Evaluation of the Interaction between the *Poincianella pyramidalis* (Tul.) LP Queiroz Extract and Antimicrobials Using Biological and Analytical Models

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Abstract

*Poincianella pyramidalis* (Tul.) LP Queiroz (Fabaceae) is an endemic tree of northeastern Brazil, occurring mainly in the Caatinga. Its medicinal use is widespread and is an important therapeutic option against diarrhea, dysentery, and respiratory and urinary infections, among other diseases. In this study we determined the chemical marker and evaluated the interaction between *P. pyramidalis* extract and a commercial antimicrobial through the use of biological and analytical models. To obtain the extract, an ethanol-water mixture (50:50 v/v) was used as solvent. It was nebulized in a spray dryer using colloidal silicon dioxide as a drying adjuvant. The extract (ENPp) was subjected to HPLC analysis to verify the presence of certain secondary metabolites. The Minimum Inhibitory Concentration (MIC) of the extract against Gram-negative bacteria was determined by broth microdilution and the MIC of synthetic antimicrobial drugs in the presence and absence of the extract. The antioxidant activity of ENPp was evaluated by the DPPH method. The compatibility between the antimicrobial and the extract was evaluated by thermal analysis (TG/DTA). The acute toxicity of the extract was evaluated in vivo in rodents. The results indicate significant additive action of the extract on synthetic antibiotics, considerable antioxidant activity and absence of toxicity. This extract shows high potential for the development of formulations for antimicrobial therapy when used with a vegetable-active ingredient.
Introduction

The emergence of antibiotics was one of the greatest advances in modern medicine. These substances play a key role in the successful treatment of infections that used to take patients’ lives, and they also help increase life expectancy. However, the widespread and indiscriminate use of antibiotics has contributed to the emergence of resistant pathogens, including multidrug-resistant strains [1,2]. This problem has been aggravated in recent decades and has recently been recognized as one of the greatest threats to human health [3,4].

A particular concern is the case of the multiresistant Gram-negative bacteria. These microorganisms, which are intrinsically resistant to different antibiotics, have an outer membrane of low permeability that restricts access of the antimicrobial agents to their targets inside the cell, and this concern, together with the resistance mechanisms of the acquired-like efflux pump, enzymatic degradation, and change in drug target site, protect the bacteria against the deleterious effects of these agents [5]. A major threat to the global level caused by these bacteria are nosocomial infections because the treatment of patients in critical condition in an intensive care unit (ICU) or the treatment of other immunosuppressed patients becomes more complex if the condition is associated with increased morbidity and mortality [6,7].

The problem of increased antimicrobial resistance becomes even more menacing when the delay in the discovery and development of new antibiotics is taken into account. The number of such drugs is still quite limited, which endangers the essential role played by antibiotics in current medical practices [8].

The aforementioned problems urgently require new therapeutic strategies. Of special importance is the search for new drugs derived from biological sources in which molecules, predominantly secondary metabolites, contribute to their development [9]. Another approach to improving the efficacy of existing antimicrobials and suppressing the emergence of multidrug-resistant strains involves the use of products that potentiate the activity of these substances [10–12]. These products can improve the effectiveness of the antibiotic in eliminating or delaying the emergence of antibiotic resistance [13].

Plant extracts are known to have antimicrobial properties and may play an important role in therapeutic treatments. For this reason, a growing number of studies in different countries have been conducted to demonstrate the effectiveness of these extracts [14–16]. Besides the direct antimicrobial activity, plant species have been tested as potential adjuvants by modifying the microbial resistance [17,18].

Combinations of antimicrobial drugs and natural products of vegetable origin, in which these products act as adjuvants, constitute a promising approach for the treatment of infections. The natural products would replace at least a part of the synthetic substances in the formulations and would eventually reduce the undesirable effects of these substances in the human body [19].

Poincianella pyramidalis (Tul.) L.P. Queiroz (Fabaceae) is an arboreal species with wide distribution in the Brazilian semiarid region. Until recently, this species was known as Caesalpinia pyramidalis Tul., but due to a taxonomic update, it came to be called P. pyramidalis [20]. Its parts, especially its bark, leaves, and flowers, are used in traditional medicine for the treatment of several diseases such as influenza, cough, diarrhea, dysentery, respiratory infections, urinary infections, and inflammation in general [21–27]. Among the biological activities of P. pyramidalis described in the literature, we can highlight the antibacterial [28,29], antifungal [30], antioxidant [31], gastroprotective [32], anti-inflammatory, antinociceptive [33] and anthelmintic [34] activities.

This work is aimed to investigate the interaction between P. pyramidalis extract and antimicrobial drugs through the use of biological and analytical models.
Material and Methods

Plant material

Bark of *P. pyramidalis* were collected on the farm “Farinha”, municipality of Pocinhos, PB, Brazil (7°07’54.53”S e 36°07’14.51”O), in January 2014. A voucher specimen (CSTR 5036) was deposited in the herbarium of the Center for Health and Rural Technology at Federal University of Campina Grande.

Preparation of extract

The plant materials were dried in an air circulation oven at 40°C. Subsequently it was ground in a knife mill with a particle size of 10 mesh. The hydroalcoholic extract obtained by extraction was assisted by ultrasound at 40°C for 60 min, using ethanol-water mixture (50:50 v/v) as solvent. Out below has been subjected to spray drying in a Mini Spray Dryer Labmaq PS-1, with onset temperature 120°C, air flow of 40 L min⁻¹, drying air flow rate 3 ml min⁻¹. The nebulized extract (ENPp) was dried with adjuvant using colloidal silicon dioxide (Aerosil 200) at 20% on dry weight basis.

Chemical assays

**Determination of total polyphenols.** The total polyphenol content of plant extracts was measured by Folin-Ciocalteu method [35]. The extracts were dissolved in distilled water to obtain a final concentration 200 μg mL⁻¹. From each solution, a 1 mL aliquot was added to 1 mL of a 1 mol L⁻¹ Folin-Ciocalteu reagent (Sigma-Aldrich). This mixture remained undisturbed for 2 min before the addition of 2 mL of 20% (w/v) Na₂CO₃ solution and left undisturbed for 10 min. Thereafter the reading was performed Spectrophotometer Shimadzu, at 757 nm. The calibration curve was obtained with a stock solution of gallic acid (Sigma-Aldrich) (1000 μg mL⁻¹), from which dilutions were made at concentrations between 1 and 40 μg mL⁻¹.

**Determination of total flavonoids.** The total flavonoids were determined by the AlCl₃ method [35]. The extracts were diluted with methanol at 1000 μg mL⁻¹. To the 5 mL of each test solution was added the same volume of 2% (w/v) AlCl₃ solution in methanol. This mixture remained undisturbed for 10 min before the UV spectrophotometric reading at 415 nm wavelength. The total flavonoids were determined by the calibration curve using quercetin (Sigma-Aldrich) as standard at concentrations between 2 and 30 μg mL⁻¹.

**Determination of condensed tannins.** The content of condensed tannins was verified through the method described by Makkar and Becker [36] wherein 0.25 mL of the sample was added to 1.5 mL vanillin (Sigma-Aldrich) dissolved in methanol (4% w/v) and subsequently in 0.75 mL of concentrated HCl (37%). After the HCl addition, the tube content was shaken in water bath at 30°C for 3–4 seconds before being read on a spectrophotometer at a 500 nm wavelength. Catechin (Sigma-Aldrich) was used as standard at concentrations between 10 and 100 μg mL⁻¹.

**Determination of saponins.** The quantification of total saponins followed the method described by Makkar et al.[37]. First, 250 μL of an 8% vanillin solution in ethanol was added to a 250 μL extract solution in 80% methanol; then 2.5 mL of 72% sulfuric acid were added. The tubes were incubated at 60°C in a water bath for 10 minutes and then transferred to an ice bath to rest for 4 minutes. The absorbance reading at 544 nm was performed against a blank consisting of the vanillin solution, 80% methanol and sulfuric acid. The calibration curve was obtained from a disogenin (Sigma-Aldrich) solution at concentrations between 100 and 500 μg mL⁻¹.

**Determination of major chemical compound.** We used a liquid chromatograph Ultra Efficiency (UPLC), Shimadzu, equipped with two pumps model LC-20AD, autosampler SIL-
20-AHT, oven column CTO-20A, detector with variable wavelength UV/Vis, model SPD-20A, controller CBM-20A, automatic computerized integrator with software LC Solution. The stationary phase was composed of a column Gemini—NX C18 (250 x 4.60 mm, 5 μm). The mobile phase consisted of an isocratic mixture of acetic acid 0.1%: metanol (90:10, v/v). Analyses were performed under controlled temperature (30°C), using a flow 1 mL min⁻¹ and injection volume of 20μL. All samples were amended with 0.45μm syringe filters diameter.

Antioxidant activity

The antioxidant activity of ENPp was originally assessed by the ability of the antioxidant substances present in the sample to capture the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). The tests were conducted using the method described by Dhar et al. [38], with adaptations. Initially, the DPPH solution was prepared at 0.200 mM in ethanol. 500 μL of this solution was added to 500 μL of diluted extract in ethanol in concentrations ranging between 50 and 3.125 μg mL⁻¹. The mixture remained at rest in the dark at room temperature for 30 minutes before the absorbance was read in a spectrophotometer at UV 517 nm. Gallic acid and quercetin were used as standards. The ability to scavenge DPPH radicals was calculated by the following equation:

\[
\text{Ability} (\%) = \frac{\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \times 100
\] (1)

where, \(\text{ABS}_{\text{control}}\) is the absorbance of the DPPH radical + ethanol; \(\text{ABS}_{\text{sample}}\) It is the absorbance of the DPPH radical + extract or standard.

