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Piper nigrum Fruit Extract as an Antibiotic Resistance Reversal Agent in MDR Bacteria

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Abstract: Antibiotic resistance development and spread in clinical pathogens is an immense threat that has already outpaced the discovery and development of novel and more effective antibiotic drugs. Recently the focus has been shifted to medicinal plants as novel therapeutic options for reversing antibiotic resistance by targeting different resistance mechanisms. *Piper nigrum* is a plant that has the potential to reverse antibiotic resistance and increase the efficacy of the current drugs. In the present study, seven different antibiotics, clindamycin, gentamicin, levofloxacin, amikacin, tigecycline, imipenem, and tetracycline, were used against antibiotic-resistant *Staphylococcus aureus* and *Salmonella typhi*. Antibiotic resistance reversal analysis was tested by the AST disc method. Increased zones of inhibition of *S. aureus* by four antibiotics, clindamycin (9 mm), gentamicin (7 mm), levofloxacin (9 mm), and amikacin (9 mm) were recorded after using *P. nigrum* extract. In addition, the use of *P. nigrum* extract also increased the zone of inhibition of *S. typhi* with amikacin (11 mm), gentamicin (10 mm), tigecycline (9 mm), levofloxacin (11 mm), and imipenem (10 mm). This study suggests that *P. nigrum* extracts can be used as natural antibiotic resistance reversal agents that increase the effectiveness of current antibiotics and can reverse antibiotic resistance.

Keywords: *Piper nigrum*; antibiotic resistance reversal; multidrug-resistant bacteria; antibiotics

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1. Introduction

Antibiotic discovery was a prodigious revolutionary event in medicine. Soon after the discovery of antibiotics, bacteria started developing resistance to the antibiotics that markedly increased with time. The severity level of hospital and community infection has increased due to multidrug-resistant (MDR) bacteria. MDR bacteria are also called "super-bugs" [1,2]. The overuse and misuse of existing drugs (antibiotics) is the biggest reason for the developing resistance in bacteria to many antibiotics. The severity level of hospital and community infection has increased due to MDR bacteria [3]. The efficacy of these most successful drugs has become threatened by rapidly developing antibiotic resistance and MDR. New antibiotics are constantly being introduced to tackle this issue [4–6]. However, developing new effective drugs is time-consuming, costly, and is already outpaced by rapidly spreading antibiotic resistance [5,7,8].

Antibiotic resistance reversal has recently gained more attention as the reuse of current antibiotics with increased efficiency can be made possible. Many synthetic, semisynthetic, and natural agents help antibiotic resistance reversal [9]. Synthetic agents, such as ethidium bromide, acriflavine, acridine dyes, and sodium dodecyl sulfate, cure plasmid-mediated resistance, but these cannot be used due to their mutagenic and toxic nature [10]. Phytol is a common acyclic isoprenoid. Pristanic acids and phytanic are their natural precursor [11].

The semi-synthetic method of drug development can generate several analogs in which functional groups of natural products are modified. This method provides the best results, with low toxicity and high bioactivity [12].

However, semisynthetic or unnatural agents cannot always be recommended for treating bacterial disease. Various plant extracts and essential oils are reported to be useful as natural antimicrobial agents [13–15]. Many plant-derived compounds are reported to exhibit promising results against MDR bacteria and can reverse antibiotic resistance. However, despite the antimicrobial activity, many plant extracts and essential oils (EO) cannot be considered clinically significant alone [9]. These antimicrobials, if administered with antibiotics, can decrease antibiotic resistance. The combination of drug and plant extract or essential oil can enhance clinical outcomes.

Plant extracts can be used to reverse antibiotic resistance. Plasmid-mediated antibiotic resistance is minimized by eliminating plasmids from bacteria and this process is called as plasmid curing [13]. Secondary compounds, like quinones (consisting of bioactive compounds major class), that are derived from plants show activity against resistance and have the ability to eliminate plasmids [16]. Herbal extracts of *Piper nigrum*, *Zingiber officinale*, *Cinnamomum verum*, *Nigella sativa, Plumbago zeylenica,* etc. contain phenol (eugenol), saponin, naphthoquinones, flavonoids, tannins, and alkaloids, which can be used as the plasmid curing agent [17]. *P. nigrum*, a member of the family Piperaceae, is used for chills, skin disorders, fever, muscle aches, flu, pain relief, asthma, diarrhea, and obesity [18]. *P. nigrum*, known as "the king of spices", helps to decrease inflammation, regulate lipid metabolism and decrease the risk of cardiovascular diseases [19]. North-Eastern people use it as herbal medicine for diseases such as headache, cold, fever, cough, diabetes, skin disease, leprosy, piles, pneumonia, and stomach-ache [20]. Piperine, a primary active compound of *Piper nigrum*, has been reported to increase drug bioavailability. The antibacterial activity of *P. nigrum* petroleum ether extract was confirmed against gram-positive and gram-negative bacterial strains. On fractionation, active petroleum ether extract yielded five compounds, including the isopiperolein B [21], 2E, 4E, 8Z-N-isobutyleicosatrienamide [22], trachyone, pellitorine, and pergumidiene. For this reason, these extracts of *P. nigrum* in petroleum ether are used in (ancient) traditional medicine, because they have antibacterial, antioxidant, hepatoprotective, and antifungal activities [23–25].

The current project focuses on exploring the potential of *P. nigrum* as a natural antibiotic reversal agent. *P. nigrum*, commonly known as black pepper, contains different therapeutic compounds, which include flavonoids, minerals, carotenoids, and vitamins. There are many biologically active compounds, which include sabinene, β-caryophyllene, α-copanene, limonene, and α-pinene [26]. *P. nigrum* can be used for the redressal of antibiotic resistance caused by multidrug-resistant bacteria.

2. Materials and Methods

2.1. Plant Material and Herbarium Number

Plant material of *P. nigrum* was procured from the local market in Lahore, Pakistan.

It was authenticated and preserved in the herbarium vide Voucher *Piper nigrum* "L.GC.Herb.Bot.3262", at the Department of Botany Government College University Lahore, Pakistan.

2.2. Plant Extract Preparation

2.2.1. Cleaning of Plant Material

Plant material was first washed with tap water three times, then dried in an oven, at 40 °C, for 48 h. When the plant material was completely dried, it was ground to obtain the powder. Then, powder was sieved through a sieve (pore size 1.17 mm).

