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Research Article
Combinational Effect of Selected Medicinal Plants and Antibiotics Against Pathogenic Bacteria

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Abstract

Background and Objective: The emergence of antibiotic resistance is a primary global health concern. As a result, there is an urgent need for new strategies to combat antibiotic-resistant bacteria. One of these essential strategies is the combination of medicinal plants and antibiotics as an alternative to using antibiotics alone which was the objective of this article. **Materials and Methods:** Nine plant materials were collected from different Egypt localities and then extracted by water. Water extracts were filtered and added with Mueller-Hinton agar during preparation. Nine test bacteria and 13 standard antibiotics were used in the disc diffusion sensitivity method. **Results:** The activity of Amikacin was increased when combined with most different plant extracts against *Escherichia coli* while antagonistic against *Pseudomonas aeruginosa*. Aztreonam, Ceftriaxone, Gentamicin and Nalidixic acid antibiotics showed antagonistic or indifferent effects when combined with most different plant extracts against *E. coli*, *Klebsiella pneumonia* and *P. aeruginosa*. The synergistic effect was achieved in Aztreonam when combined with all plant extracts, while Nalidixic acid showed antagonistic when combined with most plant extracts against *Proteus mirabilis*. The antagonistic effect was achieved in Aztreonam, Ceftriaxone and Nalidixic acid when combined with *Achillea fragrantissima*, *Artemisia monosperma* and *Leptadenia pyrotechnica*, also Aztreonam with *Lycium shawii* extract against *Salmonella typhimurium*. The *A. fragrantissima* and *A. monosperma* increase the activity of Novobiocin and Vancomycin against *Bacillus cereus* and Ampicillin and Cefazolin against *Staphylococcus aureus* but Novobiocin activity increased with most plant extracts against *S. aureus*. **Conclusion:** The combinations of antibiotics with the extracts of medicinal plants displayed varying degrees of effects, synergistic, antagonistic and indifferent according to antibiotic type, plant extract and test organism.

Key words: Antibiotic, pathogenic bacteria, medicinal plant, synergism, antagonism

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial resistance to antibiotics is a growing problem in the medical world. Bacteria can become resistant to antibiotics through several mechanisms, including mutation and horizontal gene transfer[1]. Mutations can occur naturally in bacteria while horizontal gene transfer is the process by which bacteria can acquire genes from other bacteria that confer antibiotic resistance. Through one or more processes, other bacteria become multiple drug resistant. Moreover, it leads to the selection and prevails of antibiotic-resistant bacteria[2]. To overcome the resistance of antibiotics, either by searching for a new antibiotic or increasing the potency of known antibiotics[3]. This is also achieved by new strategies, for instance, the use of bacteriophages and probiotics[4,5]. In addition to searching for new natural products with antimicrobial activities[6]. There is a huge need for new chemical entities to be discovered as antimicrobial drugs as well as for new targets to be discovered that could be used as antimicrobials. Adjuvants for antibiotics are one of the many strategies that can be used to combat antibiotic resistance[7]. The discovery of chemical adjuvants or antibiotics in combination: Combining different antibiotics can reduce the likelihood of resistance developing or may antagonistic other combinations[8,9]. On the other hand, natural products have long been the key source of new drugs against infectious diseases, especially antibiotics[10,11]. The combination of medicinal plants and antibiotics against pathogenic bacteria has been studied extensively in recent years. Studies have shown that certain medicinal plants can be used in combination with antibiotics to enhance the efficacy of the antibiotic against certain bacterial infections[12]. Combination therapy is generally an effective strategy to fight resistance and while some data on its effects are conflicting, this means not every combination of two substances will have a synergistic effect[13]. Therefore, this study deals with the *in vitro* combination of different antibiotics with selected medicinal plants to evaluate their interactions against clinically pathogenic bacteria. This provides knowledge about synergistic, interacting, antagonistic and in different interactions that would pave a new strategy for the treatment of bacterial infections, overcome antibiotic-resistant microbes, decrease using of antibiotics and thus, the negative effects they cause.

