Short Communication

Multi-Antibiotic Combinations and Antimicrobial Activities of Propolis, Fig Extracts against *Pseudomonas aeruginosa* Isolated from Patients with Pneumonia during the COVID-19 Pandemic

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Abstract

Background: The Gram-negative, non-spore-forming, aerobic bacteria *Pseudomonas aeruginosa* is highly pathogenic, due to its multidrug resistance and its high rates of mutation. The plant extracts that were used are propolis, *Ficus carica*, *Citrullus colocynthis*, etc. Propolis protects itself by producing antimicrobial-containing waxy material. *F. carica* latex is extracted from leaves and trees. *C. colocynthis* contains many bioactive elements. **Objectives:** The aim of this study is to find the most effective plant extract to either eliminate or kill *P. aeruginosa*. **Materials and Methods:** The plant extraction was done using only water, in which the plant was soaked in water that was hot enough, followed by filtration, and finally susceptibility testing was done using the disk diffusion method. **Results:** According to the observed results, among (30) plants, only *C. colocynthis* showed a significant positive result, and the inhibition zone was (15 and 12 mm). **Conclusion:** This finding underscores the potential of *C. colocynthis* as a valuable source of antimicrobial agents with the capacity to combat the highly pathogenic and antibiotic-resistant *P. aeruginosa*. The water extraction method employed in this study provides a simple and accessible approach for harnessing the antimicrobial properties of plants. Further exploration and characterization of the bioactive elements within *C. colocynthis* could pave the way for the development of novel antimicrobial strategies and contribute to the ongoing efforts in addressing antibiotic resistance.

Keywords: Antimicrobial activity, *Citrullus colocynthis*, *Ficus carica*, plant extracts, propolis, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that can mostly affect immunocompromised patients. It possesses multidrug resistance and a high mutation rate. *P. aeruginosa* can grow on medical instruments, especially on ventilation equipment.[1] Besides the high mutation rate and multidrug resistance, it can also adapt to variable conditions, including the host immune system; all these features are harbored on the pathogen's genome.[2,3] Acute infections caused by *P. aeruginosa* can be treated with monotherapy as well as combination therapy.[4,5] Studies that include plant extracts have improved recently, and that is mostly because of their importance in storage of treatments, medications, and products with health benefits and their bioactive elements (carotenoids and polyphenols.).[6,7] Plant extracts have been attracting research attention because of their advantageous biological effects, aside from the fact that plant extracts are environment-friendly and can be used as a safe option.[8-10] This study aims to

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discover the antimicrobial activity of some plants against *P. aeruginosa*.

Materials and Methods

Isolate of *P. aeruginosa*

Samples were collected from the coronavirus disease 2019 (COVID-19) department, from patients with bacteremia.[11]

Collection of the plant material

Propolis was collected from a production company in Turkey; fig latex was obtained from the *Ficus carica* tree in Erbil, and other plant extracts were collected from a spice dealer in Erbil.

Preparation of the plant extracts

Plants, except for propolis and fig, were soaked in hot water overnight.

Preparation and application of propolis and fig latex extracts

Fifty microliters each of propolis and fig latex were transferred directly to different sterilized filter paper disks, both placed on different Mueller–Hinton agar (MHA) plates that were cultured with the isolates of *P. aeruginosa* strains and incubated at 37°C for 24 h.

Preparation and application of black lime *Citrullus colocynthis* extract

Black lime was ground and applied as a powder mixed with hot water in a 1:4 ratio, left overnight, followed by centrifugation. Using a micropipette, 50 µL of the extract was transferred into a sterilized filter paper and placed on MHA cultured with *P. aeruginosa* and then incubated for 24 h at 37°C. The remaining plant extracts were prepared and applied following the same method.

Disk diffusion method

The whole procedure was done under septic conditions using an autoclave; the MHA was prepared, and along with the small circular filter papers, they were sterilized by autoclaving for 1 h at 121°C. The bacterial strains were cultured, and then the filter papers that were soaked with the extracts were placed.

Antibiotic susceptibility method

After inoculating the bacteria on MHA plates, the following antibiotics (meropenem, imipenem, ciprofloxacin, levofloxacin, gentamicin, piperacyclin, and tazobactam) disks were placed on the agar, and after 24 h incubation, the diameter of the inhibition zone is measured around each disk.

Antibiotic combination therapy

500 mg (amikacin) + 500 mg (metronidazole) 500 mg + 2 mL of distilled water.

1 g (piperacillin\ tazobactam) + (meropenem) + 1 mL distilled water.

1 g (ceftazidime) + 1 g (ciprofloxacin) + 2 mL distilled water.

80 mg (gentamycin) + 80 mg (rifampicin) + 2 mL distilled water.