The inhibitory concentration (IC₅₀) and effective concentration (EC₅₀) were estimated as described by Kroyer [39] and Prakash et al. [40]. The IC₅₀ was determined by plotting the DPPH elimination ability against the logarithm of the concentration of the sample, while the EC₅₀ was calculated using the following equation:

\[
\text{EC}_{50} = \frac{\text{IC}_{50}}{[\text{DPPH}] \mu g.mL^{-1}}
\] (2)

Microbiological assays

Were used standard strains American Type Culture Collection (ATCC) and clinical isolates of Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae whose phenotypic profile is described in Table 1. The strains were maintained on slants of tubes with Mueller-Hinton Agar, and, before testing, cultured at 37°C for 24 hours, plates with the same culture medium.

**Determination of Minimum Inhibitory Concentration (MIC).** The minimum inhibitory concentration (MIC) was determined by a microdilution method in 96-well plates using Mueller-Hinton broth [41]. Colonies of microorganisms were suspended in a 0.9% saline solution, and by a spectrophotometric method at 625 nm, the suspension adjusted to a final concentration of 5 x 10⁶ CFU mL⁻¹. Serial dilutions of the extract in the range of 1000 to 2.4 μg mL⁻¹ and antibiotics in the range of 2500 to 2.4 μg mL⁻¹ were performed. Dimethyl sulfoxide (DMSO) 10% was included as a negative control. The plates were incubated at 37 ± 1°C for 24 hours. Bacterial growth was indicated by addition of 20 μL of 0.01% aqueous resazurin solution (Sigma-Aldrich) with incubation at 37 ± 1°C for 2 h. MIC values were identified as the lowest concentration in which no bacterial growth is visible. The assays were performed in triplicate.

**Modulation of antimicrobial resistance.** Evaluation of extracts as modulators of antibiotic resistance was performed according to Coutinho et al.[42]. The MIC of the antibiotic was...
determined in presence and absence of sub-inhibitory concentrations (MIC/8) of EESb. Plates were incubated as described above and each assay was performed in triplicate.

### Evaluation of acute toxicity

The study was carried out in strict accordance with the Standard Operating Procedures (Laboratory of Pharmacology at the State University of Paraiba, Campina Grande, Brazil) approved by veterinarian, which monitored frequently the animals by physical condition assessments of the health. All efforts were exerted in order to reduce the suffering of experimental animals. The protocol was approved by the Ethics Committee on Animal Use of Faculty of Medical Sciences of Campina Grande (No: 5618092015). Disease-free albino Wistar rats (Rattus norvegicus) (6–8 weeks age and 200–220 g weight) were used for this study. The animal house were obtained of Federal University of Paraíba, João Pessoa, Paraíba, Brazil. The animals were housed in rat standard plastic cages (n = 6) with stainless steel coverlids and wood shavings. All rats underwent a period of at least 7 days of acclimatization prior to the procedure, being socialized with contact including humans. The animals were handling with care to minimize stress. The researchers confirm that the laboratory had a protocol in place for the use of humane endpoints in cases where animals became severely ill or moribund during the experiment, but no had death or behavioral changes in animals. They remained in polypropylene boxes, in single sex groups, at room temperature (22°C ± 3°C) and humidity (50% ± 20%) and 12 hrs light/dark cycles. The animals received standard laboratory pellets and water ad libitum both for the adaptation period (7 days) and during the trial, except the period of 12 hours prior to the experiment in which the access to food was restricted. Throughout the experiments, all of the animals received humane care according to the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences [43].

The animals were divided in groups of 6 males and 6 females, which received, orally, the dose of extract to 2000 mg kg\(^{-1}\). A control group was treated with saline, the same used to resuspend the extract. Immediately after administration of the extract, the animals were evaluated behaviorally carefully during the first 4 hours, as recommended by the handshake protocol and evaluation of clinical signs of OECD [44] and daily for 14 days after administration. They

### Table 1. Bacterial strains used and their phenotypic profile of antimicrobial resistance.

| Strains               | Resistance                                      |
|-----------------------|-------------------------------------------------|
| E. coli ATCC 25922    | AMC, CFL; ATM; CFO; NIT; CPM; SFM; NOR          |
| E. coli 401           | CFO; ATM; CFL; CAZ; AMP; GEN; NOR; CLI; TET; SFM. |
| E. coli 523           | CIP; CLO; NOR; SFM; TET e AMP                   |
| E. coli 534           | AMP; CFO; NOR; CAZ; ATM; TET; CPM; GEN; CFL; CLI; SFM. |
| P. aeruginosa ATCC 27853 | TOB; CFL; ATM; AMI; CPM; CFO; AMC; CFT;        |
| P. aeruginosa 106     | CFO; AMC; CFL; CFT; CAZ; SFM; NOR; GEN; CIP; TET; CPM. |
| P. aeruginosa 117     | TOB; AMI; SFM; AMP; GEN; NOR; CLI; TET.        |
| P. aeruginosa 208     | AMP; CFO; NOR; CAZ; ATM; CFT; TET; NOR; CLI; SFM; AMP; GEN. |
| K. pneumoniae ATCC 4352 | AMC; CFO; CFL; NIT; CAZ; ATM; CFT; TET; NOR; CLI; SFM; AMP; GEN. |

AMC = Amoxicillin + Clavulanic acid; CFL = Cephalothin; ATM = Aztreonam; CFT = Cefotaxime; NIT = Nitrofurantoin; CPM = Cefepime; NOR = Norfloxacin; AMP = Ampicillin; CIP = Ciprofloxacin; CFO = Ceftriaxone; CAZ = Ceftazidine; GEN = Gentamicin; CLI = Clindamycin; TET = Tetracycline; CLO = Chloramphenicol; TOB = Tobramycin; AMI—Amikacin; SFM = Sulfamethoxazole + trimethoprim.
were also observed the consumption of water and feed. Animals were sacrificed under anesthesia with ketamine/xylazine (0.5 mL of 100 mg mL⁻¹ ketamine combine with 0.05 mL of 20 mg mL⁻¹ xylazine) at a dosage of 0.55 mL/100g body weight (b.w.). After sacrifice was carried out weighing and macroscopic analysis of the viscera (liver, kidneys, spleen, lungs and heart).

**Statistical analysis**

The results of the microbiological tests were expressed as a geometric mean. It was applied to a two-way analysis of the variance, followed by the Bonferroni post-test, and was applied to toxicity testing through an analysis of the variance with Tukey’s post-test using GraphPad Prism 5.0 software.

**Thermal analysis**

The thermoanalytical profiles were obtained using a simultaneous TG-DTA analyzer, model DTG-50 (Shimadzu). Samples (5.0 ± 0.2 mg) were accommodated in a platinum crucible, and subjected to a heating program from 30 to 900°C, at 10°C min⁻¹, in inert nitrogen atmosphere (50 mL min⁻¹). The samples consisted of antibiotics and extract analyzed separately and in binary mixtures, the proportion 1:1.

The DTA module is calibrated with indium standard (mp = 156.6°C). And the calibration of TG module, was used a standard calcium oxalate monohydrate. Curves were analyzed in the TA60 software, version 2.21.

**Results and Discussion**

**Chemical assays**

The secondary metabolite content is shown in Table 2. The content of the total number of polyphenols and tannins is high compared to the total flavonoids. Although the method above is a quantitative method, it fails to predict the composition of each individual compound as well as the possible quantification of nonphenolic compounds [45].

Other studies carried out using the phytochemical with *P. pyramidalis* extracts revealed the presence of secondary metabolites such as saponins, ursolic acid, sitosterol, Cinnamic derivatives, flavonoids, quercetin, proanthocyanidins, catechin, gallic acid, and ellagic acid [29,46,47].

Identification of the major chemical compound using liquid chromatography was performed, and, based on the retention time parameter (TR), was compared with the TR values for the analyzed standards of gallic acid, catechin, quercetin, rutin, and kaempferol, with the RT values of peaks observed in the EEPp. The results indicated the presence of gallic acid,

**Table 2. Content of secondary metabolites present in *P. pyramidalis* extract obtained spectroscopy in the visible region.**

| Metabolites             | Concentration     |
|-------------------------|-------------------|
| Total polyphenols       | 36.94 ± 0.45¹     |
| Total flavonoids        | 19.09 ± 0.76²     |
| Condensed tannins       | 59.08 ± 0.69³     |
| Total saponins          | 328.43 ± 1.95⁴    |

¹Gallic acid equivalent (GAE)
²Quercetin equivalent (QE)
³Catechin equivalent (CE)
⁴Disogenin equivalent (DE).

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which can be used as a chemical marker of \textit{P. pyramidalis} (Fig 1). In a study conducted by Santanar et al. [48] with the ethanol extract of the plant, the researchers found the presence of rutin, which was absent in this study. This difference may be related to parameters such as the place and time of collection and the stage of plant development, among other factors [35].