2.2.2. Extraction and Fractionation

Liquid–liquid extraction was used for the fractionation and preparation of six different *P. nigrum* extracts, i.e., aqueous *Piper nigrum* (APN), methanolic *Piper nigrum* (MPN),

n-hexane *Piper nigrum* (n-HPN), chloroform *Piper nigrum* (CPN), ethyl acetate *Piper nigrum* (EAPN), and *n*-butanol *Piper nigrum* (n-BPN), according to the method described by Mushtaq et al. [27]. The extraction fractionation scheme is shown in Figure 1.

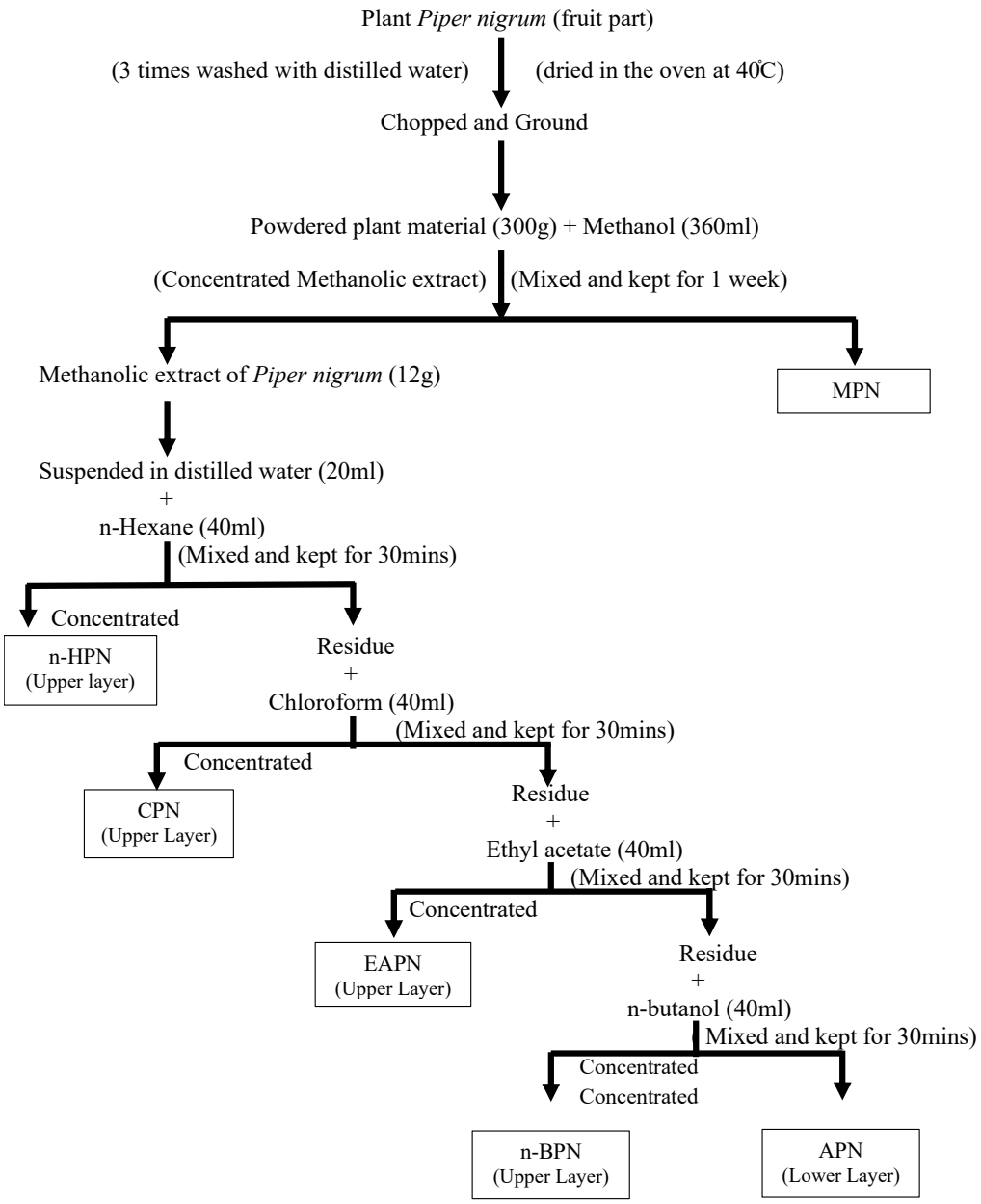

Figure 1. The schematic method used to prepare plant extracts in the different solvents.

2.2.3. Bacterial Strain

Staphylococcus aureus and *Salmonella typhi* bacterial strains were obtained from the culture collection of the Department of Microbiology, Jinnah Hospital, Lahore, Pakistan and were confirmed by standard biochemical tests [28]. *S. aureus* and *S. typhi*, with known susceptibility to the tested antibiotic, were used as controls, according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). On Mannitol Salt Agar (MSA) the strains were maintained at 4 °C temperature. Strains were subcultured on Mueller–Hinton Agar (MHA) for 24 h before each test.

2.3. Antibiotic Susceptibility Test (AST)

For the confirmation of resistant strains, an antibiotic susceptibility test (AST) was performed on Mueller–Hinton agar (MHA) plates by using the Kirby-Bauer disc diffusion method following the guidelines of the Clinical and Laboratory Standards Institute [29,30]. A well-isolated colony from the plate was aseptically emulsified in sterile 0.85% saline solution and then diluted in a 1:10 ratio, to match the turbidity of the (bacterial) suspension, with 0.5 McFarland standards. A sterile cotton swab was dipped into the bacterial suspension and streaked on the Mueller–Hinton agar (MHA) plate for a lawn of growth. Then, the plate was dried for 5 min. After this step, the antibiotic disc was placed on the surface of agar medium with the help of simple forceps and gently pressed. Then, plates were incubated at 37 °C for 24 h. After incubation, a metric ruler was used for the measurement the zones of inhibition of each antibiotic. The test organisms' zones of inhibition were compared with the standard table provided by the Clinical and Laboratory Standards Institute (2019) [29,30].