MATERIALS AND METHODS

Plant materials and extracts preparation: Nine plant materials were collected and used in this study, *Achillea fragrantissima* (areal part) desert plant collected from Saint Catherine, Sinai and Egypt. *Artemisia monosperma* Delile (aerial part), desert plant collected from North Sinai, Egypt. *Foeniculum vulgare* Mill (areal part) cultivated plant collected from the garden at Upper Egypt. *Lepidium sativum* (seeds) and *Trigonella foenum-graecum* (seeds) were obtained commercially from herbal-shop. *Lycium shawii* (areal part) desert plant collected from Rafah, North Sinai, Egypt. *Leptadenia pyrotechnica* (areal part), *Pituranthos tortuosus* (areal part) and *Senecio glaucus* (areal part) desert plant collected from Wadi Hagul, the eastern desert of Egypt. The plant materials were collected with the help of Dr. Abd El-Raheim Donia (Plant chemist) during March and April, 2019, identified and authenticated by Dr. Omran Ghaly (Plant taxonomist) after being compared to samples in the herbarium of the Desert Research Center, Cairo, Egypt. Plant materials were kept in a shaded room protected from direct exposure to sunlight for drying and then ground in the grinder. Plant materials (10 g) were mixed separately with distilled water (100 mL) by using a mixer and left for 24 hrs at room temperature. Soaked plant materials were filtered by using a gauze pad and collected unfiltered particles and then boiled with 100 mL distilled water for 2 min then filtered again to remove the large particles of the plants. Approximately 200 mL of collected filtrates were subjected to filtering again using filter papers (through Whatman No. 2 under vacuum) to obtain a clear solution and then stored at 4°C until used.

Bacterial strains: The bacteria used in the antibacterial activity include four Gram-positive bacteria, *Bacillus cereus* (isolated strain), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228) and five Gram-negative, *E. coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Proteus mirabilis* (isolated strain), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (isolated strain). Bacterial strains were obtained from microbiological collections at the Medical Laboratory Sciences Department, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University.

Antibiotic discs: Thirteen standard antibiotic discs (6 mm) were used to evaluate antimicrobial sensitivity. These antibiotics include Amikacin (AK 30 µg), Ampicillin (AP 10 µg), Aztreonam (ATM 30 µg), Cefazolin (CZ 30 µg), Ceftriaxone (CRO 30 µg), Chloramphenicol (C 30 µg), Gentamicin (GM 120 µg), Imipenem (IMI 10 µg), Nalidixic acid (NA 30 µg), Novobiocin (NO 30 µg) and Vancomycin (VA 30 µg) were obtained from Mast diagnostics, Mast Group Ltd., UK. Cefoxitin (FOX 30 µg) and Oxacillin (OX 1 µg) were produced by Oxoid, Basingstoke, UK.

Preparation of media: Mueller-Hinton (MH) agar (Scharlau, Barcelona, Spain) was used for the antibiotic sensitivity test. For the preparation of modified Mueller-Hinton (MMH) media, the clear solution of plant filtrates was separately added to MH agar during the media cooking in a water bath for a homogenized medium with subtracted the plant filtrates solution quantity from the amount of distilled water used for manufacturing preparations. All prepared media were sterilized by autoclave (ALP Co. Tokyo, Japan) at 121°C for 15 min and poured into 9 cm diameter Petri dishes. While the control (without plant extract) plates were prepared without using aqueous plant extract preparations.

Antibacterial test: The sensitivity test of antibiotics was tested by the disc diffusion (Kirby-Bauer) method[14,15]. Each test organism was sub-cultured separately on a nutrient agar (Scharlau, Barcelona, Spain) medium plate and overnight incubated. The separate fresh colonies were suspended in sterilized normal saline tubes, mixed and adjusted to 0.5 McFarland standard equivalent to approximately 1.5×10^8 CFU mL^{-1}. Each bacterial suspension was streaked on the surface of MH (standard) and MMH (with plant extract) agar plates to evaluate combination assays between antibiotics and plant extracts. Standard antibiotic discs were placed on the surface of Petri dishes and then lifted for about 1 hr to allow proper diffusion before being incubated at 37°C for 24 hrs[16]. The determinations were done in duplicate. After 24 hrs of incubation at 37°C, the plates were examined for zones of inhibition formed around the discs. The diameters (mm) of each inhibition zone produced by antibiotic alone and combinations were measured and recorded after incubation.

RESULTS

In this study, each of the selected antibiotics with a different mechanism of action was tested alone or combined with some medicinal plants extract against different bacteria strains. When antibiotics were studied alone showed a variety of degrees of antibacterial activity against the investigated test organisms (Table 1-2).

In the case of studying antibiotics against Gram-positive bacteria, Novobiocin showed the highest activity among tested antibiotics against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus cereus* respectively, while Ampicillin showed the highest activity against *Enterococcus faecalis* (Table 1). While Ampicillin showed no activity against *Bacillus cereus* and *Staphylococcus epidermidis*, Oxacillin showed no activity against *Bacillus cereus* and *Enterococcus faecalis*. In the same context, *Enterococcus faecalis* showed resistance to selected antibiotics, Cefazolin, Chloramphenicol, Novobiocin and Oxacillin (Table 1).