**Antioxidant activity**

The search for substances that might reduce the risk of developing chronic diseases caused by oxidative stress [49] is increasing. This risk includes several pathological and toxicological processes such as aging, transformation, cell killing, cancer induction, autoimmune diseases, and heart diseases, among others [50,51]. Among the most promising substances in the study are those of vegetable origin, such as polyphenols, flavonoids, alkaloids, terpenoids, carotenoids, etc. [52–54].

The scavenging capacity of the free radicals of \textit{P. pyramidalis} extract was compared through its ability to eliminate the DPPH radical. The data from these tests are shown in Table 3, expressed as a percentage of the inhibition of DPPH, IC\textsubscript{50}, and EC\textsubscript{50}. When looking at the percentage of the inhibition of DPPH, we found that the extract showed a high inhibition rate.

|              | DPPH (%) | IC\textsubscript{50} (\textmu g mL\textsuperscript{-1}) | EC\textsubscript{50} (U.A.) |
|--------------|----------|------------------------------------------------------|-----------------------------|
| Gallic acid (10 \textmu g mL\textsuperscript{-1}) | 75.28\textsuperscript{a} | 5.99 ± 0.24\textsuperscript{a} | 0.0760 ± 0.0030\textsuperscript{a} |
| Quercetin (10 \textmu g mL\textsuperscript{-1}) | 36.84\textsuperscript{b} | 13.75 ± 0.24\textsuperscript{b} | 0.1743 ± 0.0030\textsuperscript{b} |
| Extract (50 \textmu g mL\textsuperscript{-1}) | 79.71\textsuperscript{a} | 28.11 ± 0.68\textsuperscript{b} | 0.3564 ± 0.0086\textsuperscript{c} |

a, b, c—different letters in the same column mean statistically significant differences (p < 0.01).
with no statistically significant difference between it and gallic acid. On the other hand, the IC$_{50}$ and EC$_{50}$ extract was significantly higher than the standards. Its inhibition rate may be related to the fact that the extract is a complex mixture of substances as the patterns are pure substances.

There are references in the literature that mention that *P. pyramidalis* has a good number of phenolic compounds [46]. These substances are very important components of plant extracts and contribute directly to the elimination of radicals because of their hydroxyl groups [55,56]. Gallic acid, found in *P. pyramidalis* extract, is one of these compounds, and there are reports in the literature of its antioxidant properties [57–59].

### Antimicrobial and modulatory activities

As for antimicrobial activity, the ENPp had no clinically significant effect on any of the studied strains. Dall’Agnol et al. [60] and Rios and Recio [61] reported that plant extracts are considered inactive with MIC > 1 000 µg mL$^{-1}$; they are low-activity holders with CIM between 500 and 1000 µg mL$^{-1}$ and show moderate activity with MIC values between 100 and 500 µg mL$^{-1}$ and good activity when MIC is ≤ 100 µg mL$^{-1}$. The latter activity levels have good potential for the determination and purification of active compounds. The absence of antimicrobial activity presented here is consistent with the study conducted by Silva et al. [62], who detected the inactivity of the ethanol extract of *P. pyramidalis* on standard strains and clinical isolates of *E. coli*, *P. aeruginosa*, and *K. pneumoniae*.

Gallic acid, a chemical marker of ENPp, was also subjected to microbial susceptibility testing and showed no significant activity on strains tested in this study (MIC > 1000 µg mL$^{-1}$). This result corroborates the study by Chanwitheesuk et al. [63], in which the gallic acid also presented MIC > 1000 µg mL$^{-1}$ on several bacterial strains, Gram positive, Gram negative, and fungal. A similar result was observable in the study of Jayaraman et al. [64] in tests with standard and multidrug resistant strains of *P. aeruginosa*. Borges et al. [65], in trials with *E. coli*, *S. aureus*, and *Listeria monocytogenes*, obtained MIC values > 1000 µg mL$^{-1}$. However, against *P. aeruginosa*, the gallic acid showed MIC = 500 µg mL$^{-1}$. Other studies report MIC values for gallic acid that can be considered active. Sanchez-Maldonado et al. [66] obtained MIC = 490 µg mL$^{-1}$ against *E. coli*. Vaquero et al. [67], using the agar diffusion method, obtained inhibition halos against standard strains of *E. coli* (ATCC 35218 and ATCC 25922), with gallic acid concentrations ranging between 200 and 1000 µg mL$^{-1}$. The discrepancy between the findings of this and other studies may be related to the method used to determine the antimicrobial activity, as well as the phenotypic profile of the strains used.

The resistance presented by Gram-negative bacteria is possibly related to the presence of the outer membrane of the bacterial wall, which forms a semipermeable barrier composed mainly of phospholipids, lipopolysaccharides, and proteins. This barrier hinders the passage of antimicrobial drugs and is linked to the high intrinsic resistance of these bacteria [68,69]. Beyond this barrier, these microorganisms may have other mechanisms that prevent the antimicrobial from reaching the target. It is able to detect the change in the composition of the outer membrane, eliminating porins, induction efflux pumps, and enzymatic degradation of the antimicrobial [70,71].

In modulatory activity, assays have calculated a subinhibitory concentration (MIC / 8) of the extract, which are associated with antibiotics. Because the extract did not prevent efficacy, CIM considered for this calculation was the highest concentration tested and has, therefore, a subinhibitory concentration of 125 µg mL$^{-1}$. In Fig 2A, 2B, 2C and 2D, we can see that, after combination with both the extract and gallic acid, there was a significant increase in the gentamicin activity on the *E. coli* strains 401, 534, and 613. Against *E. coli* 523, chloramphenicol...
Interaction between *Poincianella pyramidalis* and Antimicrobials

**Figure A**
- Antibiotic + *P. pyramidalis*
- Antibiotic + Gallic Acid
- Antibiotic alone

**Figure B**

**Figure C**

**Figure D**

**Figure E**

**Figure F**

**Figure G**

**Figure H**

[Graphs and bars showing MIC values for different antimicrobials and conditions]
reduced MIC in both associations, while gentamicin, only in association with the extract. Moreover, the association with gallic acid caused a significant increase in nitrofurantoin (E. coli 401), ampicillin (E. coli 523), and norfloxacin (E. coli 534 e 613). In assays with P. aeruginosa strains (Fig 2E, 2F e 2G), we found the additive effect of the extract and the antibiotic in the action of gentamicin on all strains tested, ceftriaxone (P. aeruginosa 106 e 117), ciprofloxacin, and cefepime (P. aeruginosa 117). The extract also led to a significant reduction of ciprofloxacin, the MIC for P. aeruginosa 106, while the opposite effect resulted in gallic acid. Also, on this same strain, the association with gallic acid increased MIC of cephalothin. In assays with K. pneumoniae (Fig 2H), the antibiotic whose activity has intensified in both associations, was gentamicin. However, there was a significant increase in CIM clindamycin when it was added to the extract.

The best results in the interactions with the extract or gallic acid were observed in assays with gentamicin, whose CIM on all strains was reduced. This antimicrobial belongs to the class of aminoglycoside, which in turn is the class to which the most commonly used anti-infectious agents in clinical practice belong [72]. They are able to interact with different portions of rRNA, causing deleterious effects on mRNA translation process polypeptide causing inhibition of protein synthesis or production of defective proteins [72–74]. The main mechanisms of resistance to these antimicrobial agents include the enzymatic degradation (N-acetylation, adenylation, or O-phosphorylation), reduction in the intracellular concentration of the antibiotic by changes in membrane permeability and transport by active efflux; changes in the target 30S ribosomal subunit; and changes in the antimicrobial binding site [75,76].

A particularly important feature presented by the aminoglycosides is the ability to act synergistically with other drugs [76]. This feature is particularly important in the search for new therapeutic alternatives because of the emergence of resistant strains and toxicity caused by these drugs [77]. In the literature, there are studies that demonstrate the resulting synergism of the associations between β-lactams and aminoglycosides [78,79] and between aminoglycosides and natural products [80–82].

The combination of plant extracts and antibiotics is well documented in the literature. In many cases, this association led to an additive effect, resulting in improved antimicrobial activity against various multidrug-resistant strains [83–88].

The reduction in the MIC of gentamicin after association with ENPp, or its chemical marker, may be related to the promotion of drug entry into the bacterial cell. Gallic acid acts on cell membranes, leading to irreversible changes in its characteristics relating to intra- and extracellular permeability. The chemical-physical properties it is altered by changes in the hydrophobicity and decreased negative charge of the surface. It favors the occurrence of breaks or the formation of pores, with consequent loss of essential intracellular components for the bacterial life [65].

The ENPp as various types of plant extracts and phytochemicals presents a wide variety of compounds; therefore, other mechanisms may be related to the additive effects of the combination of antibiotics and ENPp. A wide variety of compounds present in the extract can act on different targets [89] and may be in one or multiple targets at once. Among the mechanisms by which the extracts can interfere with microbial growth is the inactivation of enzymes, transport and receptor proteins, and DNA/RNA, besides acting in the suppression of bacterial resistance.
mechanisms [90–92]. Moreover, the said plurality of compounds and their mechanisms of action provide a low risk for increasing resistance because they afford the greatest difficulties for microbial adaptation [93].

