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Antibacteria Activity of *Peganum harmala* and *Haloxylon salicornicum* Leaves Extracts

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Abstract

The use of antibiotics in humans and animals has been marked as a significant step in health due to their effectiveness in controlling and treating bacterial infections. The misuse and overuse of antibiotics have been identified as risk factors for bacterial resistance since microorganisms adapt and develop mechanisms to defend against antibiotics. According to the Centers for Disease Protection and Control (CDC), around 23,000 individuals die every year in the United States due to antibiotic resistance complications. As a result, a demand for alternative treatments has been a goal for scientists as the microbes adapt to selective pressure. The aim of this study is to test the antibacterial activity of leaf extracts of *Peganum harmala* and *Haloxylon salicornicum* on both Gram-positive and Gram-negative bacteria on various mediums. The results of the study showed that both *P. harmala* and *H. salicornicum* inhibited the bacterial growth in two different media. The results were also compared with different common antibiotics used in both human's and animal's fields and showed a promising outcome as alternative antibiotics.

Keywords

Peganum harmala, *Haloxylon salicornicum*, Antibiotics Resistance, Medicinal Plants, Gram-Positive, Gram-Negative

1. Introduction

The burden of bacterial infection morbidity and mortality is rapidly growing worldwide. Bacterial pathogens (both susceptible and resistant to antimicrobials)

mortality is the second leading death worldwide after cancer with around 7.7 million death cases globally [1]. It was estimated that the number of death cases were 1.7 million by antibacterial resistant bacteria and associated with nearly 5 million deaths [1] [2]. Five leading pathogens associated with more than half of bacterial mortality were *Staphylococcus aureus* (with more than one million death cases), *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (with more than half a million-death case each). In the U.S., more than 2.8 million antimicrobial-resistant infections occur each year and the number of mortalities is 35,000 cases [3] [4]. Multiple antibiotic resistance was discovered for the very first time in enteric bacteria such as *Salmonella*, *Shigella*, and *Escherichia coli* during the early 1960s [5]. The use of antibiotics in humans and animals has been increased significantly since it has been discovered [6]. Although antibiotics are agents that must be used only for bacterial infection, it has been misused for non-bacterial infection such as viral infections by many individuals. In addition, the use of antibiotics is not only constrained to the clinical settings, as prescriptions involved in the therapeutic regimens for the eradication of diseases in humans. It is also employed in livestock farming, where antibiotics can be used for disease treatment of animals, and in sub-therapeutic levels in concentrated animal feed for growth promotion, improved feed conversion efficiency, and for the prevention of diseases. The consumption of antibiotics in livestock and conservatively estimate the total consumption in 2010 at 63,151 tons and predicted to rise by 67% by 2030. This widespread use of antimicrobials in livestock contributes—by means of natural selection—to the emergence of antimicrobial-resistant bacteria (ARBs) and has significant public health implications: ARBs of animal origin can be transmitted to humans through the environment. Better understanding of the consequences of the uninhibited growth in veterinary antimicrobial consumption is needed to assess its potential effects on animal and human health [6] [7]. Due to different classes of antibiotics in the market, there are different types of antibiotic resistance mechanisms. The most common class of antibiotics is beta lactams which include penicillin and its class. This class of antibiotics triggers and inhibits the bacterial cell wall synthesis. An example of antibiotic resistant bacteria to beta lactam antibiotics is Methicillin Resistant *Staphylococcus aureus* (MRSA) in which *S. aureus* resists antibiotics in beta lactam class in which the bacteria secrete beta lactamase enzymes that break down the beta lactam antibiotic [8]. Another class of antibiotics triggers the protein synthesis such as tetracycline and aminoglycosides. Tetracycline resistance is widespread among Gram-positive and Gram-negative bacteria and can be the result of pumping the drug out of the cell before it reaches it site of action (efflux), protection of the ribosomal binding site, which decreases drug binding [9]. The last common class is fluoroquinolones which trigger bacterial DNA synthesis. Resistance to fluoroquinolones typically arises because of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and of changes in drug entry and efflux [10]. As a result, alternative antibacterial agents are the goal of many scientists over that last dec-

ades to minimize antibiotics use and antibiotics resistance.

According to the World Health Organization (WHO), 80% of the impoverished world's population continues to benefit from the utilization of traditional medicines obtained from medicinal plants [11] [12]. Additionally, the WHO has documented the names of more than 20,000 species of medicinal plants and identified them as a promising source of novel pharmaceuticals [12] [13]. Extracts from medicinal plants have been confirmed to have several biological activities, including antibacterial, anti-inflammatory, and antioxidant properties [14]. Medical plants synthesize a range of substances that are not utilized in the plant's primary metabolic processes. These compounds, termed secondary metabolites (such as alkaloids, phenols, and terpenes), have biological activities and act as a form of protection against microorganisms. Various parts of plants, including leaves, roots, flowers, fruits, etc., carry a significant concentration of bioactive compounds that have medicinal advantages [15] [16] [17].