2.4. Antibiotic Resistance Reversal Activity

The susceptibility of two isolates of *S. aureus* and *S. typhi* to different seven antibiotics was tested. For checking the reversal of antibiotic resistance, the plant extract was used. Mueller–Hinton agar (MHA) was prepared. During the pouring step, 1 mL plant extracts were added to a Petri plate and mixed with Mueller–Hinton agar (MHA). There were a total of 6 plant extracts and 6 mixed Mueller–Hinton agar (MHA) media plates prepared for each bacterial strain. Then, from pure culture, a bacterial colony was picked and emulsified in 0.85% saline solution. The turbidity of 0.85% bacterial saline suspension was matched with 0.5 McFarland standards and spread on plant-mixed Mueller–Hinton agar (MHA) media plates. Then the plate was dried for 5 min. After this step, the antibiotic disc was placed on the surface of the agar with the help of forceps and gently pressed. Cefoxitin, erythromycin, clindamycin, levofloxacin, ciprofloxacin, amikacin, and gentamicin were used for *S. aureus* strain susceptibility, and ceftazidime, tigecycline, levofloxacin, ciprofloxacin, amikacin, gentamicin, and imipenem for *S. typhi* strain susceptibility. Then plates were incubated at 37 °C for 24 h. Then, after incubation, a metric ruler was used for the measurement of the zones of inhibition of each antibiotic. The test organisms' zones of inhibition were compared with the standard table provided by the Clinical and Laboratory Standards Institute (2019) [29,30]. The difference in the zones before using plant extract (antibiotics alone) and after plant extract were used as control. All experiments were run in triplicate.

3. Results

3.1. Antibiotic Susceptibility

The antibiotic susceptibility test confirmed the antibiotic resistance of both strains. For *S. aureus,* the inhibition zones of cefoxitin, erythromycin, clindamycin, levofloxacin, ciprofloxacin, amikacin, and gentamicin were 12, 15, 20, 14, 11, 14 and 15 mm, respectively (Table 1); whereas, for *S. typhi,* the inhibition zones by ceftazidime, tigecycline, levofloxacin, ciprofloxacin, amikacin, gentamicin, and imipenem were 11, 11, 15, 14, 17, 16, and 25 mm, respectively (Table 2).

The zone of inhibition of each antibiotic for tested strains was compared with the standard table provided by CLSI [29] and found to be in the intermediate-to-resistant zone, indicating that the test organisms were antibiotic-resistant.

3.2. Antibiotic Resistance Reversal of S. aureus by P. nigrum Extracts

Antibiotic resistance reversal (prepared by fractionation in six different solvents) was investigated using these extracts in combination with antibiotics.

Table 1. Antibiotic susceptibility of *S. aureus*.

Antibiotics Tested (µg)	Zone of Inhibition for Test Strain (mm)	Zone of Inhibition According to CLSI (mm) [29,30]		
		Sensitive	Intermediate	Resistant
Cefoxitin (30)	12	≥25	-	≤20
Erythromycin (15)	15	≥23	14–22	≤13
Clindamycin (10)	20	≥21	15–20	≤14
Levofloxacin (5)	14	≥19	16–18	≤15
Ciprofloxacin (5)	11	≥21	16–20	≤15
Amikacin (30)	14	≥17	13–14	≤12
Gentamicin (10)	15	≥15	13–14	≤12

Table 2. Antibiotic susceptibility test results of *S. typhi*.

Antibiotics Tested (µg)	Zone of Inhibition for Test Strain (mm)	Zone of Inhibition According to CLSI (mm) [29,30]		
		Sensitive	Intermediate	Resistant
Ceftazidime (30)	11	≥26	23–25	≤22
Tigecycline (10)	11	≥16	-	≤12
Levofloxacin (5)	15	≥19	14–16	≤13
Ciprofloxacin (5)	14	≥21	16–20	≤15
Amikacin (30)	17	≥17	13–14	≤12
Gentamicin (10)	16	≥15	13–14	≤12
Imipenem (10)	25	≥23	20–22	≤19

3.2.1. Cefoxitin

For *S. aureus*, the zone of inhibition of cefoxitin (Cfx) was 12 mm (Figure 2), whereas the inhibition zone of *S. aureus* is ≥25 for cefoxitin according to CLSI standards (Table 1). By using plant extracts of *Piper nigrum*, the *S. aureus* zone of inhibition increased to a maximum of 18 mm with MPN + Cfx, whereas zones of inhibition of Cfx in combination with n-HPN, CPN, EAPN, n-BPN, and APN treatments increased in the range of about 1 to 2 mm (Figure 2).

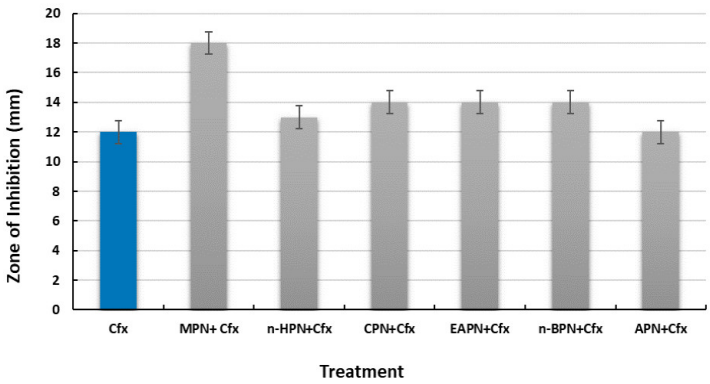

Figure 2. Antibiotic reversal activity of *P. nigrum* extracts in combination with cefoxitin (30 µg) for *S. aureus*.

3.2.2. Erythromycin

For *S. aureus*, the zone of inhibition of erythromycin (Ery) was 15 mm (Figure 3), and the inhibition zone of *S. aureus* is reported to be ≥ 23 for erythromycin (CLSI standards, Table 1). However, when erythromycin was used in combination with APN, the zone of inhibition increased by 19 mm, whereas methanolic *P. nigrum* extract (MPN) increased the zone of inhibition maximum of 20 mm when used with erythromycin. At the same time, EAPN + Ery increased the zone of inhibition by 19 mm. The zone of inhibition for all the other extracts (n-HPN, CPN, and n-BPN) increased in the range of 15 to 16 mm when combined with erythromycin.