**Acute toxicity**

During the 14 days following the statement of administration, there was no death among the animals, making it impossible to calculate the LD$_{50}$. There was also no change in the behavior of the animals. When the consumption of water and feed and the average weight of the organs of animals were evaluated, there was no statistically significant difference between the group treated with the extract and the control group (P <0.05).

**Thermal analysis**

**Differential thermal analysis (DTA).** The DTA curve of ENPp shows three processes of the sample phase transitions. The first, endothermic at 53.36°C, is possibly related to loss of water, solvent (ethanol), or volatile compounds in the sample. The following two peaks are exothermic of crystallization: the second at 348.30°C and the third at 425.17°C (Table 4).

In the DTA curve of norfloxacin, the first peak of the endothermic nature at 179.69°C was observed. The second peak endotherm occurred at 223.84°C, which corresponds to the delay of the drug fusion process that occurs between 220.00 and 221.00°C [94,95]. In the binary mixture of antibiotic + extract, we observed that the first endothermic peak occurred at a temperature of 179.36°C, which is characteristic of the drug. The second peak was in 219.51°C, which corresponds to the anticipation of the melting process of the drug. From the third, occurring in 428.63°C, the thermal sample decomposition process begins. In the mixture, there was a change in the heat peaks in advance of the drug melting temperature and suppression of peaks observed in the extract. These changes are possibly due to interactions between the extract and antibiotic (Table 4/Fig 3).

Four events were recorded in the DTA curve of ampicillin. The first, which were endothermic, recorded in temperatures of 63.52°C, related to the dehydration sample; the second event was in 218.30°C, which probably corresponds to the delayed melting process, with accompanying decomposition, of one of its anhydrous forms [95,96]; the third, at 363.24°C, was the beginning of the decomposition of ampicillin. In the curve produced with the mixture, we observed that the first two endothermic peaks of the drug were maintained at the same temperature but with less reaction heat involved in the endothermic processes. The removal of the exothermic peaks of the drug and the extract in the binary mixture curve is an indication of a strong chemical interaction between the components of the sample (Table 4/Fig 3B).

The DTA curve of cephalothin showed an endothermic peak at 167.27°C, probably related to the sample fusion process between 160.0 and 160.5°C [95], and an exotherm at 212.63°C, which corresponds to their decomposition processes [97]. With the mixture produced from the extract, a glass transition was observed at a temperature of 151.33°C and an exothermic peak at 211.66°C, both associated with the degradation of the mixture (Table 4/Fig 3C).

In the DTA curve of ciprofloxacin, two events occur, the first, of an endothermic nature, was observed at 144.45°C, corresponding to the loss of the acetylene group (C$_2$H$_2$) of the drug [98]. The second, at 318.40°C, corresponds to the melting process of the drug, which occurs between 318.0 and 320.0°C [95,99]. In the curve of the mixture, four endothermic peaks were observed. The first, at 134.90°C, resulted from the sample moisture loss and the drug group C$_2$H$_2$[98]. The second, in 296.64°C, is probably related to the anticipation of the drug fusion process. The two endotherms, at 405.53 and 430.63°C, demonstrate the sample decomposition
| Samples       | Peak 1  | Peak 2  | Peak 3  | Peak 4  | Peak 5  |
|--------------|--------|--------|--------|--------|--------|
|              | $T_{\text{peak}}/^\circ\text{C}$ | $\Delta H/\text{J/g}$ | $T_{\text{onset}}$ and $T_{\text{endset}}/^\circ\text{C}$ | $T_{\text{peak}}/^\circ\text{C}$ | $\Delta H/\text{J/g}$ | $T_{\text{onset}}$ and $T_{\text{endset}}/^\circ\text{C}$ | $T_{\text{peak}}/^\circ\text{C}$ | $\Delta H/\text{J/g}$ | $T_{\text{onset}}$ and $T_{\text{endset}}/^\circ\text{C}$ | $T_{\text{peak}}/^\circ\text{C}$ | $\Delta H/\text{J/g}$ | $T_{\text{onset}}$ and $T_{\text{endset}}/^\circ\text{C}$ | $T_{\text{peak}}/^\circ\text{C}$ | $\Delta H/\text{J/g}$ | $T_{\text{onset}}$ and $T_{\text{endset}}/^\circ\text{C}$ |
|---------------|--------|--------|--------|--------|--------|
| ENP           | 53.36  | 100.35 | 33.37-72.64 | 348.30 | 42.88 | 290.46-347.17 | 425.17 | 350.00 | 396.42-439.85 | - | - | - | - | - | - |
| NOR           | 179.69 | 6.36   | 177.15-185.30 | 223.84 | 152.22 | 220.73-230.79 | - | - | - | - | - | - | - | - | - |
| NOR + ENP     | 179.36 | 1.88   | 176.94-182.26 | 219.51 | 36.92 | 210.08-224.10 | 428.63 | 4.42 | 423.22-434.30 | 612.19 | 601.79 | 576.34-680.96 | - | - | - | - |
| AMP           | 63.52  | 12.15  | 59.68-68.27 | 218.30 | 67.97 | 213.45-227.17 | 363.24 | 125.99 | 354.77-373.46 | - | - | - | - | - | - |
| AMP + ENP     | 63.85  | 3.65   | 60.58-67.73 | 218.92 | 17.85 | 210.41-226.45 | 397.58 | 55.13 | 372.50-405.56 | - | - | - | - | - | - |
| CIP           | 144.45 | 276.67 | 119.24-157.21 | 318.40 | 462.48 | 306.38-330.48 | - | - | - | - | - | - | - | - | - |
| CIP + ENP     | 134.90 | 124.53 | 112.28-147.43 | 296.64 | 47.36 | 293.81-310.96 | 405.53 | 10.57 | 390.24-411.35 | 430.63 | 10.50 | 418.69-440.96 | - | - | - | - |
| NIT           | 272.07 | 244.79 | 288.81-271.39 | 307.84 | 8.73 | 277.92-315.87 | - | - | - | - | - | - | - | - | - |
| NIT + ENP     | 263.06 | 236.61 | 250.07-280.60 | 423.93 | 25.25 | 417.19-432.47 | - | - | - | - | - | - | - | - | - |
| CFL           | 167.27 | 22.30  | 153.32-177.02 | 212.63 | 159.74 | 204.38-220.80 | 546.06 | 202.60 | 541.67-593.37 | - | - | - | - | - | - |
| CFL + ENP     | 81.02  | 235.21 | 36.14-170.68 | 211.66 | 42.64 | 204.49-219.80 | - | - | - | - | - | - | - | - | - |
| CLI           | 51.85  | 121.54 | 34.88-67.37 | 76.84 | 30.37 | 71.36-87.41 | 207.50 | 28.64 | 206.85-215.42 | 241.92 | 41.42 | 218.79-251.80 | - | - | - | - |
| CLI + ENP     | 59.74  | 63.52  | 44.60-74.52 | 239.84 | 272.81 | 202.73-288.80 | 326.21 | 6.51 | 319.90-333.10 | - | - | - | - | - | - |
| CFO           | 80.90  | 46.28  | 61.73-90.11 | 148.22 | 32.12 | 138.06-155.45 | 270.27 | 446.45 | 261.72-285.21 | - | - | - | - | - | - |
| CFO + ENP     | 85.12  | 24.52  | 76.96-94.34 | 154.40 | 20.64 | 146.02-161.92 | 273.23 | 553.49 | 255.18-300.31 | 356.14 | 180.95 | 350.89-417.65 | 546.50 | 533.03 | 533.42-584.77 | - | - | - | - |
| GEN           | 75.05  | 350.04 | 42.44-112.06 | 252.84 | 250.41 | 242.03-278.10 | 293.71 | 194.99 | 284.83-331.94 | - | - | - | - | - | - |
| GEN + ENP     | 67.93  | 205.46 | 40.37-101.76 | 252.46 | 153.79 | 241.78-276.63 | 293.15 | 54.24 | 287.22-327.06 | - | - | - | - | - | - |
| CPM           | 50.13  | 23.94  | 33.74-62.42 | 117.38 | 25.56 | 107.28-125.85 | 182.04 | 39.98 | 158.37-195.67 | 645.78 | 473.32 | 607.25-659.49 | - | - | - | - |
| CPM + ENP     | 69.05  | 112.11 | 37.33-86.13 | 108.11 | 8.26 | 98.95-116.19 | 194.00 | 4.55 | 192.92-203.86 | - | - | - | - | - | - |
| CLO           | 184.87 | 225.63 | 164.22-217.94 | 279.09 | 130.40 | 258.62-301.24 | 576.71 | 328.00 | 522.61-617.78 | - | - | - | - | - | - |
| CLO + ENP     | 70.39  | 41.07  | 37.72-119.58 | 179.93 | 61.52 | 165.54-225.48 | - | - | - | - | - | - | - | - | - |

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Interaction between *Poincianella pyramidalis* and Antimicrobials
process. These differences point to an interaction between the substances in the mixture (Table 4/Fig 3D).