80 mg (clarithromycin) + 80 mg (gentamycin) + 2 mL distilled water.

500 mg (metronidazole) (ceftazidime) (tetracycline) + 1 mL distilled water for each.

1 g (ciprofloxacin) + 1 g (meropenem) + 2 mL of distilled water.

500 g (piperacillin + tazobactam) + zero-point 1 mL of distilled water.

80 g (meropenem) + 80 g (gentamicin) + 2 mL of distilled water.

1 g (amoxicillin) + 1 g (impenem) + 1 mL distilled water.

500 g powder (amipencillin +amikacin) + 2 mL distilled water for each one.

500 mg powder for each (tetracycline + cefazidime) + 1 mL distilled water.

In the end, we centrifuged all combinations for 5 min, and then we took the supernatant [Figure 1].

Control group

To compare the antimicrobial activities, we established a control group, without the treatment (plant extracts), and then compared the control group with the experimental group that received the treatment (plant extracts) to detect any errors in the work, also for visualizing the difference between the two groups and the antimicrobial effects on the experimental group [Figure 2].

Results

Ciprofloxacin, levofloxacin, and azithromycin show results against *Pseudomonas* isolates, in which *Pseudomonas* was resistant to most of them [Figure 3]. This group served as a control group; see Tables 1–3.

Discussion

Propolis is a substance which bees produce by mixing plant exudates with beeswax; it establishes a higher antimicrobial effect against Gram-positive bacteria than Gram-negative.[12,13] In recent studies, the propolis shows no positive results against the two strains; it's either that the Propolis was collected incorrectly or a mistake during

the procedure; also the two strains were highly resistant. In other research, propolis had antimicrobial activity against (*Staphylococcus aureus and Escherichia coli*) but showed no to little antibacterial activity against *P. aeruginosa*.[14]

In comparison with research done in 2020, Iranian propolis was tested against many bacteria which included *P. aeruginosa*; the effect of propolis against *P. aeruginosa* was less compared to that of *S. aureus*.[15] As for another research, the antimicrobial effect of Palestinian propolis against *P. aeruginosa* was too weak, varying from 0.5 to 0.6).[16] The phenolic compounds of Propolis were tested for their antimicrobial effect against *P. aeruginosa* and many other bacteria and significant activity was observed.[17] Propolis was used against clinical *P. aeruginosa* strains, in which it showed 16 positive results among 20.[18] Several studies conducted on the phytochemical features of the *F. carica* leaves showed that the leaves and the fruit itself contain high concentrations of phenol.[19] *F. carica* was used against *P. aeruginosa*. Methanol and chloroform were used in the extraction. Yet the extraction by methanol gave higher inhibition levels. Different parts of *F. carica*, such as leaf extracts, showed higher effectiveness levels with a 12 nm inhibition zone in comparison with other parts of *F. carica*.[20,21]

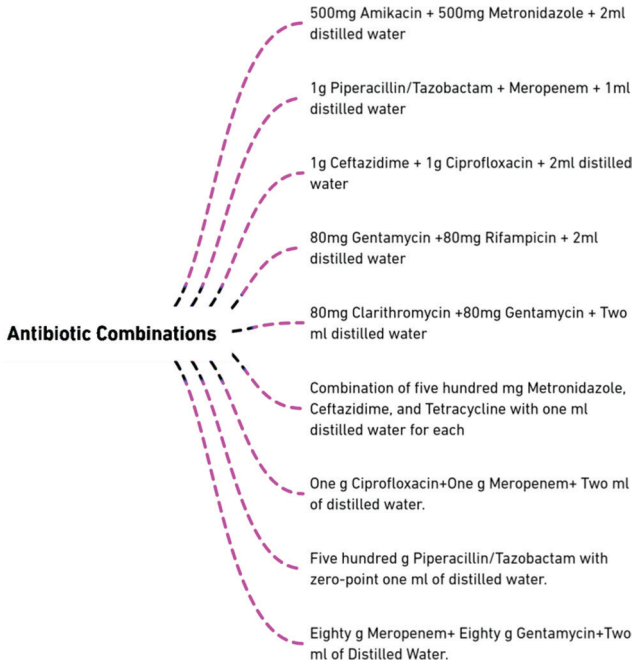

Figure 1: Antibiotic combination therapy

Compared to recent *F. carica* latex results, the *P. aeruginosa* strains were resistant to the *F. carica* latex.

