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Research Article

Evaluation of the Antibacterial and Drug Potentiating Activity of *Dysphania ambrosioides* Ethanol Extract Against MDR Strains

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Abstract

The discovery of penicillin by Alexander Fleming in 1928 marked the beginning of the antibiotic era but also led to the development of resistant bacterial strains. Bacterial resistance, worsened by the improper use of antibiotics, has become a growing concern. Phytotherapy, particularly using medicinal plants, emerges as a promising alternative for treating resistant infections. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, commonly known as "mastruz," has shown significant therapeutic potential and is the focus of this study to evaluate its effect against multidrug-resistant bacterial strains. The leaves of *D. ambrosioides* were collected, and an ethanolic extract (EEDA) was prepared from 250 g of dried leaves. Antibacterial activity was assessed using standard strains (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and resistant clinical isolates. The Minimum Inhibitory Concentration (MIC) was determined by microdilution in 96-well plates. The potentiating effect of EEDA was evaluated by combining it with gentamicin, norfloxacin, and oxacillin, using serial microdilutions and resazurin analysis. EEDA showed a significant potentiating effect when combined with gentamicin for *P. aeruginosa*, reducing the MIC. However, with norfloxacin and oxacillin, the effect was antagonistic. For *E. coli*, the combination with gentamicin also reduced the MIC, though not statistically significant. For *S. aureus*, there was no change in MIC with gentamicin and norfloxacin, and the combination with oxacillin showed an antagonistic effect. Phytochemical analysis revealed flavonoids and other secondary metabolites in EEDA, suggesting these compounds may influence antimicrobial activity. These results underscore the importance of investigating interactions between natural compounds and antibiotics to develop more effective therapeutic strategies.

***Keywords:** Bacterial resistance, Antibiotics, Phytotherapy, Medicinal plant.*

1. Introduction

In 1928, Alexander Fleming discovered *Penicillium notatum* in a London hospital when he observed the inhibition of staphylococcal growth caused by this fungus, a phenomenon known as antibiosis [1]. According to Silveira [2], the extensive use of penicillin after World War II led to the emergence of the first strains of Gram-positive bacteria resistant to penicillin antibiotics. In the 1940s, the discovery and use of penicillin significantly reduced the high mortality rates from bacterial infections, although the emergence of resistant bacteria soon became evident.

According to Silveira et al. [3], bacterial resistance is a natural response of bacteria to constant exposure to antibiotics, exacerbated by the improper and indiscriminate use of these drugs. Antimicrobials, which can be of natural, semi-synthetic, or synthetic origin, either suppress bacterial growth (bacteriostatic) or destroy bacteria (bactericidal). The inappropriate or indiscriminate use of these drugs has become a global concern [4] Brazilian folk medicine, influenced by Indigenous, European, and African cultures, is characterized by the use of medicinal plants, which have emerged as an alternative in disease treatment. In regions with limited access to commercial drugs, ethnopharmacology leads to the use of many plants without scientifically proven efficacy. However, research on these species can ensure safety and effectiveness, providing alternative treatments [5].

Medicinal plants have proven to be an effective alternative for treating infections caused by resistant bacteria. They contain chemically complex compounds with strong therapeutic potential and tend to cause fewer side effects compared to commercial drugs. Phytochemicals, which are secondary metabolites present in plants, demonstrate antimicrobial activity [6]. With advances in science and ethnopharmacological knowledge, phytotherapy and the therapeutic value of medicinal plants have received increased attention, leading to more research and the discovery of new natural compounds (secondary metabolites) for treating the population [7].

Among medicinal plants, *Dysphania ambrosioides*, commonly known in Brazil as "mastruz," stands out. This plant significantly impacts the immune system and is widely used in folk medicine as a healing and anti-inflammatory agent. *D. ambrosioides* is an angiosperm of the order Caryophyllales, belonging to the family Amaranthaceae and the genus *Dysphania*, with the binomial nomenclature *Dysphania ambrosioides* (L.) Mosyakin & Clemants, and its synonym *Chenopodium ambrosioides* [8]. Studies have demonstrated its traditional use in treating infections, highlighting its therapeutic potential in phytotherapy [9]. This plant represents an important example of how ethnopharmacological knowledge can be integrated into modern science to develop effective treatments against resistant bacterial infections.