Five events were observed in the DTA curve clindamycin, the first, of endothermic nature, which occur at temperatures of 109.00°C, concern the dehydration of the sample. The event at 148.97°C, probably related to the delayed drug fusion process, should occur in the temperature range of 141 to 143°C [95]. From the peak observed at 170.18°C, the process of drug decomposition begins. Moreover, from the curve of the mixture, only endothermic events occurred, the first at a temperature of 101.86°C, referring to the loss of moisture in the mixture. The second, at 147.23°C was probably due to the retardation of the drug fusion process. And the third, of 204.84°C, was also attributed to the delay in the onset of decomposition in the mixture. The exothermic peaks present in the drug and the mixture were removed, indicating a possible incompatibility between the drug and the nebulized extract of *P. pyramidalis* (Table 4/Fig 3E).

In the DTA curve of nitrofurantoin, there was an endothermic event at 272.07°C only. The absence of previous events indicates that the sample was completely free from humidity. The first observed event is related to the early decomposition of the drug, which should take place in temperatures above 270°C [95]. There was still a last endothermic peak at 307.84°C, ending the process of decomposition. In the mixture two exothermic events were observed. The first, at 263.06°C, related to the anticipation of the decomposition process of nitrofurantoin. And the other, in 423.83°C, corresponded to the extract degradation. These events indicate that there should probably be a strong incompatibility between the drug and the substances present in the extract (Table 4/Fig 3F).

The DTA curve of ceftriaxone showed a endothermic event at 80.90°C, attributed to the dehydration process of the sample. The second, at 148.22°C, was probably responsible for initiating the melting/decomposition process of the drug, given that ceftriaxone melts with decomposition when heated to over 155°C [95,100]. The last exothermic peak, at 270.27°C, corresponds to the continuation of the drug decomposition process [101]. The DTA curve of the binary mixture ceftriaxone + extract showed two endothermic events and three exothermic. The first endotherm, of 154.40 and 85.12°C, corresponds to the first two peaks observed in the drug, as does the first exotherm, which occurred at 273.23°C, though with a delay. The second exothermic peak, at temperature 356.14°C, is probably the first peak decomposition extract that was anticipated. And, the last peak exotherm, at 546.50°C, corresponds to the lagging end of the extract decomposition process (Table 4/Fig 3G).

The gentamicin curve showed three endothermic peaks. The first of the sample, to 75.05°C, is related to water loss. The second and third peaks occurred at 252.84°C and 299.71°C. According to the Merck Index [95], the melting point of gentamicin sulfate is between 218 and 237°C. Aquino et al. [102] reported that gentamicin sulphate, the raw material, is characterized by a range of endothermic peaks related to the fusion of the different isoforms. In the curve of the binary mixture, three endothermic peaks were observed, all three of which correspond to the drug. The first peak occurred at 67.93°C, while the second and third occurred at 252.46 and 299.15°C, respectively. The temperatures of the last two peaks came very close to the peaks of the drug (Table 4/Fig 3H).

The DTA curve of Cefepime presented three endothermic peaks and an exothermic. The first two occurred at 50.13°C and 117.38°C and are related to the sample moisture loss. The third, 182.04°C in the drug, is linked to the fusion process that occurs at close to 182°C [103].
| Samples       | Decomposition Steps | $T_{\text{Peak}}$ (°C) | $T_{\text{Onset}}$ (°C) | $T_{\text{Endset}}$ (°C) | Mass loss (%) |
|---------------|--------------------|------------------------|-------------------------|--------------------------|---------------|
| ENPp          | 1st                | 47.22                  | 46.50                   | 55.58                    | 6.36          |
|               | 2nd                | 208.99                 | 205.35                  | 251.87                   | 14.67         |
|               | 3rd                | 372.83                 | 370.02                  | 429.47                   | 58.84         |
|               | 1st                | 49.33                  | 56.42                   | 72.74                    | 7.49          |
|               | 2nd                | 213.52                 | 206.36                  | 226.62                   | 6.32          |
| NOR           | 3rd                | 327.44                 | 315.94                  | 352.93                   | 32.98         |
|               | 4th                | 402.99                 | 361.52                  | 405.21                   | 34.32         |
|               | 1st                | 61.28                  | 38.93                   | 70.41                    | 8.24          |
|               | 2nd                | 195.49                 | 191.55                  | 220.03                   | 7.28          |
| NOR + ENPp    | 3rd                | 309.93                 | 309.47                  | 350.87                   | 25.28         |
|               | 4th                | 412.53                 | 439.86                  | 454.78                   | 27.21         |
|               | 5th                | 579.67                 | 579.15                  | 628.55                   | 21.25         |
| AMP           | 1st                | 214.03                 | 209.52                  | 219.07                   | 21.54         |
|               | 2nd                | 309.64                 | 282.31                  | 363.28                   | 49.45         |
|               | 3rd                | 630.61                 | 612.64                  | 688.57                   | 25.17         |
| AMP + ENPp    | 1st                | 210.24                 | 204.34                  | 219.26                   | 12.05         |
|               | 2nd                | 324.42                 | 275.73                  | 372.71                   | 35.87         |
|               | 1st                | 135.48                 | 130.16                  | 148.40                   | 5.27          |
|               | 2nd                | 318.68                 | 313.65                  | 328.46                   | 23.34         |
| CIP           | 3rd                | 405.29                 | 397.04                  | 430.70                   | 43.86         |
|               | 1st                | 123.77                 | 122.74                  | 129.57                   | 3.72          |
|               | 2nd                | 264.45                 | 278.45                  | 299.12                   | 25.95         |
| CIP + ENPp    | 3rd                | 290.34                 | 256.13                  | 338.95                   | 35.23         |
|               | 4th                | 457.34                 | 423.66                  | 455.81                   | 20.25         |
|               | 1st                | 271.02                 | 264.20                  | 282.79                   | 6.43          |
| NIT           | 2nd                | 303.75                 | 303.21                  | 319.25                   | 42.78         |
|               | 3rd                | 376.93                 | 352.38                  | 415.19                   | 19.13         |
| NIT + ENPp    | 1st                | 247.51                 | 236.47                  | 270.91                   | 22.50         |
|               | 2nd                | 322.51                 | 319.26                  | 353.90                   | 18.26         |
|               | 1st                | 210.74                 | 206.72                  | 218.71                   | 28.349        |
| CFL           | 2nd                | 341.06                 | 298.46                  | 352.20                   | 14.764        |
|               | 3rd                | 719.30                 | 697.68                  | 791.70                   | 39.79         |
|               | 1st                | 207.79                 | 201.62                  | 219.15                   | 27.97         |
| CFL + ENPp    | 2nd                | 389.34                 | 324.64                  | 453.68                   | 11.98         |
|               | 3rd                | 610.88                 | 589.22                  | 649.52                   | 4.96          |
|               | 1st                | 46.69                  | 42.78                   | 58.64                    | 6.98          |
| CLI           | 2nd                | 250.42                 | 207.55                  | 272.20                   | 55.29         |
|               | 3rd                | 766.75                 | 755.71                  | 817.99                   | 9.05          |
|               | 1st                | 47.76                  | 36.61                   | 67.85                    | 8.49          |
| CLI + ENPp    | 2nd                | 229.07                 | 227.72                  | 259.92                   | 28.32         |
|               | 3rd                | 438.59                 | 335.54                  | 457.74                   | 34.06         |
|               | 1st                | 269.98                 | 263.73                  | 276.15                   | 18.05         |
| CFO           | 2nd                | 375.72                 | 322.28                  | 433.14                   | 30.42         |
|               | 3rd                | 717.90                 | 705.93                  | 736.42                   | 18.14         |
|               | 1st                | 68.76                  | 43.99                   | 79.59                    | 7.22          |
| CFO + ENPp    | 2nd                | 261.73                 | 249.90                  | 337.64                   | 21.17         |
|               | 3rd                | 408.28                 | 398.03                  | 466.95                   | 53.87         |

(Continued)
The last exothermic peak, at 645.78°C, is associated with the drug degradation process. In Cefepime + extract mixture, the first three peaks of the drug were maintained, occurring at 69.05, 108.11, and 186.27°C, respectively. With respect to the last peak, there was a delay in the melting point of the drug (Table 4/Fig 3I).

Chloramphenicol has two exothermic events at 184.87 and 279.09°C, probably linked to the melting and decomposition of the drug process. These results contrast with those found by Macedo [104], in which the fusion process of Chloramphenicol was observed in the 155.2°C exothermic event, characteristic of the drug decomposition that occurred at 244.1 and 257.8°C (Table 4/Fig 3J).

Thermogravimetry (TG). In the TG curves of the samples, their thermal decomposition processes were observed. The first, when it occurs at temperatures up to 100°C, refers to the loss of moisture from the sample, which, in the case of the nebulized extract of *P. pyramidalis*, was 6.36%. The second step is assigned to the main stage of decomposition. It indicates the beginning of the degradation process, which for this extract occurred at a temperature of 208.99°C, with a mass loss of 14.67% (Table 5/Fig 4). During this step, many chemical chains were broken, probably caused by carbon dioxide, other gases, and novel compounds, which join to form more stable compounds. They subsequently decompose at higher temperatures. From the second stage, there is a gradual mass loss, corresponding to the whole thermal decomposition of the sample.

At the end of the decomposition process, which generally occurs above 400°C, a mineral residue is present. This residue corresponds to the ash content of the sample, which in the case of
The drugs cephalothin, ceftriaxone, ciprofloxacin, clindamycin, and cefepime showed a three-step thermal decomposition, and the degradation stages for these samples began in 206.36°C (CFL), 263.84°C (CFO), 130.16°C (CIP), 207.55°C (CLI), and 189.01°C (CPM). The antimicrobials norfloxacin, ampicillin, and nitrofurantoin had four steps. The first step starts at temperatures of 206.36°C (NOR), 209.52°C (AMP), 236.47°C (NIT), and 244.38°C (GEN). Chloramphenicol has five stages of decomposition, the first one starting to 194.03°C (Table 5/ Fig 4).