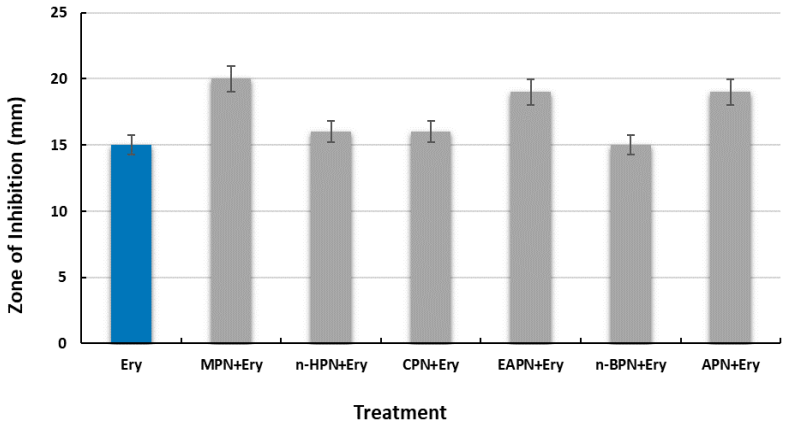

Figure 3. Antibiotic reversal activity of *P. nigrum* extracts in combination with erythromycin (15 µg) for *S. aureus*.

3.2.3. Clindamycin

The inhibition zone of *S. aureus* is ≥21 mm for clindamycin (Cli) according to CLSI standards (Table 1). For the *S. aureus* resistant strain, the zone of inhibition of Cli was recorded as 20 mm (Figure 4) without plant extract. By using APN *P. nigrum* extract in combination with Cli the zone of inhibition increased to a maximum of 29 mm. MPN + Cli increased the zone of inhibition by 27 mm, which was clinically significant. The remaining extracts of n-HPN, CPN, EAPN, and n-BPN increased the zone to 24 mm in the presence of the antibiotic clindamycin.

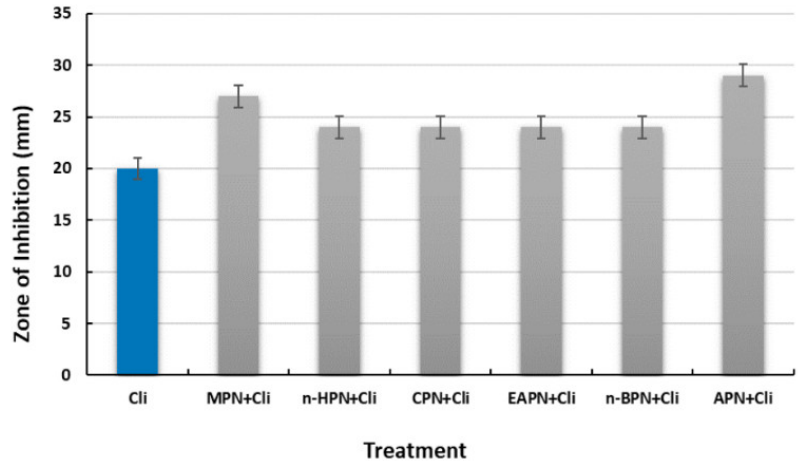

Figure 4. Antibiotic reversal activity of *P. nigrum* extracts in combination with clindamycin (10 µg) for *S. aureus*.

3.2.4. Levofloxacin

For *S. aureus*, the zone of inhibition of levofloxacin (Lxv) was 14 mm (Figure 5), whereas the inhibition zone of *S. aureus* is ≥ 19 mm for Lvx, according to CLSI standards (Table 1). By using *P. nigrum* extract, the APN + Lvx zone of inhibition increased to 23 mm. *P. nigrum* MPN + Lvx increased the inhibition zone to about 22 mm, and EAPN + Lvx increased it to 20 mm. The CPN + Lvx zone of inhibition increased to 17 mm, and the n-BPN + Lvx zone of inhibition increased to 16 mm.

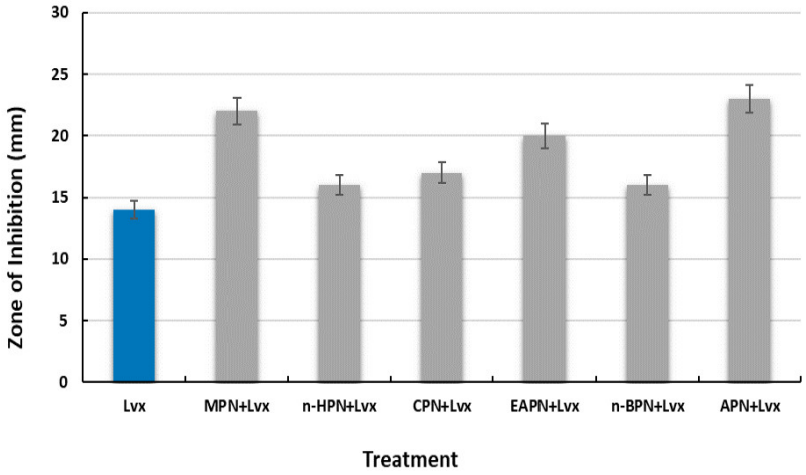

Figure 5. Antibiotic reversal activity of *P. nigrum* extracts in combination with levofloxacin (5 µg) for *S. aureus*.

3.2.5. Ciprofloxacin

The inhibition zone of *S. aureus* is ≥21 mm for ciprofloxacin (Cpfx) according to CLSI standards (Table 1), whereas, for *S. aureus,* the zone of inhibition of Cpfx was 11 mm (Figure 6). By using *P. nigrum* extracts in combination the zone of inhibition increased to a maximum of 15 mm with EAPN, which was not significant because the value falls within the resistant zone. Cpfx with MPN, n-HPN, CPN, n-BPN and APN increased the zones by just 1 to 2 mm.

