination of healthcare-associated *Acinetobacter baumannii* infection in a highly endemic region. Int J Infect Dis. 2022; 114: 11-14. doi: 10.1016/j.ijid.2021.10.011.
4. Duszynska W, Rosenthal VD, Szczesny A, Zajaczkowska K, and Fulek M. Device associated-health care-associated infections monitoring, prevention, and cost assessment at intensive care unit of University Hospital in Poland (2015-2017). BMC Infect Dis 2020; 20(1): 1-10
5. Khoshnood S, Heidary M, Asadi A, Soleimani S, Motahar M, Savari M, Saki M, Abdi M. A review on mechanism of action, resistance, synergism, and clinical implications of mupirocin against *Staphyloccus aureus*. Biomed Phrmacoter.2019; 109:1809-1818. Doi: 10.1016/j.biopha.2018.10.131.
6. Grousd, J.A, Rich H.E, Alcorn J.F. Host-pathogen interactions in Gram-positive bacterial pneumonia. Clin. Microbiol. Rev.2019; 32(3): e00107-18. Doi: 10.1128/CMR.00107-18.
7. Liu J-Y and Dickter J. Nosocomial Infections: A History of Hospital-Acquired Infections. Gastroenterol Clin North Am. 2020; 30(4): 637-652. Doi: 10.1016/j.giec.2020.06.001.
8. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus*. Virulence. 2021;12(1) :547-569. Doi: 10.1080/21505594.2021.
9. Jiang Z-Q, Wang S D, Feng DD, Zhang B, Mao S, Wu J. Epidemiological risk factors for nosocomial bloodstream infections: A four-year retrospective study in China. 2019. J Crit Care;52:92-96. Doi: 10.1016/j.jcrc.2019.04.019.
10. Ludden C, Coll F, Gouliouris T, Restif O, Blane BSc B, Blackwell GA, Kumar N, Naydenova P, Crawley C, Brown NM, Parkhill J, Peacock SJ. Defining nosocomial transmission of *Escherichia coli* and antimicrobial resistance genes: a genomic surveillance study. Lancet Microbe. 2021 ;2(9): e472-e480. Doi: 10.1016/S2666-5247(21)00117-8.
11. Joseph A, Cointe A, Mariani Kurkdjian P, Rafat C, Hertig A. Shiga Toxin-Associated Hemolytic Uremic Syndrome: A Narrative Review. Toxins (Basel). 2020; 12(2):67. Doi: 10.3390/toxins12020067.
12. Sivakumar M, Abass G, Vivekanandhan R, Anukampa, Singh DK, Bhilegaonkar K, Kumar S, Grace MR, Dubal Z. Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant *Escherichia coli* in street foods: a public health concern. J Food Sci Technol. 2021;58(4):1247-1261. Doi: 10.1007/s13197-020-04634-9.
13. Salmanov A, Vozianov S, Kryzhevsky V, Litus O, Drozdova A, Vlasenko I. Prevalence of healthcare-associated infections and antimicrobial resistance in acute care hospitals in Kyiv, Ukraine. J Hosp Infect. 2019; 102 (4): 431-437.

14. Sharma D, Misba L, & Khan A.U. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control.2019; 8(76). Doi. 10.1186/s13756-019-0533-3.
15. Bragg R, Jansen A, Coetzee M, Westhuizen W, Boucher C. Bacterial resistance to Quaternary Ammonium Compounds (QAC) disinfectants. Adv Exp Med Biol. 2014; 808:1-13. Doi: 10.1007/978-81-322-1774-9_1.
16. Wassenaar T, Ussery D, Nielsen L, Ingmer H. Review and phylogenetic analysis of qac genes that reduce susceptibility to quaternary ammonium compounds in *Staphylococcus* species. Eur J Microbiol Immunol (Bp). 2015; 5(1): 44–61. Doi: 10.1556/EUJMI-D-14-00038.
17. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, Cohen J, Findlay D, Gyssens I, Heuer OE, Kahlmeter G, Kruse H, Laxminarayan R, Liébana E, López-Cerero L, MacGowan A, Martins M, Rodríguez-Baño J, Rolain JM, Segovia C, Sigauque B, Tacconelli E, Wellington E, Vila J. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect.2015; 6: 22-29. Doi: 10.1016/j.nmni.2015.02.007
18. MacGibeny M A, and Margaret W. Preventing adverse cutaneous reactions from amplifed hygiene practices during the COVID-19 pandemic: how dermatologists can help through anticipatory guidance. Arch Dermatol Res. 2021; 313(6) :501-503. Doi: 10.1007/s00403-020-02086-x.
19. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020; 104: 246–251. Doi: 10.1016/j.jhin.2020.01.022.