This study focuses on alternative natural antibacterial agents, *Peganum harmala* and *Haloxylon salicornicum* which both commonly known having alkaloids, flavonoids and anthraquinones [18] [19]. Investigate the antibacterial activity of *Peganum harmala* and *Haloxylon salicornicum* against thirty-three bacterial isolates (Gram-positive and Gram-negative bacteria) were compare their activity with eight known antibiotics that are widely used in clinics and laboratories. In addition, the Mueller-Hinton Agar (MHA) and Luria-Bertani Agar (LB) mediums were used to exploring their influence on antibiotics' behavior.

2. Materials and Methods

2.1. Crude Extraction

Both *Peganum harmala's* and *Haloxylon salicornicum's* leaves were prepared and extracted independently by the same technique. First of all, the leaves were washed and placed in separate jars before drying for one day at room temperature. After 24 hours, the samples were collected and transferred to separate centrifuge tubes which were frozen at −80˚C. After freezing the leaves, the dried/frozen samples were separately grounded using a mortar and pestle to maximize the surface area of the leaves and extraction solvents. The crude extract then was infused with methanol (CH_3OH) to extract the components, which were subsequently distilled in a Soxhlet for six hours. A rotary evaporator was used to evaporate CH_3OH. Afterward, all crude extracts were weighed and dissolved in dimethyl sulfoxide (DMSO). Lastly, serial dilution was performed to make different concentrations of each extraction in which *P. harmala* and *H. salicornicum* were separately serially diluted by 0.5 ml in DMSO (Crude) to get the final concentration. The dilutions were in the range of 1 to 1/64 [20] [21] [22].

2.2. Bacteria Isolates

A total of thirty-three bacteria were selected for this study. Gram-positive bac-

terial species included: *Two Listeria innocua*, *Listeria ivanovii*, *two listeria monocytogenes*, *three Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Rhodococcus equi*, *Bacillus thuringiensis Israelensis*, *Bacillus thuringiensis kurstaki*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Staphylococcus epidermidis*. Gram-negative bacteria species were; *three E. coli*, *Salmonella*, *salmonella enteritidis*, *salmonella typhimurium*, *two salmonella enterica*, *campylobacter coli*, *four chromobacterium violaceum*, *Escherichia vulneris*, *Alcaligenes*, *Proteus vulgaris*, *klebsiella pneumoniae*. These species were obtained from ATCC American Type Culture Collection, Words, Carolina, and Tennessee State University.

2.3. Antimicrobial Susceptibility

The disk diffusion method of Kirby-Bauer Disk Diffusion Susceptibility Test, which is the standard technique utilized when testing pathogens that grow rapidly [23]. The method was used to determine the antibiotic resistance of 33 thirty-three bacteria isolate against two extractions (*P. harmala* and *Haloxylon salicornicum*) and the following antibiotics; Cefixime (CFM; 5 ug) Cefpodoxime (CPD; 10 ug) Doxycycline (DO; 30 ug) Azithromycin (AZM; 15 ug) Ampicillin (AMP; 10 ug) Cefepime (FEP; 30 ug) Vancomycin (VA; 30 ug) and Erythromycin (E; 15 ug). The disks were purchased from Oxoid Limited. Pure cultures of all the bacteria were grown overnight in Tryptic Soy Broth (TSB) as instructed by, at 37˚C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. The concentration was then spread evenly on Mueller-Hinton agar (Oxoid Limited) and LB Agar Luria-Bertani from Difco and BD using a sterile cotton swab [24] [25].

The eight antimicrobial disks were placed on the surface of the agar plates using an automated disk dispenser. The sterile blank paper discs from BD and Taxo, which were 6 mm in diameter, were dipped for about 1 to 2 minutes separately in each extract until fully saturated then applied on plates with serial forceps. The plates were incubated at 37˚C for 24 hours and the results of the antibiotics and the extractions were recorded by measuring the zone of inhibition by using a ruler according to Clinical and Laboratory Standards Institute guidelines [26]. The data was analyzed using R data analysis software and Microsoft Excel.

3. Results

3.1. Summary of the Average Diameter of the Zone of Inhibition

In this study, Kirby-Bauer Disk Diffusion Susceptibility method was used to investigate the antibacterial activity of *P. harmala* and *H. salicornicum* against 17 Gram-negative and 16 Gram-positive bacteria isolates, as well as to compare their activity with eight well-known antibiotics that are commonly used in clinics and laboratories around the world. The mean diameter of the zone of inhibition of the antibiotics and the extractions was calculated based on the

three experiments, as presented based on the type of media separately in Table 1 and Table 2.