The TG curves of the binary mixtures showed that the mixtures produced with all the drug extracts anticipated the degradation process, except for cefepime. However, when comparing the curves of gentamicin, with its respective binary mixture, we observed that the difference between the temperatures at which the sample began decomposition processes is very small.

**Conclusion**

The results of this study indicate that the extract of the *P. pyramidalis* bark has significant antioxidant action on the radical DPPH. The extract showed no significant antimicrobial activity against multiresistant strains; however, when it was combined with certain synthetic antibiotics, its MIC was significantly reduced. The TG/DTA curves of the antimicrobials and their binary mixtures with the extract indicate a possible physicochemical interaction between the extract and the antibiotic. Whose mixture with the extract showed no additive effect in microbiological assays. This interaction can be confirmed by additional techniques such as X-ray diffraction and FT-IR.

The nebulized extract of *P. pyramidalis* has significant potential for use as an adjuvant component formulation for use in antimicrobial therapy and is a promising alternative in combating multidrug-resistant bacteria.

**Author Contributions**

Conceived and designed the experiments: ACDM TPC. Performed the experiments: TPC FHAF FDM CPS JSS VLS. Analyzed the data: TPC FDM VLS HDMC RMRC. Contributed reagents/materials/analysis tools: ACDM DCF. Wrote the paper: TPC ACDM FDM.

**References**

1. Kumar A, Schweizer HP. Bacterial resistance to antibiotics: Active efflux and reduced uptake. Adv Drug Deliv Rev. 2005; 57: 1486–1513. doi: 10.1016/j.addr.2005.04.004 PMID: 15939505
2. Pogue JM, Kaye KS, Cohen DA, Marchaim D. Appropriate Antimicrobial Therapy in the Era of Multi-drug Resistant Human Pathogens. Clin Microbiol Infect. 2015; 21: 302–312. doi: 10.1016/j.cmi.2014.12.025 PMID: 25743999
3. Davies, S. C. Annual Report of the Chief Medical Officer. Volume Two. 2011. Infections and the rise of antimicrobial resistance. Department of Health. London. 2013.
4. World Economic Forum. Global Risks 2014. Available: http://www.weforum.org/reports/global-risks-2014-report.
5. Poole K. Multidrug resistance in Gram-negative bacteria. 2001; 4: 500–508. doi: 10.1016/S1369-5274(00)00242-3 PMID: 11587924
6. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA. 2009; 302: 2323–2329.
7. Karaiskos I, Giamarelou H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert Opin Pharmacother. 2014; 10: 1351–1370.
8. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Curr Opin Pharmacol. 2014; 18: 56–60. doi: 10.1016/j.coph.2014.09.006 PMID: 25254623
9. Taylor PW. Alternative natural sources for a new generation of antibacterial agents. Int J Antimicrob Agents. 2013; 42: 195–201. doi: 10.1016/j.ijantimicag.2013.05.004 PMID: 23796893
10. Kalana L, Wright GD. Antibiotic adjuvants: multicomponent anti-infective strategies. Expert Rev Mol Med. 2011; 13. http://dx.doi.org/10.1017/S1462339910001766 PMID: 21342612 doi: 10.1017/S1462339910001766
11. Bernal P, Molina-Santiago C, Daddaoua A, Llamas MA. Antibiotic adjuvants: identification and clinical use. Microbiol Biotechnol. 2013; 6: 445–449. doi: 10.1111/1751-7915.12044 PMID: 23445397
12. Gill EE, Franco OL, Hancock REW. Antibiotic Adjuvants: Diverse Strategies for Controlling Drug-Resistant Pathogens. Chem Biol Drug Des. 2015; 85: 56–78. doi: 10.1111/cbdd.12478 PMID: 25393203
13. Farha MA, Brown ED. Discovery of antibiotic adjuvants. Nat Biotechnol. 2013; 31: 120–122. doi: 10.1038/nbt.2500 PMID: 23392510
14. Mabona U, Viljoen A, Shikanga E, Marston A, Van Vuuren S. Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethnopharmacological screening approach. To combination studies and the isolation of a bioactive compound. J Ethnopharmacol. 2013; 148: 45–55. doi: 10.1016/j.jep.2013.03.056 PMID: 23545456
15. Madikizela B, Aderogba MA, Finnie JF, Van Staden J. Isolation and characterization of antimicrobial compounds from Terminalia phanerophlebia Engl. & Diels leaf extracts. J Ethnopharmacol. 2014; 156: 228–234. doi: 10.1016/j.jep.2014.09.003 PMID: 25218320
16. Vieira DRP, Amiral FMM, Maciel MCG, Nascimento FRF, Libério SA, Rodrigues VP. Plant species used in dental diseases: Ethnopharmacology aspects and antimicrobial activity evaluation. J Ethnopharmacol. 2014; 155: 1441–1449. doi: 10.1016/j.jep.2014.07.021 PMID: 25046882
17. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Rep. 2004; 21: 263–277. doi: 10.1039/B212695H PMID: 15042149
18. Gurb-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006; 27: 1–93. doi: 10.1016/j.mam.2005.07.006 PMID: 16105678
19. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008; 15: 639–652. doi: 10.1016/j.phymed.2008.06.008 PMID: 18599280
20. Queiroz LP. New combinations in Libidibia (DC.) Schltld. and Poincianella Britton & Rose (Leguminosae, Caesalpinoideae). Neodiversity. 2010; 5:11–12. doi: 10.13102/neod.51.3
21. Silva ACO, Albuquerque UP. Woody medicinal plants of the Caatinga in the state of Pernambuco (Northeast Brazil). Acta bot bras. 2005; 19: 17–26. doi: 10.1590/S0102-33062005000100003
22. Agra MF, Baracho GS, Nurti K, Basilio ULD, Coelho VPM. Medicinal and poisonous diversity of the flora of “Cariri Paraibano”. Brazil. J Ethnopharmacol. 2007; 111: 383–395. doi: 10.1016/j.jep.2006.12.007 PMID: 17236731
23. Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Lins Neto EMF, Melo JG, et al. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. J Ethnopharmacol. 2007; 114: 325–354. 2007. doi: 10.1016/j.jep.2007.08.017 PMID: 17900836
24. Lucena RFP, Albuquerque UP, Monteiro JM, Almeida CFBR, Florentino ATN, Ferraz JSF. Useful Plants of the Semi-Arid Northeastern Region of Brazil–A Look at their Conservation and Sustainable Use. Environ Monit Assess. 2007; 125: 281–290. doi: 10.1007/s10661-006-9521-1 PMID: 17219240
25. Lucena RFP, Nascimento VT, Araújo EL, Albuquerque UP. Local Uses of Native Plants in an Area of Caatinga Vegetation (Pernambuco, NE Brazil). Ethnobotany Research & Applications 2008; 6: 003. doi: 10.1590/S0102-695X2011000300003
26. Cartaxo SL, Souza MMA, Albuquerque UP. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. J Ethnopharmacol. 2010; 131: 326–342. doi: 10.1016/j.jep.2010.07.003 PMID: 20621178
27. Silva FS, Ramos MA, Hanazaki N, Albuquerque UP. Dynamics of traditional knowledge of medicinal plants in a rural community in the Brazilian semi-arid region. Braz J Pharmacogn. 2011; 21: 382–391. doi: 10.1590/S0102-695X2011005000054
28. Lima MRF, Luna JS, Santos AF, Andrade MCC, Santana AEG, Genet JP, et al. Antibacterial activity of some Brazilian medicinal plants. J. Ethnopharmacol. 2006; 105: 137–147. doi: 10.1016/j.jep.2005.10.026 PMID: 16356672
29. Saraiva AM, Saraiva CL, Gonçalves AM, Soares RR, Mendes FO, Cordeiro RP, et al. Antimicrobial activity and bioautographic study of antistaphylococcal components from Caesalpinia pyramidalis. Tull. Braz. J. Pharm. Sci. 2012; 48: 147–154. doi: 10.1590/S0100-676220120000100016

30. Cruz MCS, Santos PO, Barbosa JR, Melo DLFM, Alviano CS, Antoniolli AR, et al. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol. 2007; 111: 409–412. doi: 10.1016/j.jep.2006.12.005 PMID: 17234376

31. Alviano WS, Alviano DS, Diniz CG, Antoniolli AR, Alviano C, Farias LM, et al. In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch. Oral Biol. 2008; 53: 545–552. doi: 10.1016/j.archoralbio.2007.12.001 PMID: 18243157

32. Ribeiro AR, Diniz PF, Estevam CS, Pinheiro M, Albuquerque RLC Jr., Thomazzi S. Gastroprotective activity of the ethanol extract from the inner bark of Caesalpinia pyramidalis in rats. J Ethnopharmacol. 2013; 147: 383–388. doi: 10.1016/j.jep.2013.03.023 PMID: 23506986

33. Santos AC, Alilane MPR, Passos FCA, Camargo EA, Estevam CS, Santos MRV, et al. Antiinociceptive and anti-inflammatory effects of Caesalpinia pyramidalis in rodents. Braz. J. Pharmacogn. 2011; 21: 1077–1083. doi: 10.1590/S0102-696X20110050000179

34. Borges-dos-Santos RR, Santos JLL, Al-Rouk Z, David JM, David JP, Lima JWM. Biological Effect of Leaf Aqueous Extract of Caesalpinia pyramidalis in Goats Naturally Infected with Gastrointestinal Nematodes. Evid. Based. Complementary Altern. Med. 2012. doi: 10.1155/2012/510391

35. Chaves TP, Santana CP, Véras G, Brandão DO, Felixmino DC, Medeiros ACD, et al. Seasonal variation in the production of secondary metabolites and antifungal activity of two plant species used in Brazilian traditional medicine. Afr J Biotechnol. 2013; 12: 847–853. doi: 10.5897/AJB12.2579

36. Makkar HPS, Becker K, Vanillin-HCl method for condensed tannins: Effect of organic solvents used for extraction of tannins. J Chem Ecol. 1993; 4: 613–621.