In the case of antibiotics against Gram-negative bacteria study, Aztreonam has recorded the most potent activity among selected antibiotics against *Salmonella typhimurium*, *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* except its activity against *Klebsiella pneumonia* (Table 2). Cefoxitin was not active against most tested gram-negative bacteria. Also, Nalidixic acid showed any activity against *Pseudomonas aeruginosa* (Table 2). On the other hand, Ceftriaxone and Imipenem showed the highest activity against *Proteus mirabilis* and *Klebsiella pneumoniae*, respectively (Table 2).

Table 1: Effect of antibiotics on studded Gram-positive bacteria

	Antibiotic (Mean zone of inhibition (mm))					
Test organism	Ampicillin	Cefazolin	Chloramphenicol	Novobiocin	Oxacillin	Vancomycin
Bacillus cereus	00	07	21	23	00	19
Enterococcus faecalis	20	00	00	00	00	13
Staphylococcus aureus	14	28	20	31	21	20
Staphylococcus epidermidis	00	34	36	40	27	23

Diameter of antibiotic discs: 6 mm

Table 2: Effect of antibiotics on studded Gram-negative bacteria

	Antibiotic (Mean zone of inhibition (mm))						
Test organism	Amikacin	Aztreonam	Cefoxitin	Ceftriaxone	Gentamicin	Imipenem	Nalidixic acid
Escherichia coli	20	39	16	36	34	29	28
Klebsiella pneumoniae	24	08	00	15	19	29	10
Proteus mirabilis	20	38	08	40	25	28	22
Pseudomonas aeruginosa	25	25	00	16	23	24	00
Salmonella typhimurium	17	44	08	36	30	30	23

Diameter of antibiotic discs: 6 mm

Table 3: Combination effects between antibiotics and medicinal plants on *Escherichia coli*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Amikacin	Aztreonam	Ceftriaxone	Gentamicin	Nalidixic acid
Control	20	39	36	34	28
Achillea fragrantissima	23	37	32	30	25
Artemisia monosperma	23	37	33	32	28
Foeniculum vulgare	22	41	38	30	28
Lepidium sativum	20	38	30	34	25
Leptadenia pyrotechnica	26	34	31	28	27
Lycium shawii	22	36	34	32	23
Pituranthos tortuosus	22	38	31	28	24
Senecio glaucus	24	36	32	34	25
Trigonella foenum-graecum	24	38	34	34	28

Diameter of antibiotic discs: 6 mm

Table 4: Combination effects between antibiotics and medicinal plants on *Klebsiella pneumonia*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Amikacin	Aztreonam	Cefoxitin	Ceftriaxone	Nalidixic acid
Control	24	08	00	15	10
Achillea fragrantissima	22	09	00	12	12
Artemisia monosperma	21	07	00	15	14
Foeniculum vulgare	24	12	00	13	14
Lepidium sativum	20	00	00	9	10
Leptadenia pyrotechnica	24	10	00	16	10
Lycium shawii	24	11	00	18	11
Pituranthos tortuosus	24	07	00	12	11
Senecio glaucus	23	13	00	19	11
Trigonella foenum-graecum	23	08	00	15	11

Diameter of antibiotic discs: 6 mm

Table 5: Combination effects between antibiotics and medicinal plants on *Pseudomonas aeruginosa*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Amikacin	Aztreonam	Cefoxitin	Ceftriaxone	Nalidixic acid
Control	25	25	00	16	00
Achillea fragrantissima	24	26	00	15	07
Artemisia monosperma	24	23	00	15	00
Foeniculum vulgare	20	26	00	17	00
Lepidium sativum	22	24	00	15	00
Lycium shawii	19	25	00	14	00
Pituranthos tortuosus	18	26	00	18	00
Leptadenia pyrotechnica	19	23	00	15	00
Senecio glaucus	18	26	00	18	00
Trigonella foenum-graecum	24	25	00	11	00

Diameter of antibiotic discs: 6 mm

The findings of the present study showed that combinations of antibiotics with the extracts of medicinal plants displayed varying degrees of effects, synergistic, antagonistic and indifferent effects (Table 3-11).