3.2. The Influence of LB and MH Media on the Antibiotics Behavior

The investigation has demonstrated that the medium has a substantial impact on the zone of inhibition. Overall, all tested antibacterial, including the two extractions, exhibited a greater zone of inhibition when LB media was used. The Mueller-Hinton Agar (MH) and Luria-Bertani Agar (LB) mediums were utilized to investigate whether they affected the behavior of the antibiotics. The antibacterial activity of *P. harmala* and *H. salicornicum* has been demonstrated in both Gram-negative and Gram-positive bacteria in both media tested. Furthermore, there was a difference in the interaction between the antibiotics and the medium, which affected the zone of the inhibition size. According to the results of the ANOVA test, there was a statistically significant difference ($p < 0.05$) Figure 1 & Figure 2.

3.3. The Effect of Antibacterial Agents on Bacterial Growth in LB Media

The effects of antibacterial agents (Antibiotics and both *P. harmala* and *H. salicornicum*) were determined by zone of inhibition. Among the eight different antibiotics, Cefepime and Doxycycline showed the greatest inhibition in both Gram-positive in LB medium with over (20 mm) inhibition zone. For *H. salicornicum*, it showed better activity in Gram-negative (12 mm) than in Gram-positive (9 mm) while *P. harmala* showed better activity in Gram-positive (10 mm) than in Gram-negative (7 mm). Both of the plant's extracts showed better activity than both Vancomycin and Erythromycin in Gram-positive and Gram-negative bacteria. The rest of antibiotics different activities between Gram-positive and Gram-negative as shown in Figure 3 & Figure 4.

3.4. The Effect of Antibacterial Agents on Bacterial Growth in MH Media

In MH medium, only Cefepime inhibited both Gram-positive and Gram-negative over 20 mm. Both *P. harmala* and *H. salicornicum* also showed better inhibition than Vancomycin and Erythromycin. Like LB medium, *P. harmala* had a greater zone of inhibition in Gram-positive and *H. salicornicum* showed a greater zone of inhibition in Gram-negative. The overall results showed that inhibition zone in both Gram-positive and Gram-negative was statistically significant ($p < 0.05$) in both media Figure 5 & Figure 6.

3.5. Effects of Different Concentrations of *P. harmala* and *H. salicornicum* on *Strep. pyogenes* and *Chromobacterium violaceum* 2

This experiment was carried out to examine whether various concentrations of

Table 1. Summary of the average diameter of the zone of inhibition of antibiotics measured in mm on LB media.

Gram Stain/Bacteria	LB Media									
	AMP 10 mcg	AZM 15 mcg	CFM 5 mcg	CPD 10 mcg	DO 30 mcg	E 15 mcg	FEP 30 mcg	HX 10.163 ug/ml	PG 0.3 ug/ml	VA 30 mcg
Negative										
1-*E. coli* (25,922)	17.00	9.00	20.00	22.00	22.00	0.00	26.00	11.00	7.00	0.00
2-*E. coli* (11,775)	17.00	19.00	20.00	22.00	22.00	7.00	29.00	8.00	6.00	0.00
3-*Salmonella* (1078)	20.00	18.00	20.00	20.00	20.00	0.00	28.00	8.00	0.00	0.00
4-*Salmonella enteritidis* (13,076)	20.00	20.00	20.00	22.00	20.00	0.00	26.00	10.00	0.00	0.00
5-*Salmonella typhimurium* (13,311)	18.00	13.00	19.00	22.00	18.00	0.00	27.00	6.00	0.00	0.00
6-*Salmonella enterica* (BAA 1674)	21.00	10.00	24.00	24.00	25.00	0.00	27.00	8.00	0.00	0.00
7-*Klebsiella pneumoniae* (700,603)	0.00	9.00	0.00	0.00	11.00	0.00	14.00	10.00	6.00	0.00
8-*Salmonella enterica* (53,647)	0.00	19.00	15.00	15.00	12.00	9.00	25.00	11.00	7.00	0.00
9-*Campylobacter coli* (33,559)	18.00	16.00	20.00	20.00	19.00	0.00	28.00	10.00	6.00	0.00
10-CV1	0.00	20.00	0.00	0.00	26.00	15.00	22.00	20.00	13.00	0.00
11-CV2	0.00	21.00	0.00	0.00	27.00	17.00	27.00	23.00	18.00	0.00
12-CV3	9.00	21.00	0.00	7.00	27.00	15.00	13.00	23.00	20.00	8.00
13-CV6	0.00	22.00	0.00	0.00	28.00	14.00	21.00	21.00	15.00	6.00
14-*E. coli* (C 294,546)	15.00	8.00	17.00	20.00	21.00	0.00	26.00	11.00	6.00	0.00
15-*E. vulneris* (33,821)	16.00	24.00	23.00	24.00	25.00	8.00	28.00	13.00	7.00	0.00
16-*Alcaligenes* (W 470,179-186)	0.00	20.00	13.00	0.00	21.00	12.00	20.00	15.00	8.00	0.00
17-*Proteus vulgaris* (W 470,179-538)	18.00	0.00	25.00	25.00	7.00	0.00	22.00	0.00	0.00	0.00
Positive										
18-*L. innocua* (33,090)	15.00	20.00	18.00	20.00	20.00	0.00	25.00	6.00	6.00	0.00
19-*L. ivanovii* (19,119)	17.00	16.00	18.00	20.00	20.00	0.00	27.00	7.00	6.00	0.00
20-*Staphylococcus aureus* (49,444)	20.00	17.00	20.00	21.00	20.00	0.00	28.00	7.00	6.00	0.00
21-*Staphylococcus aureus* (25,923)	15.00	9.00	20.00	21.00	22.00	0.00	29.00	6.00	6.00	0.00
22-*Staphylococcus aureus* (25,925)	16.00	11.00	20.00	22.00	22.00	0.00	28.00	7.00	6.00	0.00
23-*Listeria monocytogenes* (19,115)	15.00	10.00	18.00	21.00	22.00	0.00	28.00	10.00	6.00	0.00
24-*Enterococcus faecalis* (13,379)	16.00	9.00	18.00	19.00	20.00	0.00	25.00	10.00	6.00	0.00

