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SYNERGISTIC EFFECT OF MEDICINAL PLANTS AND ANTIBIOTICS ON PATHOGENIC BACTERIA

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ABSTRACT

Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. Accordingly, searches for new antimicrobial agents are frequent, and medicinal plants have been considered interesting by researchers since they are frequently used in folk medicine as remedies for many infectious diseases. However, the aim of the current study was to verify and validate synergism between antibacterial drugs and medicinal plants. There was a significant synergistic effect between each of *Rhamnus frangula*, *Commiphora molmol*, *Curcuma aromatica* and amikacin, gentamicin and vancomycin against Gram-positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus* and *S. epidirmidis*. However, *Rhamnus frangula* showed the highest synergistic activity with amikacin against *Bacillus subtilis* and *Staphylococcus aureus*. On the other hand, the medicinal plants showed a remarkable synergistic effect with drugs against the Gram-negative bacteria; *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella sonnei*. Interestingly, *Rhamnus frangula* showed the highest synergistic activity with Imipenem against all the investigated Gram-negative bacteria. However, *Calligonum comosum* showed

a significant synergistic activity with the antibiotic drugs against *Proteus vulgaris* and *Shigella sonnei*.

Keywords: Synergistic Effect, Medicinal plants, Antibiotics, Pathogenic Bacteria.

INTRODUCTION

The World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases caused by bacteria and fungi. The discovery of antibiotics was an essential part in controlling bacterial infections that once destroyed humankind. Different antibiotics exhibit their inhibitory activity on different pathogenic organisms. Emerge and spread of resistance to the currently available antibiotics is a worldwide concern.

The increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs **[1]**. Recently, clinically important bacteria are characterized by multiple antibiotic resistance - the consequence of past decades of antimicrobial use and misuse **[2]**. Consequently, the drug resistance presents an ever increasing global health threat that includes all major microbial pathogens and antimicrobial drugs **[3, 4]**.

Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents **[5]**. *Staphylococcus aureus* is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. Methicillin-resistant and multidrug resistant staphylococci have become a major nosocomial pathogen **[6]**. Accordingly, the importance of identifying new effective antimicrobial agents cannot be over-emphasized. Among the potential sources of new agents, medicinal plants have long been investigated **[7]**.

The synergism between antimicrobial agents and plant extracts is a novel concept and has been recently reported **[8-11]**.On the other hand, few studies have found that the efficacy of antimicrobial agents can be improved by combining them with crude plant extracts against different pathogens including *S. aureus*, *P. aeruginosa*, *E. Coli* **[12-15]**. In the current study, we evaluated the possible synergism between some medicinal plants; *Rhamnus frangula*, *Calligonum comosum, Curcuma aromatica*, *Ferula assa-foetida, Commiphora molmol*, and *Alkanna tinctoria* and certain known antibacterial drugs including amikacin, colistin sulphate, gentamicin, vancomycin, nalidixic acid, cerfuroxime and azithromycin which utilized against certain pathogenic bacteria.

MATERIALS AND METHODS

Plant materials and extract preparation: The plant materials used in this study, namely; *Alkanna tinctoria, Calligonum comosum, Curcuma aromatica, Commiphora molmol, Ferula assa-foetida* and *Rhamnus frangula* were obtained from herbal-shop. Ten grams of each grounded plant was mixed with 200 ml of sterilized distilled water by using mixer and then left in room temperature for 24 hr. The large particles of the plant was removed by using the gauze pad and the filtrate was firstly centrifuged at 3000 rpm for 10 minutes and secondly filtered by using filter papers till a clear solution was obtained and then storedin the refrigerator until used **[16]**.

Bacterial Strains: Three Gram- positive (*Bacillus subtilis, Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*) and five Gram-negative (*Escherichia coli, Proteus mirabilis, Proteus vulgaris, Salmonella typhimurium, Shigella sonnei*) were used as test organisms. All strains were clinical isolates from urine, surgical wound and ear swab, a generous gift from King Fahad Medical City, and stored in microbiological collection at the Laboratory of Microbiology (College of Applied Medical Sciences, Prince Sattam Bin AbdulAziz University, Al-Kharj, KSA).

Antimicrobial Drugs: Ten drugs were evaluated for antibacterial synergism assays. These included Amikacin (30 µg), Azithromycin (15 µg), Cefuroxime (30 µg), Colistin sulphate (25 µg), Gentamicin (120 µg), Imipenem (10 µg), Nalidixic acid (30 µg), Penicillin G (10 units) and Vancomycin (30 µg). All these drugs were produced by Mast diagnostics, Mast Group Ltd, Merseyside, UK.

Antibacterial Tests: The bacterial suspensions were prepared from overnight cultures by the direct colony method on Nutrient agar plates (Scharlau, Barcelona, Spain). Colonies were taken directly from the plate and suspend into 5ml of sterile 0.9 % saline. Each isolate suspension was standardized and matched with 0.5 McFarland standards to give a resultant concentration of 1.5×10^8cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby–Bauer diffusion technique **[17],** and according to Clinical and Laboratory Standards Institute guidelines **[18]**. The plant filtrates were separately added to Mueller-Hinton agar during the cooking of medium. The plates were then left to for about one hour to allow proper diffusion before swabbing with the resultant saline suspension of each test organism. The antibiotic discs were transferred onto the plates and incubated at 37 °C for 24 h **[19]**.