According to activity against *E. coli* test bacteria, the activity of Amikacin increased when combined separately with different plant extracts (*Leptadenia pyrotechnica*, *Senecio glaucus*, *Trigonella foenum-graecum*, *Achillea fragrantissima*, *Artemisia monosperma*, *Foeniculum vulgare*, *Lycium shawii* and then *Pituranthos tortuosus*). While most plant extracts (*Achillea fragrantissima*, *Artemisia monosperma*, *Lepidium sativum*, *Leptadenia pyrotechnica*, *Lycium shawii*, *Pituranthos tortuosus* and *Senecio glaucus*) reduced the activity of Aztreonam, Ceftriaxone, Gentamicin and Nalidixic acid antibiotics when combined separately against *E. coli* (Table 3).

On the other hand, *Achillea fragrantissima* extract increases the activity of Amikacin, while reducing the activity effect of Ceftriaxone, Gentamicin, Nalidixic acid and Aztreonam against *E. coli*. *Artemisia monosperma* extract increased the activity of Amikacin while reducing the activity effect of Aztreonam, Ceftriaxone and Gentamicin but has no effect added to Nalidixic acid against *E. coli*.

Table 6: Combination effects between antibiotics and medicinal plants on *Proteus mirabilis*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Aztreonam	Ceftriaxone	Gentamicin	Imipenem	Nalidixic acid
Control (without plant extract)	38	40	25	28	22
Achillea fragrantissima	42	42	22	40	18
Artemisia monosperma	42	42	25	54	20
Foeniculum vulgare	42	38	26	30	20
Lepidium sativum	43	45	20	30	21
Leptadenia pyrotechnica	44	40	24	26	23
Lycium shawii	44	42	28	32	19
Pituranthos tortuosus	41	40	27	30	19
Senecio glaucus	44	38	25	28	17
Trigonella foenum-graecum	42	40	24	50	22

Diameter of antibiotic discs: 6 mm

Table 7: Combination effects between antibiotics and medicinal plants on *Salmonella typhimurium*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Aztreonam	Ceftriaxone	Gentamicin	Imipenem	Nalidixic acid
Control (without plant extract)	44	36	30	30	23
Achillea fragrantissima	38	32	30	34	17
Artemisia monosperma	36	32	28	29	20
Foeniculum vulgare	44	34	28	32	21
Lepidium sativum	42	42	30	28	22
Leptadenia pyrotechnica	38	32	32	31	21
Lycium shawii	40	36	29	30	21
Pituranthos tortuosus	34	33	28	31	17
Senecio glaucus	38	32	28	30	19
Trigonella foenum-graecum	42	37	29	32	24

Diameter of antibiotic discs: 6 mm

Table 8: Combination effects between antibiotics and medicinal plants on *Bacillus cereus*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Ampicillin	Cefazolin	Novobiocin	Oxacillin	Vancomycin
Control	00	07	23	00	19
Achillea fragrantissima	07	09	33	00	26
Artemisia monosperma	00	08	27	00	22
Foeniculum vulgare	00	07	24	00	18
Lepidium sativum	00	07	23	00	17
Leptadenia pyrotechnica	00	07	24	00	18
Lycium shawii	00	00	23	00	16
Pituranthos tortuosus	00	07	25	00	18
Senecio glaucus	00	07	25	00	17
Trigonella foenum-graecum	00	07	23	00	18

Diameter of antibiotic discs: 6 mm

Table 9: Combination effects between antibiotics and medicinal plants on *Staphylococcus aureus*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Ampicillin	Cefazolin	Novobiocin	Oxacillin	Vancomycin
Control	14	28	31	21	20
Achillea fragrantissima	18	38	40	20	22
Artemisia monosperma	17	33	38	20	20
Foeniculum vulgare	16	28	36	22	20
Lepidium sativum	15	29	34	21	20
Leptadenia pyrotechnica	14	32	18	20	18
Lycium shawii	15	32	34	20	19
Pituranthos tortuosus	16	29	38	19	20
Senecio glaucus	16	30	34	21	19
Trigonella foenum-graecum	14	30	32	19	18

Diameter of antibiotic discs: 6 mm

Table 10: Combination effects between antibiotics and medicinal plants on *Enterococcus faecalis*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Ampicillin	Cefazolin	Chloramphenicol	Novobiocin	Oxacillin
Control	20	00	00	00	00
Achillea fragrantissima	24	07	29	21	00
Artemisia monosperma	25	07	26	20	00
Foeniculum vulgare	25	07	27	20	00
Lepidium sativum	19	07	27	20	00
Leptadenia pyrotechnica	23	14	29	19	00
Lycium shawii	20	07	26	12	00
Pituranthos tortuosus	25	18	30	19	00
Senecio glaucus	25	10	29	12	00
Trigonella foenum-graecum	21	11	28	13	00