Continued

25-*Enterococcus faecium* (335,651)	18.00	10.00	20.00	21.00	22.00	0.00	27.00	10.00	6.00	0.00
26-*Rhodococcus equi* (6939)	17.00	14.00	20.00	22.00	20.00	0.00	27.00	11.00	0.00	0.00
27-*L. innocua* (NCTC 11,288)	0.00	16.00	0.00	10.00	17.00	0.00	0.00	11.00	14.00	0.00
28-*Listeria monocytogenes* (NCTC 11,994)	0.00	12.00	0.00	0.00	18.00	0.00	0.00	11.00	17.00	0.00
29-*Bacillus thuringiensis* Israelensis BTI	0.00	13.00	0.00	0.00	27.00	19.00	0.00	10.00	19.00	16.00
30-*Bacillus thuringiensis* kurstaki BTK	0.00	13.00	0.00	0.00	27.00	20.00	0.00	7.00	17.00	17.00
31-*Strep. pyogenes* (W 470,179-570)	28.00	33.00	33.00	34.00	34.00	24.00	28.00	12.00	16.00	16.00
32-*Strep. pneumonia* (W 470,179-498)	27.00	18.00	13.00	26.00	35.00	25.00	28.00	8.00	16.00	17.00
33-*Staph. epidermidis* (W 470,176-544)	16.00	20.00	13.00	18.00	15.00	0.00	25.00	10.00	16.00	14.00

P. harmala and *H. salicornicum* had a varied impact on *pyrogen* and *Chromobacterium violaceum* 2 bacteria in different media. The findings indicate that there is a positive correlation between the concentration of the extractions and the size of the zone of inhibition. Also, the type of medium impacts the size of the zone diameter (Table 3 & Table 4).

4. Discussion

Antibacterial Activity of *P. harmala* and *H. salicornicum* against Gram-Positive and Gram-Negative Bacteria in LB and MH Medium

The study's findings reveal that the leaf extracts of *P. harmala* and *H. salicornicum* exhibit antibacterial activity compared to antibiotics that are well-known and widely used. The effects of *P. harmala* and *H. salicornicum* on gram-negative bacteria were compared with the impact of the eight antibiotics (different antibiotics classes). The obtained results suggested that the *H. salicornicum* extract had significant antibacterial activity, particularly against the microorganisms *Chromobacterium violaceum*, *E. vulneris*, *Alcaligenes*, and *E. coli*. Furthermore, the *H. salicornicum* inhibition zone average was higher than the averages for Vancomycin (VA; 30), Erythromycin (E; 15 ug), Ampicillin (AMP; 10 ug), and *P. harmala*. Similarly, *P. harmala* exhibits antibacterial action, and its averages were higher than the (VA; 30) and Erythromycin (E; 15 ug) inhibitory zone averages, respectively.

The effects of *P. harmala* and *H. salicornicum* on gram-positive bacteria were the total opposite of their impact on gram-negative bacteria, with *P. harmala* extract demonstrating more antibacterial activity than *H. salicornicum* extract in

Table 2. Summary of the average diameter of the zone of inhibition of antibiotics measured in mm on MH media.