37. Makkar HPS, Siddhuraju P, Becker K. Plant Secondary Metabolites. Totowa, New Jersey: Humana Press; 2007.

38. Dhar P, Bajpai PK, Tayade AB, Chaurasia OB, Srivastava RB, Singh SB. Chemical composition and antioxidant capacities of phytococktail extracts from trans-Himalayan cold desert. BMC Complement Altern Med 2013; 13: 259. doi: 10.1186/1472-6882-13-259 PMID: 24098968

39. Kroyer GT. Red clover extract as antioxidant active and functional food ingredient. Innov Food Sci Emerg Technol. 2004; 5: 101–105. doi: 10.1016/S1466-8564(03)00040-7

40. Prakash D, Upadhyay G, Singh BN, Singh HB. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chem. 2007. 104: 783–790. doi: 10.1016/j.foodchem.2006.12.029

41. CLSI–Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility Testing. Twenty Second Informational Supplement. ninth ed. Document M100–S22. Pensilvania. USA: NIH.

42. Coutinho HDM, Costa JGM, Lima EO, Falcão-Silva VS, Siqueira-Júnior JP. Herbal therapy associated with antibiotic therapy: Potentiation of the antibiotic activity against methicillin-resistant Staphylococcus aureus by Turnera ulmifolia L. BMC Complement Altern Med 2009; 9: 13–17. doi: 10.1186/1472-6882-9-13 PMID: 19426487

43. OECD, OECD Guidance Document on Acute Oral Toxicity Testing, OECD, Paris, France, 2000.

44. Garber J, Barbee R, Bielitzki J, Clayton L, Donovan J, et al. Guide for the care and use of laboratory animals. Washington DC: National Academic Press. 2010. 220 p.

45. Roby MAH, Sarhan MA, Selima KAH, Khalel KI. Evaluation of antioxidant activity. total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. Ind Crops Prod. 2013; 43: 827–831. doi: 10.1016/j.indcrop.2012.08.029

46. Monteiro JM, Lins Neto EMF, Amorim ELC, Strattmann RC, Araújo EL. Albuquerque UP, Tannin concentration in three simpatric medicinal plants from caatinga vegetation. Rev Árvore. 2005; 29: 999–1005. doi: 10.1590/S0100-67622005000600020

47. Santos CA, Passos AMPR, Andrade FC, Camargo EA, Estevam CS, Santos MRV, Thomazzi SM. Antiinociceptive and anti-inflammatory effects of Caesalpinia pyramidalis in rodents. Braz J Pharmacogn. 2011; 21: 1077–1083 doi: 10.1590/S0100-676220120000100020

48. Santana DG, Santos CA, Sachs DC, Nogueira PCL, Thomazzi SM, Estevam CH, et al. Beneficial effects of the ethanol extract of Caesalpinia pyramidalis on the inflammatory response and abdominal hyperalgesia in rats with acute pancreatitis. J Ethnopharmacol. 2012; 142: 445–455. doi: 10.1016/j.jep.2012.05.015 PMID: 22626927

49. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc. 1998; 75: 199–212. doi: 10.1007/s11746-998-0032-9
50. Halliwell B. Murcia MA. Chirico S. Aruoma OI. Free radicals and antioxidants in food and in vivo: What they do and how they work. Crit Rev Food Sci Nutr. 1995; 35: 7–20. doi: 10.1080/10408399509527682 PMID: 7748482

51. Zwart LL. Meerman JHN. Commandeur JNM. Vermeulen NPE. Biomarkers of free radical damage: Applications in experimental animals and in humans. Free Radic Biol Med. 1996; 26: 202–26. doi: 10.1016/S0891-5849(96)00196-8 PMID: 8980655

52. Procházková D. Boušková I. Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011; 82: 513–523. doi: 10.1016/j.fitote.2011.01.018 PMID: 21277359

53. González-Burgos E. Gómez-Serranillos MP. Terpene Compounds in Nature: A Review of Their Potential Antioxidant Activity. Curr Med Chem. 2012; 19: 5319–5341. doi: 10.2174/09298671280333335 PMID: 22963623

54. Choi DY. Lee YJ. Hong JT. Lee HJ. Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Res Bull. 2012; 87: 144–153. doi: 10.1016/j.brainresbull.2011.11.014 PMID: 22155297

55. Hatano T. Edamatsu R. Mori A. Fujita Y. Yasuhara E. Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 1989; 37: 2016–2021.

56. Duh PD. Tu YY. Yen GC. Antioxidant Activity of Water Extract of Hamg Jyur (Chrysanthemum morifolium Ramat). Food Sci Technol. 1999; 32: 269–277. doi: 10.1006/fstl.1999.0548

57. Strlič M. Radović T. Kolar J. Pihlar B. Anti- and Prooxidative Properties of Gallic Acid in Fenton-Type Systems. J Agric. Food Chem. 2002; 50: 6313–6317. doi: 10.1021/jf025636x PMID: 12381109

58. Yen GC. Duh PD. Tsai HL. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 2002; 79: 307–313. doi: 10.1016/S0308-8146(02)00145-0

59. Yilmaz Y. Toledo RT. Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin. Epicatechin. and Gallic Acid. J. Agric. Food Chem. 2004; 52: 255–260. doi: 10.1021/jf030117h PMID: 14733505

60. Dall’Agnol R. Ferraz A. Bernardi AP. Albring DC. Sarmento L. Lamb L. et al. Antimicrobial activity of some Hypericum species. Phytomedicine. 2003; 10: 511–516. doi: 10.1078/094471103322331476 PMID: 13678236

61. Rios JL. Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005; 100: 80–84. doi: 10.1016/j.jep.2005.04.025 PMID: 15964727

62. Silva CHTP. Peixoto Sobrinho TJP. Saraiva AM. Pisciottano MNC. Amorim ELC. Phytochemical profile and antibacterial activity of bark and leaves of Caesalpinia pyramidalis Tul. and Sapium glanduloso (L.) Morong. J Med Plants Res. 2012; 6: 4766–4771. doi: 10.5897/JMPR12.830

63. Chanwittheesuk A. Teerawutgulrag A. Kilburn JD. Rakariyatham N. Antibacterial activity of Gallic acid from Caesalpinia mimosoides Lamk. Food Chem. 2007; 100: 1044–48. doi: 10.1016/j.foodchem.2005.11.008

64. Jayaraman P. Sakharkar MK. Lim CS. Tang TH. Sakharkar KR. Activity and interactions of antibiotic and phytochemical combinations against Pseudomonas aeruginosa in vitro. Int. J. Biol. Sci. 2010; 6: 556–68. doi: 10.7890/j.1365-2672.2011.05141.x PMID: 21895894

65. Borges A. Ferreira C. Saavedra MJ Simões M. Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. Microb Drug Resist. 2013; 19: 256–65. doi: 10.1089/mdr.2012.0244 PMID: 23480526

66. Sánchez-Maldonado AF. Schieber A. Ganzle MG. Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. J. Appl. Microbiol. 2011; 111: 1176–1184. doi: 10.1111/j.1365-2672.2011.05141.x PMID: 21895894

67. Vazquez S. Gómez-Jiménez P. Antibacterial effect of phenolic compounds from different wines. Food Control. 2007; 18: 93–101.