Diameter of antibiotic discs: 6 mm

Table 11: Combination effects between antibiotics and medicinal plants on *Staphylococcus epidermidis*

	Antibiotic (Mean zone of inhibition (mm))				
Test organism	Cefazolin	Chloramphenicol	Novobiocin	Oxacillin	Vancomycin
Control	34	36	40	27	23
Achillea fragrantissima	42	39	40	30	25
Artemisia monosperma	38	38	44	29	23
Foeniculum vulgare	30	32	38	26	20
Lepidium sativum	34	33	38	26	20
Leptadenia pyrotechnica	34	32	38	25	22
Lycium shawii	28	26	32	23	19
Pituranthos tortuosus	34	34	38	28	22
Senecio glaucus	31	30	40	27	23
Trigonella foenum-graecum	31	30	37	23	19

Diameter of antibiotic discs: 6 mm

Foeniculum vulgare extract slightly increases the activity of Amikacin, Aztreonam and Ceftriaxone while it weakens the activity of Gentamicin but has no effect when added to Nalidixic acid against *E. coli*. *Leptadenia pyrotechnica* extract increased the activity of Amikacin, while activity reduced the activity of Gentamicin, Ceftriaxone and Aztreonam but has no effect added to Nalidixic acid against *E. coli* (Table 3). There was an antagonistic effect between Ceftriaxone and *Lepidium sativum* followed by *Achillea fragrantissima* followed by *Artemisia monosperma* against *E. coli*, respectively. While the antagonistic effect was achieved between Gentamicin with *Achillea fragrantissima*, *Foeniculum vulgare* and against the same test organism. Also, an antagonistic effect was observed between Nalidixic acid with *Lepidium sativum* and *Achillea fragrantissima* against *E. coli* (Table 3).

Regarding to activity against *Klebsiella pneumonia* test bacteria, the activity of Aztreonam was increased when combined with *Senecio glaucus*, *Foeniculum vulgare* and *Lycium shawii*. Also, Ceftriaxone activity increased when combined with *Senecio glaucus* and *Lycium shawii*. While Nalidixic acid activity increased with the combination of *Artemisia monosperma* and *Foeniculum vulgare*. In other words, *Senecio glaucus* and *Lycium shawii* increase the activity of Aztreonam and Ceftriaxone. *Foeniculum vulgare* increases the activity of Aztreonam and Nalidixic acid against *Klebsiella pneumonia* (Table 4).

The activity of Cefoxitin was no activity alone or even in combination with medicinal plants against *Klebsiella pneumonia*.

On the other hand, *Lepidium sativum* decreases the activity of Ceftriaxone, Amikacin and Aztreonam against *Klebsiella pneumonia* (Table 4).

As for the activity against *Pseudomonas aeruginosa*, there were no synergistic effects between selected antibiotics and medicinal plants that were noticed. Moreover, the antagonistic effects were achieved between Amikacin and each of *Pituranthos tortuosus*, *Senecio glaucus*, *Lycium shawii*, *Leptadenia pyrotechnica*, *Foeniculum vulgare* and *Lepidium sativum* against *Pseudomonas aeruginosa* (Table 5). There is no activity of Cefoxitin and Nalidixic acid alone or even in combination with tested medicinal plants against *Pseudomonas aeruginosa* (Table 5).

According to activity against *Proteus mirabilis* test bacteria, the activity of Aztreonam increased when combined separately with all selected plant extracts (*Leptadenia pyrotechnica*, *Lycium shawii*, *Senecio glaucus*, *Lepidium sativum*, *Achillea fragrantissima*, *Artemisia monosperma*, *Foeniculum vulgare*, *Trigonella foenum-graecum* and then *Pituranthos tortuosus*). *Lycium shawii* enhances the activity of Aztreonam, Imipenem, Ceftriaxone and Gentamicin (Table 6).

Artemisia monosperma increases the activity of Aztreonam and Imipenem but decreases the activity of Nalidixic acid against *Proteus mirabilis*.

Trigonella foenum-graecum increases the activity of Imipenem against *Proteus mirabilis*. But regarding antagonistic effects, Nalidixic acid activity decreases when combined with *Senecio glaucus*, *Achillea fragrantissima*, *Lycium shawii*, *Pituranthos tortuosus*, *Artemisia monosperma* and *Foeniculum vulgare* (Table 6).