In new research, the leaves extracted by methanol were effective against *P. aeruginosa*, and when mixed with antibiotics, it was even more effective.[22] The phytochemical elements found in *F. carica* were used to aid in the making of calcium oxide nanoparticles; these CaOPs established a noticeable antimicrobial effect against *P. aeruginosa*.[23]

A study that was done in 2023 approved that the extract of *F. carica* branch had an effective antimicrobial effect, in comparison with a recent study in which *F. carica* latex had been used, as the results of using *F. carica* latex against *P. aeruginosa* were negative.[24]

Begum *et al.*[25] proved that the *F. carica* established good results against *P. aeruginosa*. This variation in results

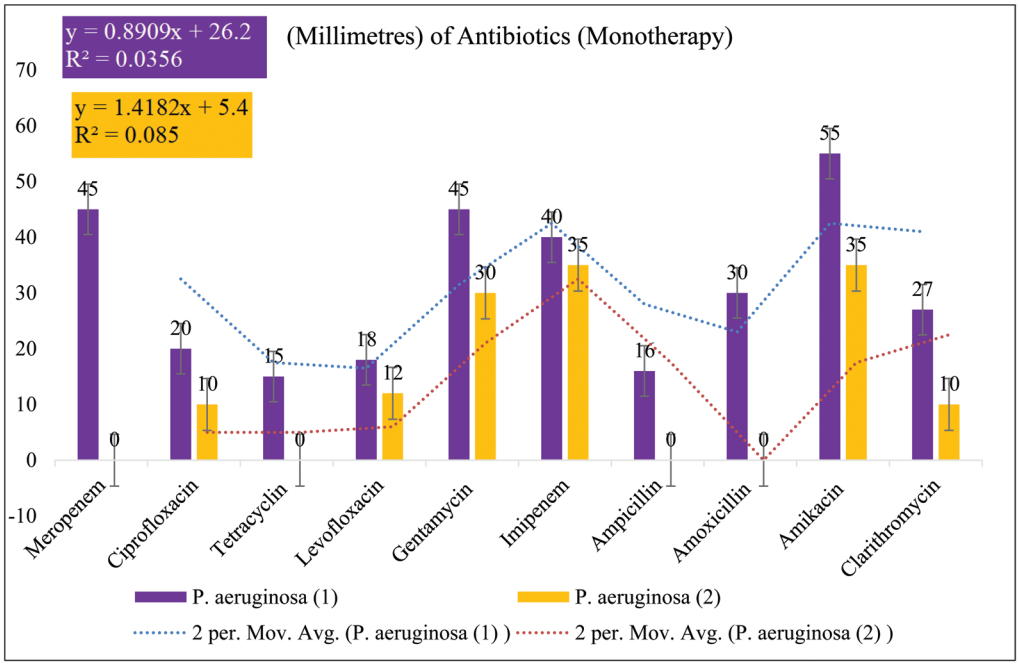

Figure 2: Results in (mm) of antibiotics (monotherapy) against the two strains of *P. aeruginosa*

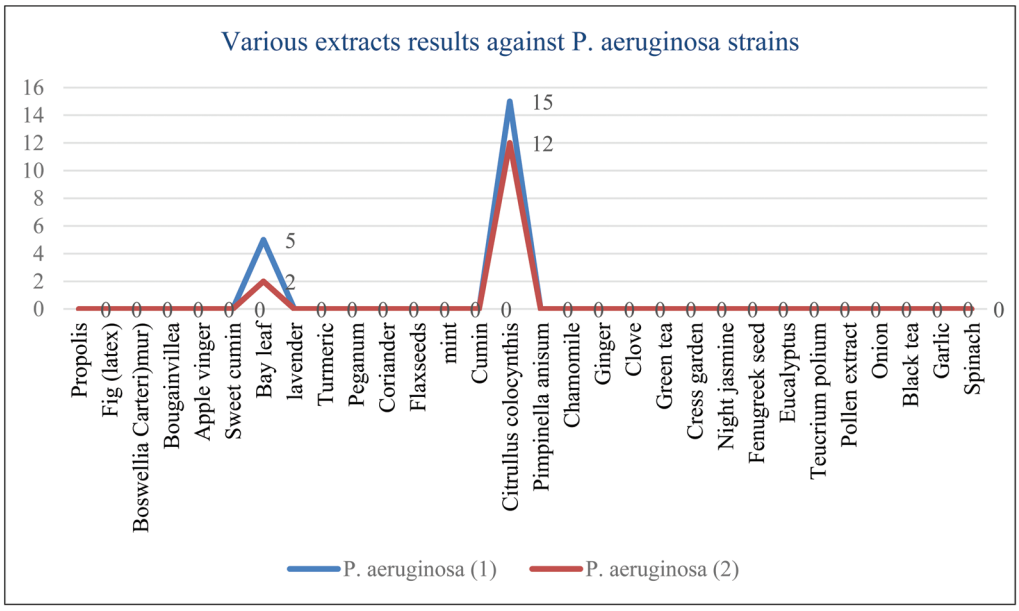

Figure 3: The results of plant extracts in (mm) against the two strains of *P. aeruginosa*, among all the parameters only two plant extracts showed positive results, which are *Citrullus colocynthis* and bay leaf