Gram Stain/Bacteria	MH Media									
	AMP 10 mcg	AZM 15 mcg	CFM 5 mcg	CPD 10 mcg	DO 30 mcg	E 15 mcg	FEP 30 mcg	HX 10.163 ug/ml	PG 0.3 ug/ml	VA 30 mcg
Negative										
1-*E. coli* (25,922)	15.00	13.00	16.00	20.00	19.00	0.00	25.00	8.00	7.00	0.00
2-*E. coli* (11,775)	16.00	11.00	17.00	21.00	17.00	0.00	24.00	7.00	7.00	0.00
3-*Salmonella* (1078)	20.00	14.00	15.00	18.00	15.00	0.00	25.00	6.00	0.00	0.00
4-*Salmonella enteritidis* (13,076)	18.00	13.00	16.00	20.00	15.00	0.00	24.00	7.00	0.00	0.00
5-*Salmonella typhimurium* (13,311)	17.00	13.00	16.00	18.00	16.00	7.00	27.00	9.00	0.00	0.00
6-*Salmonella enterica* (BAA 1674)	22.00	15.00	25.00	25.00	19.00	9.00	30.00	7.00	0.00	0.00
7-*Klebsiella pneumoniae* (700,603)	0.00	12.00	9.00	0.00	10.00	0.00	19.00	7.00	0.00	0.00
8-*Salmonella enterica* (53,647)	0.00	13.00	20.00	20.00	10.00	10.00	25.00	7.00	7.00	0.00
9-*Campylobacter coli* (33,559)	16.00	13.00	18.00	20.00	15.00	0.00	28.00	7.00	0.00	0.00
10-CV1	0.00	22.00	0.00	0.00	23.00	17.00	25.00	17.00	13.00	0.00
11-CV2	0.00	20.00	0.00	0.00	22.00	17.00	25.00	20.00	15.00	0.00
12-CV3	0.00	15.00	0.00	0.00	18.00	12.00	8.00	18.00	13.00	6.00
13-CV6	0.00	23.00	0.00	0.00	25.00	17.00	17.00	18.00	13.00	7.00
14-*E. coli* (C 294,546)	15.00	8.00	17.00	19.00	18.00	0.00	23.00	8.00	0.00	0.00
15-*E. vulneris* (33,821)	15.00	10.00	20.00	20.00	16.90	10.00	26.00	13.00	7.00	0.00
16-*Alcaligenes* (W 470,179-186)	0.00	22.00	12.00	0.00	16.00	13.00	15.00	12.00	8.00	0.00
17-*Proteus vulgaris* (W 470,179-538)	21.00	0.00	25.00	23.00	0.00	0.00	22.00	0.00	0.00	0.00
Positive										
18-*L. innocua* (33,090)	13.00	10.00	16.00	17.00	15.00	0.00	25.00	7.00	7.00	0.00
19-*L. ivanovii* (19,119)	13.00	11.00	15.00	17.00	15.00	0.00	24.00	7.00	7.00	0.00
20-*Staphylococcus aureus* (49,444)	14.00	12.00	16.00	20.00	16.00	0.00	25.00	7.00	7.00	0.00
21-*Staphylococcus aureus* (25,923)	14.00	12.00	17.00	20.00	15.00	0.00	28.00	7.00	0.00	0.00
22-*Staphylococcus aureus* (25,925)	14.00	13.00	18.00	22.40	16.00	7.00	28.00	8.00	7.00	0.00
23-*Listeria monocytogenes* (19,115)	15.00	13.00	18.00	20.00	18.00	0.00	27.00	9.00	0.00	0.00
24-*Enterococcus faecalis* (13,379)	14.00	14.00	17.00	20.00	17.00	7.00	28.00	7.00	0.00	0.00

Continued

25-*Enterococcus faecium* (335,651)	15.00	14.00	19.00	20.00	17.00	0.00	28.00	7.00	7.00	0.00
26-*Rhodococcus equi* (6939)	15.00	12.00	16.00	20.00	15.00	0.00	27.00	7.00	0.00	0.00
27-*L. innocua* (NCTC 11,288)	0.00	10.00	0.00	0.00	13.00	0.00	10.00	10.00	12.00	0.00
28-*Listeria monocytogenes* (NCTC 11994)	0.00	8.00	0.00	0.00	14.00	0.00	0.00	10.00	16.00	0.00
29-*Bacillus thuringiensis* Israelensis BTI	0.00	15.00	0.00	0.00	20.00	19.00	0.00	7.00	18.00	16.00
30-*Bacillus thuringiensis* kurstaki BTK	0.00	16.00	0.00	0.00	20.00	20.00	0.00	9.00	18.00	15.00
31-*Strep. pyogenes* (W 470,179-570)	24.00	25.00	27.00	27.00	25.00	25.00	24.00	11.00	17.00	19.00
32-*Strep. pneumonia* (W 470,179-498)	29.00	25.00	12.00	26.00	27.00	25.00	27.00	7.00	12.00	17.00
33-*Staph. epidermidis* (W 470,176-544)	14.00	20.00	12.00	20.00	13.00	0.00	24.00	7.00	13.00	14.00

Table 3. Four different concentrations of *P. harmala* on *S. pyogenes*.

Concentration	0.15 ug/ml	0.0375 ug/ml	0.0093 ug/ml	0.0046 ug/ml
LB	12 mm	10 mm	9 mm	7 mm
MH	13 mm	12 mm	10 mm	9 mm

Table 4. Four different concentrations of *H. salicornicum* on *Chromobacterium violaceum* 2.