In the case of *Salmonella typhimurium*, there was little synergistic effect between Ceftriaxone and *Lepidium sativum* and between Imipenem and *Achillea fragrantissima* against *Salmonella typhimurium* (Table 7). The selected antibiotics affected antagonistically against *Salmonella typhimurium* by the presence of most tested medicinal plant extracts this clearly appears when Aztreonam, Ceftriaxone and Nalidixic acid antibiotics combined separately with *Senecio glaucus*, *Pituranthos tortuosus*, *Leptadenia pyrotechnica*, *Artemisia monosperma* and *Achillea fragrantissima*. While the activity of Gentamicin and Imipenem is not affected when combined with most selected medicinal plant extracts against *Salmonella typhimurium* (Table 7).

In the case of the *Bacillus cereus* test organism, the activity of Novobiocin and Vancomycin increased when separately combined with *Achillea fragrantissima* and *Artemisia monosperma* against *Bacillus cereus* (Table 8). Antagonistic effect by combination of Vancomycin with *Lycium shawii*. However, the antibiotics Ampicillin, Cefazolin and Oxacillin have little activity or no activity alone or combined separately with the selected plant extracts against *Bacillus cereus* Gram-positive bacteria (Table 8).

Concerning *Staphylococcus aureus* bacteria, the activity of Novobiocin increased when combined with all selected plants except when combined with *Leptadenia pyrotechnica* showed an antagonistic effect against *Staphylococcus aureus* (Table 9). The activity of Ampicillin and Cefazolin increased when combined with *Achillea fragrantissima* and *Artemisia monosperma*. The activity of Cefazolin increased when combined with *Leptadenia pyrotechnica* and *Lycium shawii*. On the other hand, Oxacillin and Vancomycin showed not affected markedly when combined with selected plant extract (Table 9).

Interestingly regarding *Enterococcus faecalis* test bacteria, Chloramphenicol and Novobiocin activity increased markedly with the presence separately of all selected medicinal plants (*Achillea fragrantissima*, *Artemisia monosperma*, *Pituranthos tortuous*, *Foeniculum vulgare*, *Lepidium sativum*, *Leptadenia pyrotechnica*, *Lycium shawii*, *Pituranthos tortuosus*, *Senecio glaucus* and *Trigonella foenum-graecum*) against *Enterococcus faecalis*. In addition, synergistic effect in the case of Ampicillin when combined with *Achillea fragrantissima*, *Artemisia monosperma*, *Pituranthos tortuous* plant extract against the same test organism. On the other hand, *Leptadenia pyrotechnica*, *Pituranthos tortuosus* and *Senecio glaucus* plant extracts increase the activity of Ampicillin, Cefazolin, Chloramphenicol and Novobiocin antibiotics against *Enterococcus faecalis* (Table 10). While Oxacillin showed no activity alone or combined separately with all plant extracts against *Enterococcus faecalis* (Table 10).

Regarding *Staphylococcus epidermidis* test organisms, *Achillea fragrantissima* extract little increases the activity of Cefazolin, Chloramphenicol and Oxacillin. Also, *Artemisia monosperma* extract little increases the activity of Cefazolin and Novobiocin against *Staphylococcus epidermidis* Gram-positive bacteria (Table 11).

Otherwise, the antagonistic effect was achieved by the combination of Chloramphenicol, Vancomycin with the most medicinal plants including *Foeniculum vulgare, Lepidium sativum Senecio glaucus* and *Trigonella foenum-graecum*. *Lycium shawii* and extracts antagonistic with all antibiotics including Cefazolin, Chloramphenicol, Novobiocin, Oxacillin and Vancomycin. In addition to *Foeniculum vulgare* and *Lepidium sativum* reduce the activity of Vancomycin against *Staphylococcus epidermidis* bacteria (Table 11).