20. Chen Z, Guo J, Jiang Y, Shao Y. High concentration and high doses of disinfectants and antibiotics used during the COVID-19 pandemic threaten human health. Environ Sci Eur. 2021; 33 (1): 11. Doi:10.1186/s12302-021-00456-4.
21. Dhama K, Patel S K, Kumar R, Masand R, Rana J, Yatoo M.I.T, Tiwari R, Sharun K, Mohapatra RK, Natesan S, Dhawan M, Ahmad T, Emran TB, Malik YS, Harapan H. The role of disinfectants and sanitizers during COVID-19 pandemic: advantages and deleterious effects on humans and the environment. Environ Sci Pollut Res Int. 2021; 28(26) :34211-34228. Doi:10.1007/s11356-021-14429-w.
22. Silva L.N, Zimmer K.R, Macedo A.J, Trentin D.S. Plant Natural Products Targeting Bacterial Virulence Factors. Chem. Rev. 2016; 116: 9162–9236. Doi.: 10.1021/acs.chemrev.6b00184.
23. Riau A.K, Aung T.T, Setiawan M, Yang L, Yam G.H.F, Beuerman R.W, Venkatraman SS, Mehta JS. Surface Immobilization of Nano-Silver on Polymeric Medical Devices to Prevent Bacterial Biofilm Formation. Pathogens. 2019; 8(3):93. Doi:10.3390/pathogens8030093.
24. Newman DJ and Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2014. J Nat Prod. 2016; 79(3): 629–661. Doi:10.1021/np200906s.
25. Badduri N, Gupta NV, Gowda DV, Manohar M. Formulation and development of topical anti-acne formulation of spirulina extract. Int. J. Appl. Pharm. 2018; 10 (6): 229-33. Doi:10.22159/ijap.2018v10i6.26334.
26. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 2019 ; 9(11) :258. Doi:10.3390/metabo9110258.
27. Bhatia P, Sharma A, George A.J, Anvitha D, Kumar P, Dwivedi V.P, Chandra NS. Antibacterial activity of medicinal plants against ESKAPE: An update. Heliyon. 2021; 7(2): e06310. Doi: 10.1016/j.heliyon.2021.
28. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, and Pardesi KR. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front. Microbiol. 2019; 10: 539–563. Doi: 10.3389/fmicb.2019.00539.
29. Harich M, Maherani B, Salmieri S, and Lacroix M. Antibacterial activity of cranberry juice concentrate on freshness and sensory quality of ready to eat (RTE) foods. Food Control. 2017; 75: 134-144. Doi: 10.1016/j.foodcont.2016.11.038.
30. Zeouk I, Sifaoui I, López-Arencibia A, Reyes-Batlle M, Bethencourt-Estrella C. J, Bekhti K, Lorenzo-Morales J, Jiménez IA, Piñero JE. Sesquiterpenoids and flavonoids from Inula viscosa induce programmed cell death in kinetoplastids. Biomed. Pharmacother. 2020; 130: 110518. Doi: 10.1016/j.biopha.2020.110518.
31. Fongang Fotsing Y- S, Bankeu Kezetas J-J, Gaber B, Iftikhar A and Lenta N- B. Extraction of Bioactive Compounds from Medicinal Plants and Herbs. 2022; Chapiter in Natural Medicinal Plants. http://dx.doi.org/10.5772/intechopen.98602.
32. Yeo V.L, Chia Y.Y, Lee C.H, Sheng Sow H, Sum Yap W. Effectivensess of Maceration Periods with Different Extraction Solvent on in-vitro Antimicrobial Activity from Fruit of Momordica charantia L. J App Pharm Sci. 2014; 4(10): 16-23. https://japsonline.com/admin/php/uploads/1153_pdf.pdf.
33. Zeouk I, Balouiri M, Bekhti K. Antistaphylococcal Activity and Phytochemical Analysis of Crude Extracts of Five Medicinal Plants Used in the Center of Morocco against Dermatitis. Int J Microbiol. 2019: 1803102. Doi: 10.1155/2019/1803102.
34. Magill S. S, O'Leary E, Janelle S. J, Thompson D L, Dumyati G, Nadle J, Wilson LE, Kainer MA, Lynfield R, Greissman S, Ray SM, Beldavs Z, Gross C, Bamberg W, Sievers M, Concannon C, Buhr N, Warnke L, Maloney M, Ocampo V, Brooks J, Oyewumi T, Sharmin S, Richards K, Rainbow J, Samper M, Hancock EB, Leaptrot D, Scalise E, Badrun F, Phelps R, Edwards JR. Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals. N Engl J Med. 2018; 379(18):1732–1744. Doi:10.1056/NEJMoa1801550.