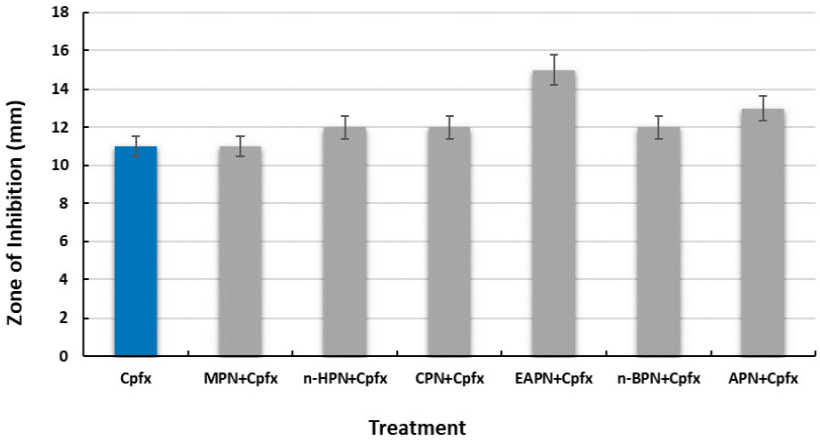

Figure 6. Antibiotic reversal activity of *P. nigrum* extracts in combination with ciprofloxacin (5 µg) for *S. aureus*.

3.2.6. Amikacin

For *S. aureus,* the zone of inhibition of amikacin (Amk) was 14 mm (Figure 7), whereas the inhibition zone of *S. aureus* is ≥17 mm for Amk, according to CLSI standards (Table 1). By using APN *P. nigrum* extract with amikacin, the maximum zone of inhibition obtained

was 23 mm, which was significant because the value falls in the sensitive zone. The CPN zone of inhibition increased by 20 mm and the n-HPN zone of inhibition increased by 17 mm. The MPN, EAPN, and n-BPN zones of inhibition increased by just 1 to 2 mm.

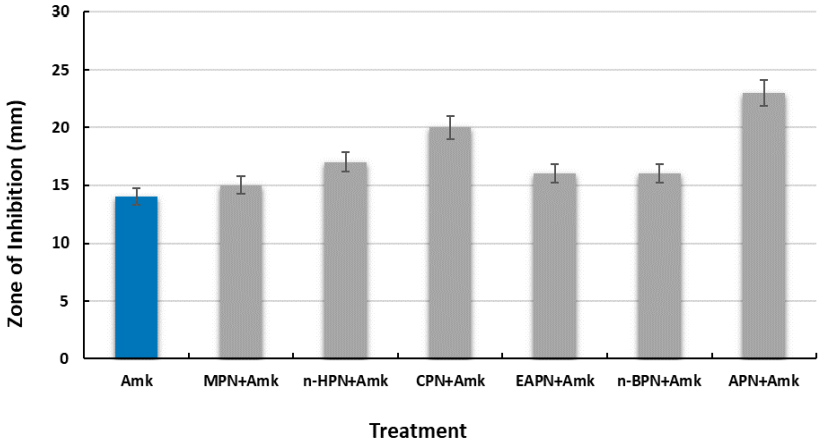

Figure 7. Antibiotic reversal activity of *P. nigrum* extracts in combination with amikacin (30 µg) for *S. aureus*.

3.2.7. Gentamicin

According to CLSI standards, the zone of inhibition of *S. aureus* by gentamicin (Gent) is ≥15 mm (Table 1). In the present work, *S. aureus* also showed a 15 mm zone of inhibition by Gent (Figure 8). Using plant extracts, the *P. nigrum* extract zone of inhibition increased to a maximum of 22 mm with EAPN, which was very significant; whereas the MPN and APN zones of inhibition increased to 20 mm and the n-BPN zone of inhibition increased to 18 mm. The n-HPN, CPN, and n-BPN zones of inhibition increased to 17 mm.

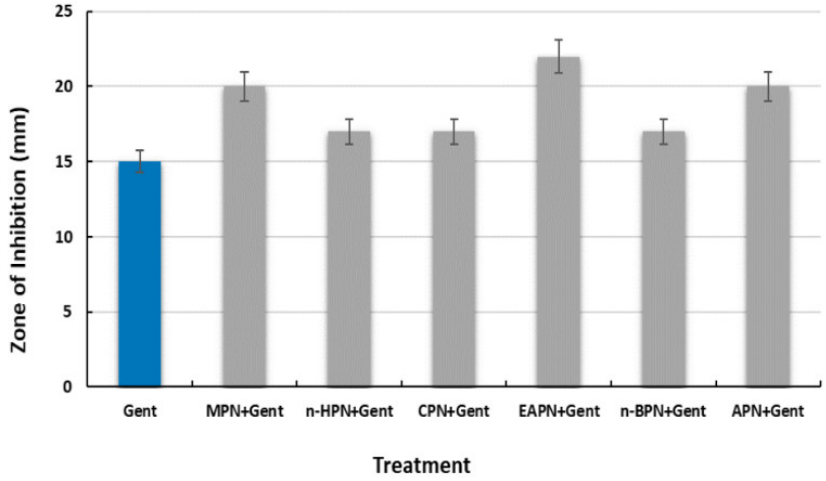

Figure 8. Antibiotic reversal activity of *P. nigrum* extracts in combination with gentamicin (10 µg) for *S. aureus*.

All the values on the Figures 2 and 4–8 are means of three parallel replicates. Error bars indicate the standard error of the mean.

3.3. Antibiotic Resistance Reversal of S. typhi by P. nigrum Extracts

3.3.1. Amikacin

In the case of *S. typhi*, the zone of inhibition of amikacin (Amk) was 17 mm (Figure 9), whereas the inhibition zone of *S. typhi* is ≥17 mm for amikacin according to CLSI standards (Table 2). By using plant extracts of *P. nigrum*, the zone of inhibition increased to a maximum

of 28 mm with MPN, which was significant because the value falls within the sensitive zone. The plant extract n-HPN zone of inhibition increased to 24 mm, and the APN zone of inhibition increased to 23 mm; the n-HPN, CPN, and EAPN zones of inhibition increased to 19 mm.