However, the antibiotic discs transferred onto plates without plant filtrate were used as control. All determinations were done in duplicate. The plates were examined for zones of inhibition formed around the discs **[17]**.

RESULTS

All the antibiotics used in this study showed an antibacterial activity against the investigated test organisms (Tables 1, 2). However, colistin-sulphate and penicillin G showed no activity against *Bacillus subtilis* and *Staphylococcus aureus*. Also, nalidixic acid and cefuroxime were not active against *E. coli*. *Proteus mirabilis* and *P. vulgaris* showed no sensitivity to colistin-sulphate (Table 2).

The obtained results of the current study revealed that the activity of the antibiotics was remarkably increased with presence of medicinal plants (Tables 3-10). There was a significant synergistic effect between each of *Rhamnus frangula***,** *Commiphora molmol*, *Curcuma aromatica* and the investigated antibacterial drugs against *Bacillus subtilis, Staphylococcus aureus, S. epidirmidis* (Tables 1, 3-5). However, the medicinal plant *Rhamnus frangula* showed the highest synergistic activity with amikacin against the Gram-positive bacteria; *Bacillus subtilis, Staphylococcus aureus,* and *S. epidirmidis.*

On the other hand, the medicinal plants showed a remarkable synergistic effect with drugs against the Gram-negative bacteria; *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Salmonella typhimurium, Shigella sonnei* (Tables 2, 6-10). Interestingly, the medicinal plant *Rhamnus frangula* showed the highest synergistic activity with Imipenem against all the investigated Gram-negative bacteria. However, *Calligonum comosum* showed a significant synergistic activity with the antibiotic drugs against *Proteus vulgaris* and *Shigella sonnei* (Tables 8, 10).

Table 1: Effect of antibiotics on *Bacillus subtilis, Staphylococcus aureus,* and *S. epidermidis*.

Antibiotic / Bacteria	Inhibition zone (mm)				
	Amikacin (30)	**Colistin sulphate (25)**	**Gentamicin (120)**	**Penicillin G (10)**	**Vancomycin (30)**
Bacillus subtilis	24.00	00.00	26.00	00.00	15.00
Staphylococcus aureus	14.00	00.00	19.00	00.00	11.00
Staphylococcus epidirmidis	17.00	07.00	20.00	08.00	11.00

Table 2: Effect of antibiotics on *Escherichia coli, Proteus mirabilis* and *P. vulgaris.*

Antibiotic / Bacteria	Inhibition zone (mm)				
	Nalidixic acid (30)	**Imipenem (10)**	**Cefuroxime (30)**	**Azithro-mycin (15)**	**Colistin sulphate (25)**
Escherichia coli	00.00	25.00	00.00	14.00	11.00
Proteus mirabilis	18.00	29.00	27.00	08.00	00.00
Proteus vulgaris	17.00	28.00	27.00	18.00	00.00
Salmonella typhimurium	20.00	29.00	21.00	21.00	12.00
Shigella sonnei	24.00	29.00	24.00	26.00	13.00

Table 3: Effect of medicinal plants and antibiotics on *Bacillus subtilis*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Amikacin (30)	Colistin sulphate (25)	Gentamicin (120)	Penicillin (10)	Vancomycin (30)
Alkanna tinctoria	17.00	00.00	19.00	00.00	19.00
Calligonum comosum	33.00	11.00	39.00	16.00	28.00
Commiphora molmol	48.00	19.00	32.00	17.00	27.00
Curcuma aromatica	25.00	00.00	36.00	00.00	34.00
Ferula assa-foetida	43.00	22.00	44.00	40.00	32.00
Rhamnus frangula	58.00	21.00	47.00	23.00	44.00

Table 4. Effect of medicinal plants and antibiotics on *Staphylococcus aureus*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Amikacin (30)	Colistin sulphate (25)	Gentamicin (120)	Penicillin (10)	Vancomycin (30)
Alkanna tinctoria	11.00	00.00	14.00	00.00	13.00
Calligonum comosum	31.00	12.00	22.00	10.00	23.00
Commiphora molmol	17.00	00.00	23.00	07.00	18.00
Curcuma aromatica	24.00	08.00	30.00	00.00	20.00
Ferula assa-foetida	16.00	00.00	26.00	00.00	15.00
Rhamnus frangula	54.00	18.00	35.00	22.00	26.00

Table 5. Effect of medicinal plants and antibiotics on *Staphylococcus epidermidis*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Amikacin (30)	Colistin sulphate (25)	Gentamicin (120)	Penicillin (10)	Vancomycin (30)
Alkanna tinctoria	12.00	00.00	15.00	13.00	10.00
Calligonum comosum	29.00	21.00	27.00	17.00	19.00
Commiphora molmol	24.00	08.00	28.00	23.00	19.00
Curcuma aromatica	28.00	13.00	32.00	20.00	23.00
Ferula assa-foetida	22.00	10.00	33.00	27.00	22.00
Rhamnus frangula	49.00	16.00	34.00	20.00	16.00