DISCUSSION

In the current study, antibiotics studied alone showed a variety of degrees of antibacterial activity against the investigated test organisms, these results agreed with many results in previous studies. The emergence of antibiotic resistance is a major global health concern. Antibiotic resistance occurs when bacteria become resistant to commonly used antibiotics, making it difficult or impossible to treat infections caused by these bacteria[17]. The activity of Novobiocin in this study showed against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus cereus*. Novobiocin is effective against three Gram-positive bacteria including *S. epidermidis*, *S. aureus* and *B. cereus* by inhibiting DNA gyrase enzyme according to binding the ATP-binding site in the ATPase subunit. These three bacteria did not have a

bacteria outer membrane which acts as a permeability barrier. Ampicillin showed the highest activity against *Enterococcus faecalis* agreed with other studies[18-20]. Ampicillin interference with cell wall synthesis by attachment to Penicillin-binding Proteins (PBPs). Most strains of *Enterococcus faecalis* did not have β-lactamase enzyme production, therefore it is sensitive to Ampicillin. Also, in this study Aztreonam has recorded activity against *Salmonella typhimurium*, *E. coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Because Aztreonam has a high affinity for PBPs, it inhibits bacterial cell wall formation and several of these Gram-negative species did not have modified (extended) β-lactamase enzyme. Ceftriaxone and Imipenem showed activity against *Proteus mirabilis* and *Klebsiella pneumoniae*, respectively. Ceftriaxone (third generation of cephalosporins) inhibits the synthesis of mucopeptide bacterial cell wall and it is stable to β-lactamases. By deactivating PBPs, imipenem prevents the cross-linking of peptidoglycan during the production of the cell wall. All these results come to agreement with previous studies[21,22]. Generally, antibiotics are frequently used to treat bacterial infections, which has caused the emergence and spread of resistant bacterial strains. Therefore, it is crucial to discover new antimicrobial agents or treatment techniques that can effectively treat infectious diseases brought on by drug-resistant bacteria[6]. Along with the search for new natural products with antimicrobial activities[6], A novel idea, the interaction between plant extracts and antibiotics has the potential to be either beneficial (synergistic or additive interaction) or harmful (antagonistic or toxic outcome)[23]. Medicinal plants are widely used in traditional medicine for their antimicrobial properties. The use of medicinal plants and antibiotics in combination has been proposed as an alternative to the use of antibiotics alone for the treatment of pathogenic bacteria[24]. In recent years, there has been increasing interest in using combinations of medicinal plants and antibiotics as an alternative to using antibiotics alone. This approach has several potential advantages over traditional antibiotic therapy, including reduced toxicity and improved efficacy against certain bacterial species[25]. This study focused on the efficacy and evaluated the *in vitro* effects of combinations of medicinal plants and antibiotics on bacterial growth and antibiotic resistance. The current study's results revealed synergistic, antagonistic and in different effect interactions as a result of the combination of plant extracts with antibiotics. One of the aminoglycosides in the amikacin family is applied to the treatment of several pathogenic bacteria. An enzymatic alteration carried out by one of the three types of phosphotransferases, acetyltransferase and an enzymatic modification by aminoglycoside-modifying enzymes, which are divided into acetyl-transferases, phosphotransferases and nucleotidyl transferases, is the main defense mechanism against the aminoglycoside group. Amikacin interferes with the reading of the genetic code when it binds to the 30S bacterial ribosome subunit. The main amikacin resistance mechanism is acetylation by the aminoglycoside 6 -N-acetyltransferase type Ib [AAC(6)-Ib], a Gram-negative bacterial gene that codes for this enzyme[26].

In the current study, the activity of Amikacin was increased when combined with most different plant extracts on *E. coli*, this agreed with the study that tested Amikacin against *Acinetobacter* species that also exhibited efflux pump inhibitory activities[27]. Aztreonam is a synthetic antibiotic and it inhibits bacterial cell wall synthesis due to a high affinity to penicillin-binding protein[28]. The synergistic effect was achieved in Aztreonam when combined with all plant extracts, against *Proteus mirabilis* in this study which coincides with the synergistic effects results of plant extracts and Aztreonam, this can be explained by suppression of microbial enzymes and ribosomal structure interference consequently the reductions in protein synthesis and seems related to the synergism profile[29]. The different leaf extracts of some species of *Lycium* (*L. barbarum* and *L. chinense*). Leaves contain important amounts of flavonoids and showed relevant antioxidant activity and antibacterial effects against both Gram-negative and positive bacteria[30]. In this study, *Lycium shawii* increased the activity of Aztreonam, Ceftriaxone, Gentamicin and Imipenem against *Proteus mirabilis*. The synergistic effect remarkably increased in the case of Novobiocin and Vancomycin combined with *Achillea fragrantissima* and *Artemisia monosperma* against *Bacillus cereus*. Also, the synergistic effect was achieved in Ampicillin, Cefazolin and Novobiocin combined with *Achillea fragrantissima* and *Artemisia monosperma* and Novobiocin with all plant extracts against *Staphylococcus aureus*. Several studies approved that, the tested antibiotics, others in the same groups (for instance beta-lactams, aminoglycosides) or even other groups synergistic with extracted plant extracts against gram-positive and negative bacteria regardless of the activity of its extracts[31,32]. These synergistic effects may be caused by certain plant extract constituents changing the efflux pump's activity or they may result from their constituents permeabilizing and depolarizing the bacterial cytoplasmic membrane, which allows the antibiotic to enter the bacterial cell more easily[33,34]. These findings suggested that a combination of plant extract and antibiotics may be effective in battling newly developed drug-resistant bacteria. Amikacin, Aztreonam, Ceftriaxone, Gentamicin and Nalidixic acid showed indifferent effects with most plant extracts