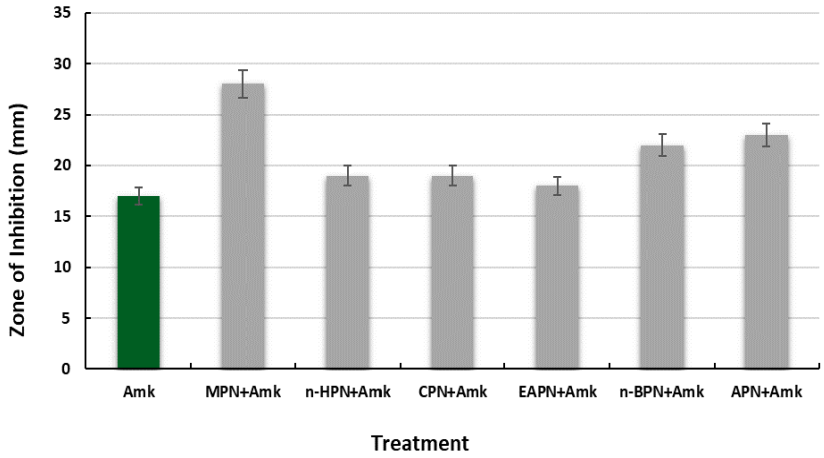

Figure 9. Antibiotic reversal activity of *P. nigrum* extracts in combination with amikacin (30 µg) for *S. typhi*.

3.3.2. Gentamicin

In the case of *S. typhi*, the zone of inhibition of gentamicin (Gen) was 16 mm (Figure 10), whereas the inhibition zone of *S. typhi* recorded according to the CLSI standard is ≥15 mm (Table 2). By using *P. nigrum* extracts along with Gen, the zone of inhibition increased to a maximum of 26 mm with MPN, which was very significant. CPN and EAPN extracts, when supplemented with gentamicin, increased the zones of inhibition to about 18 mm, whereas the remaining extracts, n-HPN, n-BPN, and APN in combination with gentamycin, increased the inhibition zones to between 17 and 18 mm.

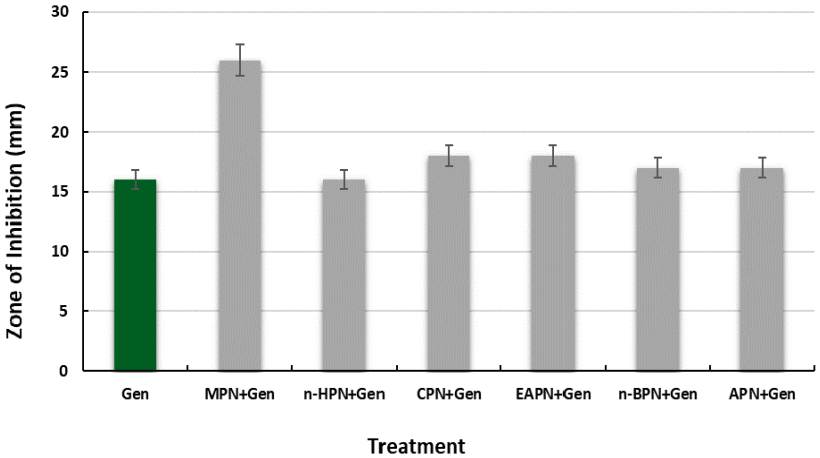

Figure 10. Antibiotic reversal activity of *P. nigrum* extracts in combination with gentamicin (10 µg) for *S. typhi*.

3.3.3. Ciprofloxacin

In the case of *S. typhi*, the zone of inhibition of ciprofloxacin (Cpfx) was 14 mm (Figure 11) and the inhibition zone is ≥21 mm for *S. typhi* by ciprofloxacin according to CLSI standards (Table 2). By using *P. nigrum* extract + Cpfx, the zone of inhibition was increased to a maximum of 20 mm with MPN. EAPN + Cpfx zones of inhibition increased to about 19 mm, while the n-HPN + Cpfx zone of inhibition increased to about 18 mm. The

zones of inhibition for the remaining combinations, i.e., CPN + Cpfx, n-BPN + Cpfx, and APN + Cpfx, were increased to about 16–17 mm.

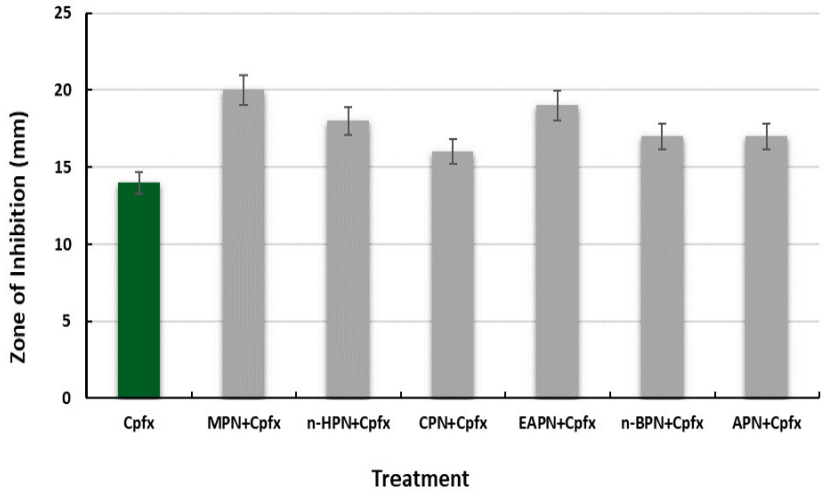

Figure 11. Antibiotic reversal activity of *P. nigrum* extracts in combination with ciprofloxacin (5 µg) for *S. typhi*.

3.3.4. Tigecycline

In the case of *S. typhi*, the zone of inhibition of tigecycline (TGC) was 11 mm (Figure 12), whereas the inhibition zone of *S. typhi* is ≥16 mm for tigecycline (TGC) according to CLSI standards (Table 2). By using *P. nigrum* extracts with tigecycline the zone of inhibition was increased up to a maximum of 20 mm with EAPN, n-HPN, and n-BPN. The MPN, CPN, n-BPN, and APN zones of inhibition were about 18 mm.

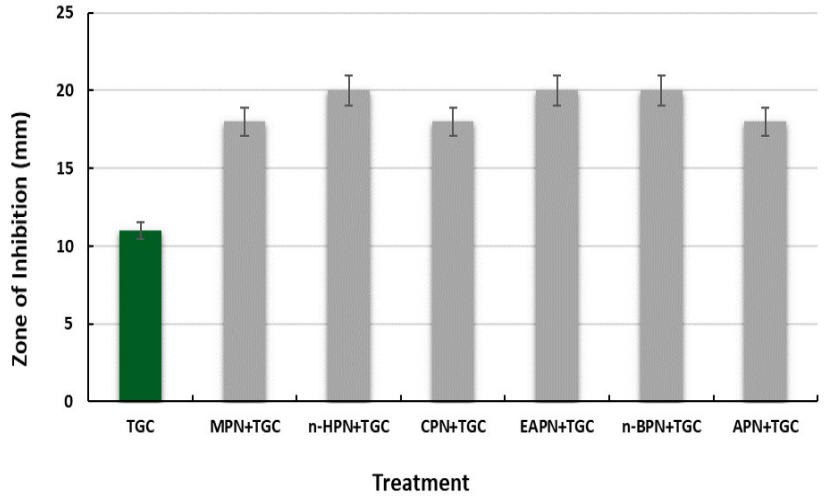

Figure 12. Antibiotic reversal activity of *P. nigrum* extracts in combination with tigecycline (10 µg) for *S. typhi*.