Concentration	5.081 ug/ml	1.27 ug/ml	0.317 ug/ml	0.158 ug/ml
LB	13 mm	12 mm	9 mm	7 mm
MH	13 mm	13 mm	10 mm	10 mm

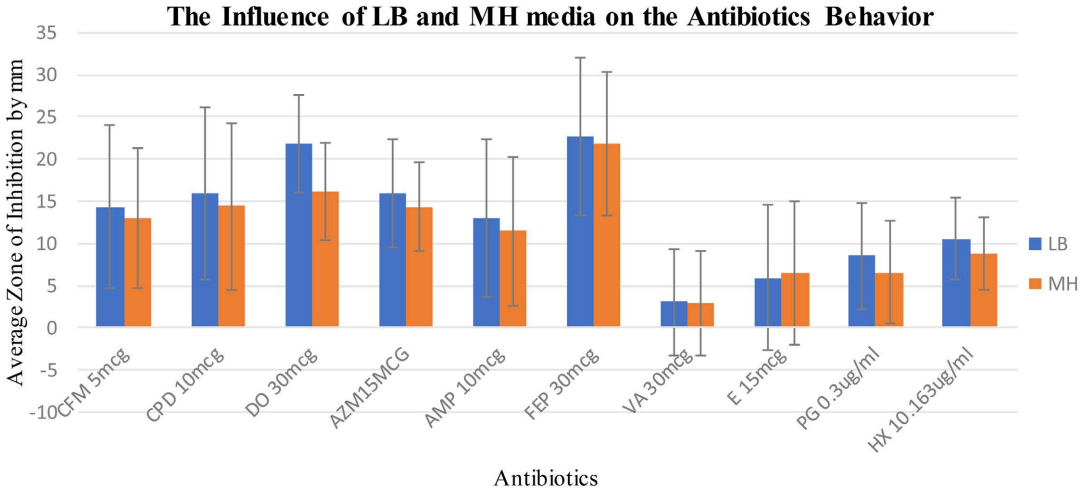

Figure 1. Antibiotic action on two different media differentiates ($p < 0.05$).

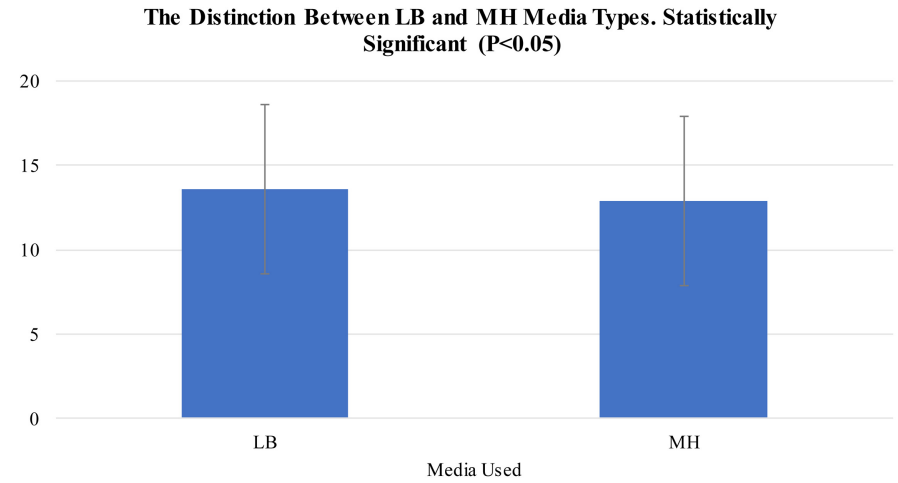

Figure 2. The distinction between LB and MH media types.