35. Saleem Z, Godman B, Azmi Hassali M, Khurshid Hashmi F, Azhar F, Rehman I. Point prevalence surveys of health-care-associated infections: a systematic review. Pathog Glob Health. 2019; 113(4): 191-205. Doi:10.1080/20477724.2019.1632070.
36. Balouiri M, Sadiki M, Ibnsouda S.K. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6(2): 71-79. Doi: 10.1016/j.jpha.2015.11.005.
37. St-Pierre A, Blondeau D, Bourdeau N, Bley J, Desgagné-Penix I. Chemical composition of black spruce (*Picea mariana*) bark extracts and their potential as a natural disinfectant. Ind Biotechnol. 2019; 15 (3): 219-231. Doi:10.1089/ind.2019.0007.
38. Springthorpe VS and Sattar SA. Carrier tests to assess microbicidal activities of chemical

disinfectants for use on medical devices and environmental surfaces. J AOAC Int. 2005; 88(1) :182–201. Doi:10.1093/jaoac/88.1.182.
39. Espigares E, Bueno A, Fernández-Crehuet M, Espigares M. Efficacy of some neutralizers in suspension tests determining the activity of disinfectants. J Hosp Infec. 2003; 55(2) :137-140. Doi:10.1016/S0195-6701(03)00238-X.
40. Ainsworth K.M and Gillespie E.A. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007; 2(4): 875-7. Doi:10.1038/nprot.2007.102.
41. Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittelforschung. 1996; 46(11): 1086-9.
42. Arshad N, Zitterl-Eglseer K, Hasnain S, and Hess M. Effect of *Peganum harmala* or its β-carboline alkaloids on certain antibiotic-resistant strains of bacteria and protozoa from poultry. Phytother Res. 2008; 22 (11): 1533-1538. Doi:10.1002/ptr.2528.
43. Senhaji S, Lamchouri F, Toufik H. Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant *Anabasis aretioïdes* Coss. and Moq. (Chenopodiaceae)", Biomed Res Int. 2020: 16. Doi.: 10.1155/2020/6152932.
44. Ghafari R, Mouslemanie N, Nayal R. Antibacterial activity of *Rubia tinctorum* linn. Root extracts. Int J Pharm Sci Res. 2018; 9(9): 3914-3918. Doi: 10.13040/IJPSR.0975-8232.9(9).3914-18.
45. Djebari S, Wrona M, Boudria A, Salafranca J, Nerin C, Bedjaoui K, Madani K. Study of bioactive volatile compounds from different parts of *Pistacia lentiscus L*. extracts and their antioxidant and antibacterial activities for new active packaging application. Food Control. 2021; 120: 107514. Doi: 10.1016/j.foodcont.2020.107514.
46. Edziri H, Mastouri M, Matieu M, Zine M, Gutman L, and Aouni M. Biological activities of Peganum harmala leaves. Afr J Biotechnol. 2010; 9 (48): 8199–8205. Doi:10.5897/AJB10.564.
47. Senhaji F, Lamchouri F, Boulfia M, Lachkar N, Bouabid K, and Toufik H. Mineral composition, content of phenolic compounds and in vitro antioxidant and antibacterial activities of aqueous and organic extracts of the seeds of *Peganum harmala L.* S Afr J Bot. 2022; 147: 697-712. Doi: 10.1016/j.sajb.2022.03.005.
48. Benhammou N, Bekkara F. A, and Panovska T. K. Antioxidant and antimicrobial activities of the Pistacia lentiscus and *Pistacia atlantica* extracts. Afr J Pharm Pharmacol. 2008; 2(2): 022-028. Doi: 10.5897/AJPP.9000056.
49. Abachi S, Khademi F, Fatemi H, and Malekzadeh F. Study of antimicrobial activity of selected Iranian plant extracts on vancomycin-resistant *Staphylococcus epidermidis*. IOSR J Dent Med Sci. 2013; 4 (1): 59–63. Doi:10.9790/0853-041596.
50. Essaidi I, Snoussi A, Ben Haj Koubaier H, Casabianca H, Bouzouita N. Effect of acid hydrolysis on alizarin content, antioxidant and antimicrobial activities of *Rubia tinctorum* extracts. Pigment Resin Technol. 2017; 46(5): 379–384. Doi:10.1108/PRT-11-2015-0116.
51. Khlifi D, Sghaier R. M, Amouri S, Laouini D, Hamdi M, Bouajila J. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba, Ruta chalpensis L*. and *Peganum harmala L*. Food Chem Toxicol. 2013; 55: 202-208. Doi: 10.1016/j.fct.2013.01.004.
52. Ait Abderrahim L, Taïbi K, and Ait Abderrahim C-H. Assessment of the Antimicrobial and Antioxidant Activities of *Ziziphus lotus* and *Peganum harmala*. Iran J Sci Technol Trans A Sci .2019 ; 43 (2) : 409-414. Doi :10.1007/s40995-017-0411-x.
53. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12: 564-582. Doi:10.1128/CMR.12.4.564.
54. Akabli T, Lamchouri F, Senhaji S, Toufik H. Molecular docking, ADME/Tox prediction, and in vitro study of the cell growth inhibitory activity of five β-carboline alkaloids. Struct Chem. 2019; 30: 1495–1504. Doi: 10.1007/s11224-019-01308-x.
55. Belhachat D, Aid F, Mekimene L, and Belhachat M. Phytochemical screening and in vitro antioxidant activity of *Pistacia lentiscus* berries ethanolic extract growing in Algeria. Med J Nutrition Metab. 2017; 10 (3): 273-285. Doi: 10.3233/MNM-17169.
56. Hemma R, Belhadj S, Ouahchia C, and Saidi F. Antioxidant activity of *Pistacia lentiscus* methanolic extracts. Revue agrobiologia. 2018 ; 8(1) :845-852. http://agrobiologia.net/online/wp-content/uploads/2018/06/845-852 HEMMA_et_al_2-col.pdf/
57. Kákoniová D, Vaverková Š, Lišková D, Urgeová E, and Juráková Z. The possibility to enhance flavonoids production in *Rubia tinctorum L*. callus cultures. Nova Biotechnol. 2009; 9(2): 191–197. Doi:10.36547/nbc.1277.
58. Cushnie TT and Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011; 38 (2): 99-107. Doi: 10.1016/j.ijantimicag.2011.02.014.
59. Shuping L, Xuemei C, Changhong W. A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus Peganum. J Ethnopharmacol 2017; 203: 127-162. Doi: 10.1016/j.jep.2017.03.049.
60. Nadia M, Rima A, Rima R, Khouloud A, Abdelouahab B. Evaluation of the Subacute Toxic Effects of the Alkaloids of the Seeds of *Peganum harmala L* in the Liver, Kidney and Ovary of Female Rats. Trop J Nat Prod Res. 2022 ; 6(10):1632-1637. Doi :10.26538/tjnpr/v6i10.12.
61. Eltawaty SIA, Suliman MB, El-HddadS. Chemical Composition, and Antibacterial and Antifungal Activities of Crude Extracts from *Pistacia lentiscus L*. Fruit. Trop J Nat Prod Res. 2023 ; 7(9):4049-4054. Doi :10.26538/tjnpr/v7i9.30
62. Tagousop C. N, Ekom S. E, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. BMC Complement Altern Med. 2018; 18(1): 1-10. Doi:10.1186/s12906-018-2321-7.
63. Lundén J, Autio T, Markkula A, Hellström S, Korkeala H. Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. Int J Food Microbiol. 2003; 82(3): 265-272. Doi:10.1016/S0168-1605(02)00312-4.
64. Derksen G. C, Niederländer H. A, van Beek T. A. Analysis of anthraquinones in Rubia tinctorum L. by liquid chromatography coupled with diode-array UV and mass spectrometric detection. J Chromatogr A. 2002; 978 (1-2): 119-127. Doi:10.1016/S0021-9673(02)01412-7.

65. Hamed M. M, Refahy L. A, Abdel-Aziz M. S. Evaluation of antimicrobial activity of some compounds isolated from *Rhamnus cathartica L.* Orient J Chem. 2015; 31(2): 1133-1140. Doi:10.13005/ojc/310266.
66. Malmir M, Serrano R, Silva O. Anthraquinones as potential antimicrobial agents-A review. In: A. Méndez-Vilas, editor. Antimicrobial research: Novel bioknowledge and educational programs. Badajoz: Formatex Research Center 2017: 55-61. https://www.researchgate.net/publication/319620317_Anthraquinones_as_potential_antimicrobial_agents-A_review.
67. Sanhueza L, Melo R, Montero R, Maisey K, Mendoza L, Wilkens M. Synergistic interactions between phenolic compounds identified in grape pomace extract with antibiotics of different classes against *Staphylococcus aureus* and *Escherichia coli*. PloS one. 2017; 12(2): e0172273. Doi 10.1371/journal.pone.0172273.
68. Gaysinsky S, Davidson PM, Bruce BD, Weiss J. Stability and antimicrobial efficiency of eugenol encapsulated in surfactant micelles as affected by temperature and pH. J Food Prot. 2005; 68 (7): 1359-1366. Doi:10.4315/0362-028X-68.7.1359.
69. Bolfoni MR, Ferla MDS, Sposito ODS, Giardino L, Jacinto RDC, Pappen FG. Effect of a surfactant on the antimicrobial activity of sodium hypochlorite solutions. Braz Dent J. 2014; 25: 416-419. Doi:10.1590/0103-6440201300049.