3.3.5. Levofloxacin

In the case of *S. typhi*, the zone of inhibition of levofloxacin (Lvx) was 15 mm (Figure 13), whereas the inhibition zone of *S. typhi* is ≥ 17 mm for levofloxacin according to CLSI standards (Table 2). Using plant extracts of *P. nigrum*, the zone of inhibition increased to a maximum of 26 mm with MPN, which is clinically significant because the value falls in the sensitive zone. The EAPN zone of inhibition was increased to 22 mm and the CPN and n-HPN zones of inhibition were increased to 21 mm. The n-BPN and APN zones of inhibition were increased to 20 mm.

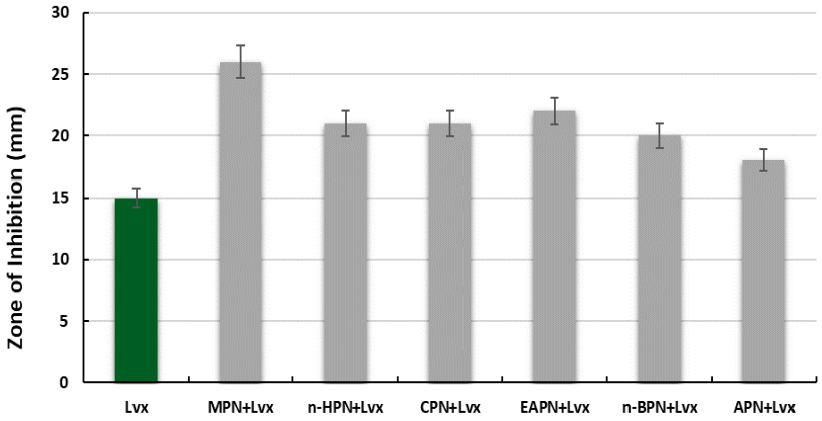

Figure 13. Antibiotic reversal activity of *P. nigrum* extracts in combination with levofloxacin (5 µg) for *S. typhi*.

3.3.6. Imipenem

In the case of *S. typhi,* the zone of inhibition of imipenem (Imp) was 25 mm (Figure 14), whereas the inhibition zone of *S. typhi* is ≥23 mm for imipenem according to CLSI standards (Table 2). EAPN extract with Imp showed the maximum zone of inhibition (35 mm), which was significant because the value falls within the sensitive zone. The APN zone of inhibition was increased to about 29 mm while the MPN and n-BPL zones of inhibition were increased to about 28 mm. The remaining extracts', viz., n-HPN, CPN, and n-BPN, zones of inhibition were increased only to between 25 and26 mm.

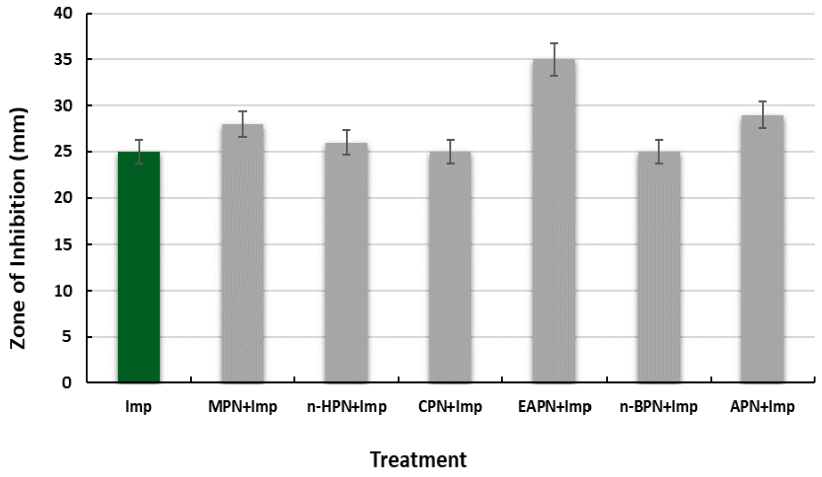

Figure 14. Antibiotic reversal activity of *P. nigrum* extracts in combination with Imipenem (10 µg) for *S. typhi*.

3.3.7. Cefotaxime

The inhibition zone of *S. typhi*, according to CLSI is ≥26 mm for cefotaxime (Table 2). In the case of *S. typhi,* the zone of inhibition of cefotaxime (Ctx) was 6 mm (Figure 15). Using extracts of *P. nigrum* supplemented with cefotaxime, MPN, n-HPN, CPN, EAPN, n-BPN, and APN did not show any increase in the zone of inhibition for *S. typhi*.

All the values on the Figures 9–15 are means of three parallel replicates. Error bars indicate the standard error of the mean.

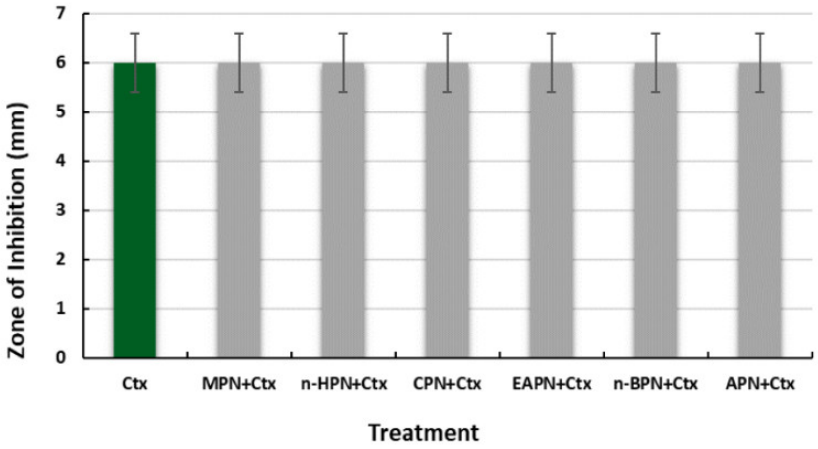

Figure 15. Antibiotic reversal activity of *P. nigrum* extracts in combination with cefotaxime (30 µg) for *S. typhi*.