Table 6. Effect of medicinal plants and antibiotics on *Proteus mirabilis*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Nalidixic acid (30)	Imipenem (10)	Cefuroxime (30)	Azithro-mycin (15)	Colistin sulphate (25)
Alkanna tinctoria	23.00	31.00	33.00	12.00	08.00
Calligonum comosum	26.00	37.00	32.00	15.00	17.00
Commiphora molmol	21.00	32.00	33.00	00.00	00.00
Curcuma aromatica	13.00	27.00	29.00	08.00	10.00
Ferula assa-foetida	25.00	34.00	31.00	11.00	09.00
Rhamnus frangula	41.00	39.00	35.00	19.00	19.00

Table 7. Effect of medicinal plants and antibiotics on *Proteus vulgaris*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Nalidixic acid (30)	Imipenem (10)	Cefuroxime (30)	Azithro-mycin (15)	Colistin sulphate (25)
Alkanna tinctoria	27.00	35.00	29.00	22.00	09.00
Calligonum comosum	38.00	40.00	40.00	29.00	18.00
Commiphora molmol	18.00	29.00	28.00	00.00	00.00
Curcuma aromatica	20.00	31.00	26.00	12.00	00.00
Ferula assa-foetida	23.00	28.00	36.00	18.00	19.00
Rhamnus frangula	48.00	57.00	49.00	39.00	15.00

Table 8. Effect of medicinal plants and antibiotics on *Salmonella typhimurium*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Nalidixic acid (30)	Imipenem (10)	Cefuroxime (30)	Azithro-mycin (15)	Colistin sulphate (25)
Alkanna tinctoria	21.00	35.00	22.00	15.00	09.00
Calligonum comosum	22.00	32.00	24.00	13.00	14.00
Commiphora molmol	19.00	27.00	16.00	12.00	10.00
Curcuma aromatica	22.00	33.00	19.00	18.00	13.00
Ferula assa-foetida	18.00	40.00	18.00	18.00	15.00
Rhamnus frangula	32.00	38.00	27.00	34.00	19.00

Table 9. Effect of medicinal plants and antibiotics on *Shigella sonnei*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Nalidixic acid (30)	Imipenem (10)	Cefuroxime (30)	Azithro-mycin (15)	Colistin sulphate (25)
Alkanna tinctoria	29.00	50.00	30.00	18.00	12.00
Calligonum comosum	51.00	55.00	45.00	38.00	27.00
Commiphora molmol	26.00	36.00	26.00	15.00	13.00
Curcuma aromatica	25.00	38.00	24.00	26.00	15.00
Ferula assa-foetida	26.00	50.00	24.00	20.00	22.00
Rhamnus frangula	23.00	34.00	25.00	00.00	10.00

Table 10. Effect of medicinal plants and antibiotics on *Escherichia coli*.

Antibiotic / Medicinal plant	Inhibition zone (mm)				
	Nalidixic acid (30)	Imipenem (10)	Cefuroxime (30)	Azithro-mycin (15)	Colistin sulphate (25)
Alkanna tinctoria	00.00	32.00	00.00	08.00	09.00
Calligonum comosum	00.00	30.00	00.00	10.00	13.00
Commiphora molmol	00.00	25.00	00.00	07.00	10.00
Curcuma aromatica	07.00	29.00	08.00	12.00	13.00
Ferula assa-foetida	00.00	28.00	00.00	08.00	14.00
Rhamnus frangula	17.00	38.00	11.00	19.00	21.00

DISCUSSION

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant bacterial strains. Accordingly, it is extremely important to find new antimicrobial agents or new methods that are effective for treatment of infectious diseases caused by drug-resistant bacteria **[20]**. The synergism between antibiotics and plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome). Despite the abundant literature about the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been used for clinical use as antibiotics **[21]**.

The results of the current study revealed that there was a synergistic effect resulting from combination of antimicrobial drugs with plant extracts. The *Rhamnus frangula*, *Calligonum comosum*, and *Curcuma aromatica* exhibited synergism with

amikacin, gentamicin and vancomycin drugs against *Bacillus subtilis*, *Staphylococcus aureus*, and *S. epidirmidis*. However, there was a significant synergistic effect for *Rhamnus frangula* with nalidixic acid, imipene and cerfuroxime against *Proteus mirabilis* and *P. vulgaris*. The results obtained in the current study were consistent with previous studies showed the synergistic effect of antibiotics due to combination of different antimicrobial drugs with different crude plant extracts **[10, 11, 13, 15]**. The synergistic effect between antimicrobial agents and plant extracts may be developed due to the possible activities of substances found in plant extract(s) on ribosome structure and bacterial enzymes inhibition **[10**]. However, the obtained results of this study revealed the necessity of more investigation and justification of synergism between antibiotics and plants to overcome the antimicrobial drug-resistance.

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