68. Hancock REW. The bacterial outer membrane as a drug barrier. Trends Microbiol. 1997; 5: 37–42. doi: 10.1016/S0966-842X(97)81773-8 PMID: 9025294

69. Savage PB. Multidrug-resistant bacteria: overcoming antibiotic permeability barriers of gram-negative bacteria. Ann Med. 2001; 33: 167–171. doi: 10.1080/0785389019002073 PMID: 11370769

70. Denyer SP. Maillard JY. Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. J Appl Microbiol. 2002; 92 Suppl: 3S–4S. doi: 10.1046/j.1365-2672.92.3s1.19.x PMID: 12006111

71. Denyer SP. Stewart GSAB. Mechanism of action of disinfectants. Int Biodeterior Biodegradation. 1998; 41: 261–268. doi: 10.1016/S0964-8305(98)00023-7

Interaction between Poincianella pyramidalis and Antimicrobials
72. Kotra L.P. Haddad J. and Mobashery S. Aminoglycoside: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother. 2000; 44: 3249–3256. doi: 10.1128/AAC.44.12.3249-3256.2000 PMID: 11083623

73. Noller HF. Ribosomal RNA and translation. Annu Rev Biochem. 1991; 60: 191–227.

74. Dunkle JA. Vinal K. Desai PM. Zelinskaya N. Savic M. West DM. et al. Molecular recognition and modification of the 3OS ribosome by the aminoglycoside-resistance methyltransferase NpmA. Proc Natl Acad Sci U S A. 2014; 111: 6275–6280. doi: 10.1073/pnas.1402789111 PMID: 24717845

75. Mingeot-Leclercq MP. Glupczyński Y. Tulkens PM. Aminoglycosides: Activity and Resistance. Antimicrob Agents Chemother. 1999; 43: 727–737. PMID: 10103173

76. Shakil S. Khan R. Zarrilli R. Khan AU. Aminoglycosides versus bacteria—a description of the action, resistance mechanism, and nosocomial battleground. J Biomed Sci. 2008; 15: 5–14. doi: 10.1128/AAC.44.12.3249-3256.2000 PMID: 17657587

77. Avent M. L. Rogers B. A. Cheng A. C. and Paterson D. L. Current use of aminoglycosides: indications. pharmacokinetics and monitoring for toxicity. Int Med J. 2011; 41: 441–449. doi: 10.1111/j.1445-5994.2011.02452.x PMID: 21309997

78. Giamarellou H. Zisis NP. Tagari G. Bouzos J. In vitro synergistic activities of aminoglycosides and new beta-lactams against multiresistant Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1984; 25: 534–536. doi: 10.1128/AAC.25.4.534 PMID: 6428310

79. Wu YL. Scott EM. Po AL. Tariq VN. 1999. Ability of azlocillin and tobramycin in combination to delay or prevent resistance development in Pseudomonas aeruginosa. J Antimicrob Chemother. 44: 389–392. PMID: 10511408

80. Sousa EO. Rodrigues FFG. Campos AR. Lima SG. Costa JGM. Chemical composition and synergistic interaction between aminoglycosides antibiotics and essential oil of Lantana montevidensis Briq. Nat Prod Res. 2013; 27: 942–945. doi: 10.1080/17435804.2012.678351 PMID: 22475025

81. Barreto HM. Lima IS. Coelho KMRN. Osório LR. Mourão RA. Santos BHC. et al. Effect of Lippia origanoides H.B.K. essential oil in the resistance to aminoglycosides in methicillin resistant Staphylococcus aureus. Eur J Integr Med. 2014; 6: 560–564. doi: 10.1016/j.eujim.2014.03.011

82. Coutinho HDM. Rodrigues FFG. Nascimento EMM. Costa JGM. Falcão-Silva VS. Siqueira-Júnior JP. Synergism of Gentamicin and Norfloxacin with the Volatile Compounds of Lippia microphylla Cham. (Verbenaceae). J Essent Oil Res. 2011; 23: 24–28. doi: 10.1080/10412905.2011.9700443

83. Aqil F. Ahmad I. Owais M. Evaluation of anti-methicillin-resistant Staphylococcus aureus (MRSA) activity and synergy of some bioactive plant extracts. Biotechnol J. 2006; 1: 1093–1102. doi: 10.1002/biot.20060130 PMID: 17004300

84. Coutinho HDM. Costa JGM. Falcão-Silva VS. Siqueira-Júnior JP. Lima EO. Effect of Momordica charantia L. in the resistance to aminoglycosides in the methicillin-resistant Staphylococcus aureus. Comp Immunol Microbiol Infect Dis. 2010a; 33: 467–471. doi: 10.1016/j.cimid.2009.08.001 PMID: 19732954

85. Coutinho HDM. Costa JGM. Falcão-Silva VS. Siqueira-Júnior JP. Limao EO. Effect of Eugenia uniflora and Eugenia jambolanaum. J Med Food. 2010b; 13: 1024–1026. doi: 10.1089/jmf.2009.0158 PMID: 20482280

86. Bitu VCN. Fecundo HTF. Costa JGM. Coutinho HDM. Rodrigues FFG. Santana NM. et al. Chemical composition of the essential oil of Lippia gracilis Schauer leaves and its potential as modulator of bacterial resistance. Nat Prod Res. 2014; 28: 399–402. doi: 10.1080/174861419.2013.853810 PMID: 24479735

87. Figueiredo FG. Lucena BFF. Matias EFF. Tintino SR. Leite NF. Andrade JC. et al. Chemical composition and evaluation of modulatory of the antibiotic activity from extract and essential oil of Myracrodruon urundeuva. Pharm Biol. 2014; 52: 560–565. doi: 10.3109/13880209.2013.853810 PMID: 24251788

88. Tintino SR. Souza CES. Guedes GMM. Costa JI. Duarte FM. Chaves MCO. et al. Modulatory antimicrobial activity of Piper arboreum extracts. Acta Bot Croat. 2013; 73: 281–289. doi: 10.2478/abtc-2013-0026

89. Juven BJ. Kanner J. Schved F. Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Appl Bacteriol. 1994; 76: 626–631. doi: 10.1111/j.1365-2672.1994.tb01661.x PMID: 8027009

90. Imming P. Sinning C. Meyer A. Drugs. their targets and the nature and number of drug targets. Drug Discov. 2006; 5: 821–834. doi: 10.1038/ird2132 PMID: 17016423

91. Schmidt B. Ribnicky DM. Poulev A. Logendra S. Cefalu WT. Raskin I. A natural history of botanical therapeutics. Metabolism. 2008; 57 (Suppl 1): S3–S9. doi: 10.1016/j.metabol.2008.03.001 PMID: 18555851
92. Wagner H. Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine. 2009; 16: 97–110. doi: 10.1016/j.phymed.2008.12.018 PMID: 19211237

93. Matias EEF. Alves EF. Santos BS. Souza CES. Ferreira JVA. Lavor AKLS. et al. Biological activities and chemical characterization of Cordia verbenacea DC as tool to validate the ethnobiological usage. Evid. Based Complement. Alternat. Med. 2013. 1–7. doi: 10.1155/2013/164215

94. Sustar B. Bukovec N. Bukovec P. Polymorphism and stability of Norfloxacin. (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinil)-3-quinolinocarboxylic acid. J Therm Anal. 1993; 40: 475–48. doi: 10.1007/BF02546616

95. O’Neil MJ. Heckelman PE. Dobbelaar PH. Roman KJ. Kenny CM. Karaffa LS. The Merck Index. 15th ed.; RSC Publishing. 2013.

96. Baraldi C. Tinti A. Ottanic S. Gamberinia MC. Characterization of polymorphic ampicillin forms. J Pharm Biomed Anal. 2014; 100: 329–340. doi: 10.1016/j.jpba.2014.08.021 PMID: 25194347

97. Otsuka M. Kaneniwa N. Effect of grinding on the crystallinity and chemical stability in the solid state of cephalothin sodium. Int J Pharm. 1990; 62: 65–73. doi: 10.1016/0378-5173(90)90031-X

98. Sadeek SA. El-Shwiniy WH. Zordok WA. El-Didamony AM. Spectroscopic, structure and antimicrobial activity of new Y(III) and Zr(IV) ciprofloxacin. Spectrochim Acta A Mol Biomol Spectrosc. 2011; 78: 854–867. doi: 10.1016/j.saa.2010.12.048 PMID: 21208824

99. Dorofeev VL. Arzamastsev AP. Veselova OM. Melting point determination for the analysis of drugs of the fluoroquinolone group. Pharmaceutical Chemistry Journal. 2004; 38: 333–335. doi: 10.1023/B:PHAC.0000048148.54165.55

100. Owens HM. Dash AK. Ceftriaxone sodium: comprehensive profile. Profiles Drug Subst Excip Relat Methodol. 2003; 30: 21–57. doi: 10.1016/S0099-5428(03)30002-4 PMID: 22469539

101. Masoud MS. Ali AE. Elasala GS. Synthesis, spectral, computational and thermal analysis studies of metalloceftaxone antibiotic. J Mol Struct. 2015; 1084: 259–273. doi: 10.1016/j.molstruc.2014.11.049

102. Aquino RP. Auriemma G. Mencherini T. Russo P. Porta A. Adami R. et al. Design and production of gentamicin/dextran microparticles by supercritical assisted atomisation for the treatment of wound bacterial infections. Int J Pharm. 2013; 440: 188–194. doi: 10.1016/j.ijpharm.2012.07.074 PMID: 22917746

103. Ferdous S. Sultan Z. Bashar T. Rahman A. Islam S. In vitro and In vivo Studies of Drug-Drug Interaction between Metformin and Cefepime. Pharm Anal Acta. 2015; 6:348. doi: 10.4172/2153-2435.1000348

104. Macedo RO. Araújo CFS. Nascimento TG. Macêdo AMC. Applications of Thermogravimetry in the quality control of chloramphenicol tablets. J Therm Anal Calorim. 1999; 56: 1323–1327. doi: 10.1023/A:1010102422381

105. Flörke OW. Graettsch HA. Brunk F. Benda L. Paschen S. Bergna HE. et al. Silica. Ullmann’s Encyclopedia of Industrial Chemistry. Wiley Online Library. 2008. doi: 10.1002/14356007.a23_583.pub3