against *Klebsiella pneumonia*. The indifferent effect confirmed other antibiotics with the plant extracts against *Bacillus cereus*. In the other study, the combination between some aqueous plant extracts and ampicillin: Cefotaxime, amikacin and ciprofloxacin antibiotics increase the activity or indifferent effects against multidrug-resistant bacteria[35]. The antagonistic effect was achieved in Amikacin when combined with all plant extracts, while Aztreonam, Ceftriaxone, Gentamicin and Nalidixic acid showed indifferent effects with tested plant extracts against *Pseudomonas aeruginosa*.

The antagonistic effect was achieved in Aztreonam, Ceftriaxone and Nalidixic acid when combined with *Achillea fragrantissima*, *Artemisia monosperma* and *Leptadenia pyrotechnica*, also Aztreonam antagonistic with *Lycium shawii* extract, while indifferent effect achieved in Gentamicin and Imipenem when combined with the most plant extracts against *Salmonella typhimurium*. Nalidixic acid showed antagonistic when combined with most plant extracts against *Proteus mirabilis*. Novobiocin combined with *Leptadenia pyrotechnica* showed remarkable antagonistic activity), but an indifferent effect was achieved with Oxacillin and Vancomycin combined with all plant extract against *Staphylococcus aureus*. This recorded in many studies[36]. However, to explain synergy effects between plant extract bioactive constituents and antibiotics can be produced by the following antimicrobial interactions in one of four ways, (1) By inhibiting several steps in a biochemical pathway, (2) By inhibiting the enzymes that break down antimicrobials, (3) By interfering with the cell wall or (4) By increasing the uptake of other antimicrobials. Additionally, antagonistic behavior is expected to take place in one of three ways, (1) A combination of bacteriostatic and bactericidal antimicrobials is present, (2) Antimicrobials act on the same site (the same mechanism of actions) or (3) Antimicrobials interact with one another[34].

As the bacteria have evolved to resistant antibiotics and this resistance increases day after day. This study concluded that the way to overcome bacterial resistance is necessary to study the synergistic and antagonistic effects of studied antibiotics with selected plants, either to increase the efficiency of the antibiotic or to avoid resistance to pathogenic bacteria. This means the possibility of knowing which plants can improve the effectiveness of antibiotics or prevent the consumption of certain plants at the time of using these antibiotics. On the other hand, it is necessary to know the active compounds in plant extracts to determine the compounds responsible for these effects to use or avoidance of use it.

CONCLUSION

This study concluded that the combinations between antibiotics and plant extract have three interactions: Synergistic, additive and antagonistic. These interactions depend on several parameters including the type of microbes (Gram-positive/negative) and their resistance, antibiotic groups, class and their mechanism of action, plant constituents and concentrations of their active materials. We should not ignore these interactions although they may change over time according to changes in the parameters. Because it has been discovered that synergistic combinations can boost antimicrobial effects and it also prevent potentially harmful antagonistic interactions which will help to overcome the resistance of pathogenic microorganisms. Therefore, more research is required to fully comprehend this strategy's potential advantages and risks.

SIGNIFICANCE STATEMENT

This study introduces understanding the combinations between antibiotics and plant extract which have three interactions: Synergistic, additive and antagonistic, these combinations might overcome the resistance of pathogenic microorganisms and reduce antibiotic side effects. This study explained that the selected antibiotics like Amikacin, Aztreonam, Ceftriaxone, Gentamicin and Nalidixic acid showed different effects when combined with tested plant extracts against specified tested bacteria. On the other hand, some selected plants like *Lycium shawii*, *Achillea fragrantissima*, *Artemisia monosperma* and *Leptadenia pyrotechnica* affected the activity of some tested antibiotics against specified tested bacteria. In terms of future knowledge, use, management and research potential, the findings of this study should be a priority.

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