4. Discussion

P. nigrum is a plant of medicinal importance. The extracts of *P. nigrum* in different solvents by fractionation contain many active components [18,21–23] that can be used for antibiotic resistance reversal.

Erythromycin belongs to the macrolides class of antibiotics, clindamycin belongs to the lincosamide group of antibiotics, amikacin belongs to aminoglycosides, and gentamicin belongs to the aminoglycosides class of antibiotics, which inhibit bacterial growth by inhibiting protein synthesis [30,31]. In the present work, the use of *P. nigrum* extracts supplemented with different antibiotics on *S. aureus* growth revealed that the plant extracts increased the zone of inhibition to a significant level. According to the phytochemical analysis of *P. nigrum*, it contains flavonoids, glycosides, alkaloids, and tannins that have resistance reversal activity [9] and antioxidant activity [32].

Levofloxacin is a DNA synthesis inhibitor, and it belongs to the fluoroquinolones class, 2nd generation [31]. Levofloxacin resistance is developing due to environmental factors [30]. However, this resistance can be reversed by using compounds from natural plant sources. Our results showed that, with the help of *P. nigrum* extracts, MPN, and APN, in combination with levofloxacin, *S. aureus* growth inhibition increased to a significant level, indicating the antibiotic reversal activity of this extract. However, ciprofloxacin belongs to fluoroquinolones, which inhibit DNA synthesis in bacterial cells and the growth of bacteria [31]. By using plant extracts, the zone of inhibition of *S. aureus* was not increased as much, so these extracts did not reverse the resistance of ciprofloxacin in *S. aureus* [9,30] because these extracts have a very low effect on the DNA synthesis process. Cefoxitin belongs to the class of beta-lactam antibiotics. It kills bacteria by inhibiting the process of cell wall synthesis [31]. No significant increase in the cefoxitin zone of inhibition of *S. aureus* was observed compared to other antibiotics.

The effect of these extracts supplemented with different antibiotics was also evaluated for antibiotic resistance reversal with *Salmonella typhi*. Amikacin and gentamicin belong to the aminoglycosides. Tigecycline is a broad-spectrum drug belonging to the antibiotic class glycylcycline, derived from the tetracycline class; it inhibits bacterial growth by inhibiting the protein synthesis mechanism in the bacterial cell [31]. Using *P. nigrum* extracts in combination with these antibiotics, the zone of inhibition of *S. typhi* significantly increased. *P. nigrum* contains volatile compounds, monoterpenes and sesquiterpenes, which have antibacterial, antioxidant, antifungal, anti-asthmatic, and anti-carcinogenic properties. These plant extracts help antibiotics in the killing of bacteria [33].

Tigecycline susceptibility decreases due to the development of resistance by ribosomal protection and an active efflux pump mechanism [31]. By using *P. nigrum* extracts with tigecycline, the zone of inhibition was increased with EAPN, n-HPN, n-BPN, MPN, CPN, n-BPN, and APN. Therefore, the significant results obtained in the present study suggest

that these plant extracts may affect the efflux pump mechanism in *S. typhi*. It has been suggested that these plant extracts block the efflux pump and facilitate the drug's ability to kill bacteria [34]. According to the phytochemical analysis of *P. nigrum*, it contains many components, which include flavonoids, glycosides, alkaloids, and tannins, that help in the reversal of antibiotic resistance [9] by blocking the efflux pump in the bacterial cell.

Ciprofloxacin belongs to fluoroquinolones, which inhibit DNA synthesis in bacterial cells and the growth of bacteria [31]. The present study suggested that *P. nigrum* extracts have a very low effect on the reversal of ciprofloxacin resistance in *S. typhi*. Levofloxacin is also a DNA synthesis inhibitor and belongs to the fluoroquinolones class, 2nd generation [31].

Levofloxacin resistance has been developing due to environmental factors [30]. However, using natural plant sources, resistance can be reversed. This result suggested that, with the help of plant extracts, the zone of inhibition increased to a significant level [35]. Our results showed that, with the help of MPN and EAPN *P. nigrum* extracts in combination with levofloxacin, *S. typhi* growth inhibition increased significantly, indicating the antibiotic reversal activity of these extracts; this could be because *P. nigrum* contains ingredients that help the antibiotic kill bacteria by inhibiting DNA synthesis [9,36].

Imipenem belongs to the carbapenems group of antibiotics, which inhibit bacterial growth by inhibiting cell wall synthesis. Imipenem is a broad-spectrum antibiotic [31]. The present study suggested that these *P. nigrum* extracts, in combination with imipenem, help in the reversal of antibiotic resistance in *S. typhi*.

All antibiotics have different mechanisms of action for killing bacteria. *P. nigrum* extracts have different effects with each antibiotic due to the different compounds present in each extract. *P. nigrum* phytochemical analysis explained that flavonoids, glycosides, alkaloids, and tannins are present in the plants; these compounds have resistance reversal [9] and antioxidant activity [32], which helps antibiotics to kill bacteria by blocking the efflux pumps of the resistant bacteria [34].

The use of plant extracts in combination with antibiotics can reverse antibiotic resistance and it helps us to use old antibiotics and increase the effectiveness of current drugs. A new kind of drug could be developed in which plant extract along with antibiotics can be used for the treatment of bacterial diseases.

5. Conclusions

The extracts of *P. nigrum* could reverse antibiotic resistance in *S. typhi* and *S. aureus* for selective antibiotics when used in combination with them. The extracts of *P. nigrum* have successfully reversed resistance to the antibiotics clindamycin, gentamicin, levofloxacin, amikacin, tigecycline, levofloxacin, and imipenem. Further elaborate phytochemical analysis and detailed active component analysis needs to be carried out. This will not only help with the use of old antibiotics and increase the effectiveness of current drugs but also to develop a new kind of combinational drug therapy that can be used for the treatment of bacterial diseases.

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