Table 1: Antibiotics tested against *Pseudomonas*

Antibiotics	*Pseudomonas* strains	Susceptibility (%)	Resistance (%)
Ciprofloxacin	*Pseudomonas 1*	0	100
Levofloxacin	*Pseudomonas 1*	0	100
Azithromycin	*Pseudomonas 1*	5	95
Ciprofloxacin	*Pseudomonas 2*	0	100
Levofloxacin	*Pseudomonas 2*	0	100
Azithromycin	*Pseudomonas 2*	5	95

Table 2. Susceptibility testing against strains of *Pseudomonas aeruginosa* to *F. carica*, propolis, and *Citrullus colocynthis*

Plant extract	Inhibition zone *Pseudomonas 1*	Inhibition zone *Pseudomonas 2*
Propolis	None	None
Fig milk	None	None
Citrullus colocynthis	15 mm	12 mm

may mostly be caused by the use of different methods to prepare the extracts.[25] *C. colocynthis* herb contains an enormous number of elements that improve well-being. It contains flavonoids which act as an antimicrobial.[26,27] This study establishes the activity of *C. colocynthis* against *P. aeruginosa* and other bacteria. The ethanol and aqueous extracts discriminate limited antimicrobial effects against *P. aeruginosa*.[28] In another study, *C. colocynthis* has been used, in it had no noticeable effect on *P. aeruginosa*.[29]

In a recent study, the antimicrobial effect of black lime was tested, in which the results showed that the aqueous *C. colocynthis* extract against *P. aeruginosa* was positive, the inhibition zone for the first strain was 15 mm, while for the second, it was 12 mm. In research that was established in 2021, they used fatty amido benzoic acid made from *C. colocynthis* seeds oil, and its antimicrobial effect against *P. aeruginosa* was tested. The results showed that the FBA had a very little effect against *P. aeruginosa*.[30]

In a comparative study, they used ethanolic, methanolic, and ethyl acetate extracts of *C. colocythis*, as the ethanol inhibited one strain, whereas the ethyl acetate extract inhibited one to six strains.[31,32] The ethanol extract of *C. colocynthis* fruit pulp inhibited Gram-positive bacteria in a higher rate in compression with Gram-negative bacteria.[33] Ethanolic and aqueous extracts of *C. colocynthis* were used against various Gram-negative and Gram-positive bacteria including *P. aeruginosa*, the extract showed a little to moderate effect on *P. aeruginosa*.[34,35]

Conclusion

The investigation into the antimicrobial efficacy of various plant extracts against *P. aeruginosa* has revealed promising results. Among the 30 plant extracts examined, *C. colocynthis* emerged as the most effective, exhibiting substantial inhibition zones of 15 and 12 mm. This finding underscores the potential of *C. colocynthis* as a valuable source of antimicrobial agents with the capacity to combat the highly pathogenic and antibiotic-resistant *P. aeruginosa*. The water extraction method employed in this study provides a simple and accessible approach for harnessing the antimicrobial properties of plants. Further exploration and characterization of the bioactive elements within *C. colocynthis* could pave the way for the development of novel antimicrobial strategies and

Table 3: Antibiotic combinations effect on *Pseudomonas aeruginosa* strain1e and strain 2

Antibiotic combinations	*P. aeruginosa 1* means (mm)	*P. aeruginosa 2* means (mm)	*P* value
Piperacillin + tazobbactam	40	45	0.857
Meropenem + gentamycin	30	22	0.06
Amoxcillin + imipenem	20	30	0.112
Amipencillin + amikacin	35	32	0.211
Tetracyclin + cefazidime	10	9	0.006**
Amikacin + metronidazole	32	30	0.697
(Piperacillin + tazobactam) + meropenem	37	35	0.150
Ceftazidime + ciprofloxacin	20	19	0.343
Gentamycin + rifampicin	30	15	0.011*
Clarithromycin + gentamycin	25	20	0.065
Metronidazole + ceftazidime + tetracycline	12	14	0.005**
Ciprofloxacin + meropenem	40	30	0.865
Imipenem + amikacin	25	23	0.144
Amikacin + ceftazidime	35	25	0.014*
Levofloxacin + imipenem	30	29	0.011*

The values are expressed by mean + SE. Superscript asterisk symbols denoted significant differences when compared with the NC group *significant at $P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. # denoted the significant differences when compared to PC group.
SE, standard error

contribute to the ongoing efforts in addressing antibiotic resistance.

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Conflicts of interest
There are no conflicts of interest.

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