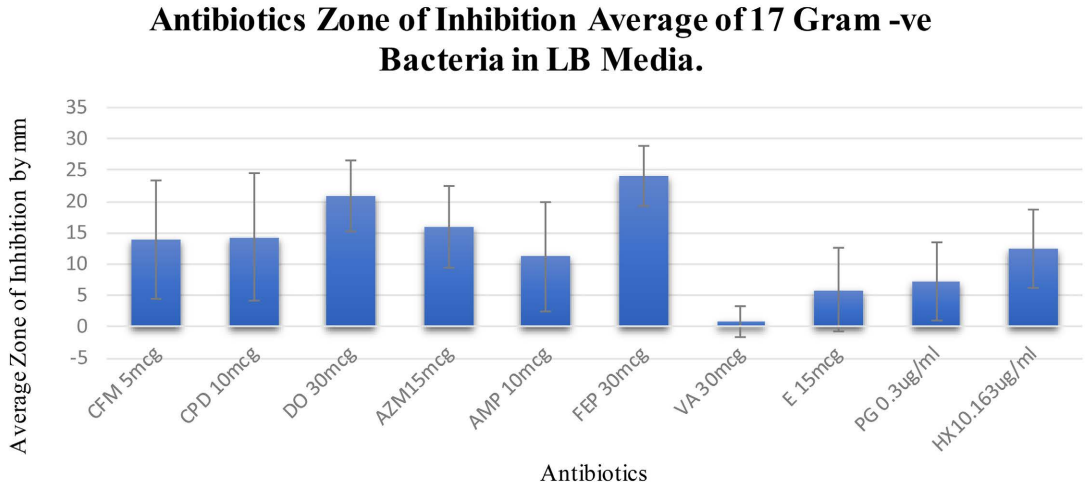

Figure 3. The effects of *P. harmala* and *H. salicornicum* on Gram-negative bacteria in LB media were compared with the impact of eight antibiotics. It is statistically significant ($p < 0.05$).

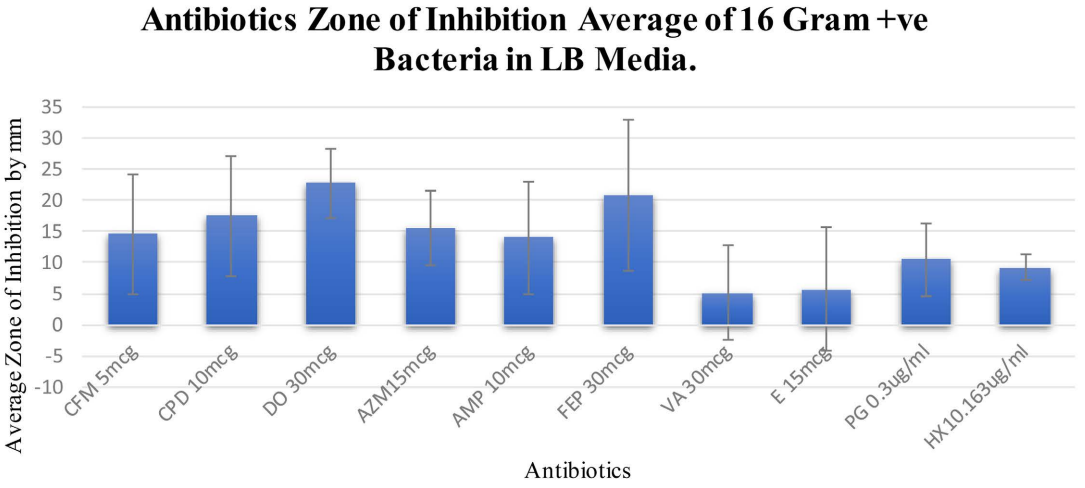

Figure 4. The effects of *P. harmala* and *H. salicornicum* on Gram-positive bacteria in LB media were compared with the impact of eight antibiotics. It is statistically significant ($p < 0.05$).

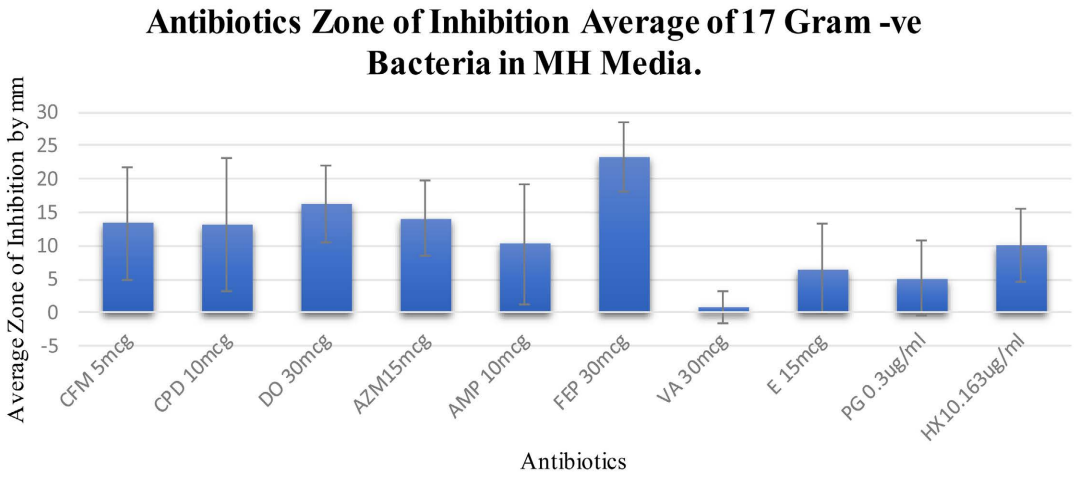

Figure 5. The effects of *P. harmala* and *H. salicornicum* on Gram-negative bacteria in MH media were compared with the impact of eight antibiotics. It is statistically significant ($p < 0.05$).

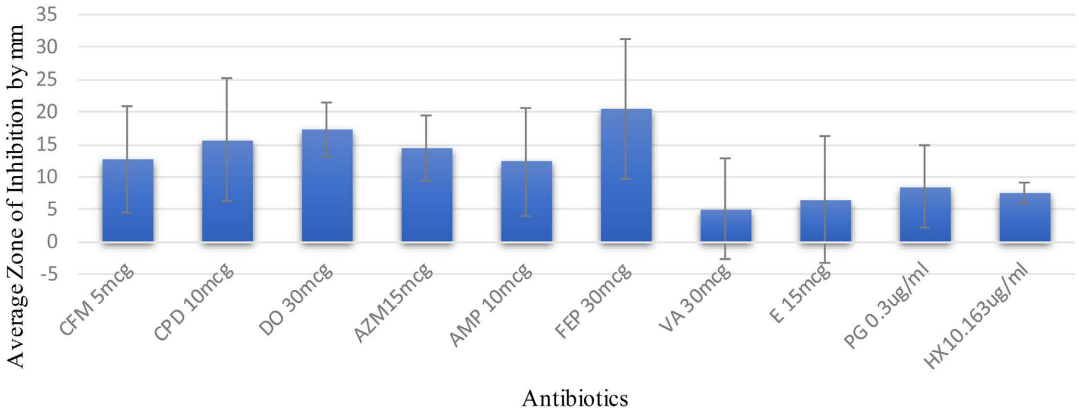

Figure 6. The effects of *P. harmala* and *H. salicornicum* on Gram-positive bacteria in MH media were compared with the impact of eight antibiotics. It is statistically significant ($p < 0.05$).

the case of gram-positive bacteria when assessed statistically, the inhibition zone average of *P. harmala* extract is greater than the inhibition zones of Vancomycin (VA; 30), Erythromycin (E; 15 ug), Ampicillin (AMP; 10 ug), and *H. salicornicum* extract. It was shown that *P. harmala* exhibited significant antibacterial activity, especially against *listeria monocytogenes*, *Bacillus thuringiensis Israelensis*, *Bacillus thuringiensis kurstaki*, *Staph. epidermidis*, *Strep. pyogenes*, *Staph. pyogenes*, *Staph. epidermidis*, *and Bacillus thuringiensis*. As previously stated, the antibacterial activity of *H. salicornicum* is shown by its averages being more significant than the (VA; 30) and Erythromycin (E; 15 ug) inhibitory zone averages on gram-positive bacteria.

The outcomes of the investigation suggest that both *P. harmala* and *H. salicornicum* extracts have antibacterial activity against bacteria of the gram-positive and gram-negative types. That means that *P. harmala* and *H. salicornicum* act in

the same way as broad-spectrum antibiotics when compared to broad-spectrum antibiotics such as Cefixime (CFM; 5 ug), Cefpodoxime (CPD; 10 ug), Doxycycline (DO; 30 ug), and Ampicillin (AMP; 10 ug).

As a result of the research, it was discovered that there is a statistically significant difference between the bacterial culture medium Mueller-Hinton (MH) and Luria-Bertani (LB). When the statistical study was completed, it was discovered that Mueller-Hinton (MH) and Luria-Bertani (LB) influenced antibiotic behavior because the antibiotics gave different zone of inhibition diameters for the same bacteria in both mediums as well as different bacteria growth densities. The findings were very significant ($p < 0.05$).

Many studies have been conducted on the antibacterial activity *of P. harmala* and *H. salicornicum*, both in vitro and in vivo. In general, all of these studies provide support to the study findings. According to Darabpour *et al*. (2011), *P. harmala's* active phytochemicals demonstrated a broad spectrum and significant antibacterial activity against clinical bacterial pathogens, including *Bacillus anthracis*, *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *and Streptococcus pyogenes* Gram-positive bacterial species. Gram-negative bacteria included *Pseudomonas aeruginosa*, *Brucella melitensis*, *Proteus mirabilis*, *Salmonella typhi*, *Escherichia coli*, *and Klebsiella pneumoniae* [27]. In addition, it was observed in 2023 by Rugaie and colleagues that a *H. salicornicum* extract had a strong inhibitory effect on *Staphylococcus aureus*, *S. pyogenes* and *Escherichia coli* bacteria, when the disc diffusion technique was utilized [19]. By comparing the results, it adds credence to the findings of this research.

5. Conclusion and Summary

The antibacterial investigation found that *P. harmala* and *H. salicornicum* had a clear impact on Gram-positive and Gram-negative bacteria. They include active phytochemicals and have antibacterial activity as a broad-spectrum antibiotic. However, due to several limitations on the study, further researches should be conducted. Further research into these plants' extracts *in vivo* is recommended for more advanced studies. Other recommendations for future studies include using other parts of *P. harmala* and *H. salicornicum*, such as the seed and root, and the addition of different species.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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