The article aims to observe the effect of the ethanolic extract of *D. ambrosioides* (EEDA) on multidrug-resistant bacterial strains, evaluating the effect of secondary metabolites in combination with antimicrobials. It seeks to determine whether EEDA can potentiate the treatment of these strains when used with antimicrobials.

2. Materials and Methods

2.1. Collection of plant material and preparation of extract

The leaves of *Dysphania ambrosioides* were collected from the medicinal plant garden at the Regional University of Cariri – URCA, Crato, CE, Brazil, at 09:00 ± 00:30 h under the coordinates 07°14'19.2'' S and 39°24'52.8'' W. A specimen was pressed, identified, and deposited in the Herbário Anchieta - PACAAGP under voucher number 116226. For the ethanolic extract of *Dysphania ambrosioides* (EEDA), 250 g of dried leaves were crushed and placed in a container with 96% ethanol. After 72 hours, the material was filtered and concentrated using a rotary evaporator.

2.2. Antibacterial activity

According to the methodology of Bezerra, et.al [9], standard bacterial strains were used to determine the Minimum Inhibitory Concentration (MIC), being *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923. While clinical bacterial isolates of *E. coli* 06, *P. aeruginosa* 24 and *S. aureus* 10, with a drug resistance profile. As for the culture medium for the antibacterial assays, Brain Heart Infusion (BHI) was prepared according to the measures recommended by the manufacturer. While the drugs used to evaluate the potentiating capacity of EDDA were gentamicin from the aminoglycoside class, Norfloxacin, belonging to the fluoroquinolone class and oxacillin sodium is an antibiotic that belongs to the penicillin group.

2.2.1. Minimum inhibitory concentration (MIC)

The MIC is the minimum concentration responsible for completely inhibiting bacterial growth. Adapted from the methodology of Bezerra et al. [9] and Gomes et al. [10], a 1000 µL solution containing 100 µL of inoculum and 900 µL of 10% BHI liquid culture medium was prepared in eppendorf tubes. Subsequently, this solution was distributed in 96-well plates filled in the numerical direction by adding 100 µL to each well. Subsequently, microdilutions were performed in series with 100 µL of EEDA at concentrations ranging from 1,024 µg/mL to 1 µg/mL, so that the plates were incubated for 24 hours at 37 °C. To read the MIC, 20 µL of a resazurin solution were added to each well so that redox reactions would occur in the wells where there was still bacterial growth. After 1 h, a change in the color of the wells was observed, where the change from blue to red corresponds to microbial growth and blue remains the absence of growth.

2.2.2. Potentiating effect of antibiotics

To measure the modulating effect of EEDA, the methodology proposed by Coutinho et al. [] was used, with modifications, in which, after MIC tests with resistant bacteria, the results were used to determine the subinhibitory concentrations to be used with antibiotics in concentrations ranging from 1,024 µg/mL to 1 µg/mL. Thus, 1,162 µL of 10% BHI were used for the tests, with 150 µL of the inoculum of each strain and the ethanolic extract with a volume corresponding to a subinhibitory concentration, while the control group was prepared with only 1,350 µL of BHI (10%) and 150 µL of bacterial suspension. Subsequently, a serial microdilution with the antibiotic was performed, with 100 µL of each drug up to the penultimate well. The plates were incubated (24 hours at 37 °C) and read by adding 20 µL of resazurin [9].

2.3. Statistical analysis

All experiments were conducted in triplicate. The results were presented as means with their corresponding standard errors (±SEM). Data were then analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test with a 95% confidence level. P-values were classified as < 0.0001 (**** = extremely significant), 0.0001 to 0.001 (*** = highly significant), 0.001 to 0.01 (** = very significant), 0.01 to 0.05 (* = significant), and >0.05 (ns = not significant). All analyses were carried out using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

3. Results and Discussions

EEDA did not show direct antibacterial activity against standard and multiresistant pathogenic strains, with MIC > 512 µg/mL. With the observed results, it was possible to create the following graph, presenting the microorganisms tested and their MIC for each antimicrobial and with the use of EEDA. It was possible to create Figure 1.

Based on the results presented in Figure 1, it was observed that the *Pseudomonas aeruginosa* strain treated with the ethanolic extract of *Dysphania ambrosioides* leaves (EEDA) in combination with gentamicin showed a significant modifying effect on the antibiotic ($p < 0.0001$). However, this effect was not observed when EEDA was combined with the other two antibiotics tested. Specifically, oxacillin maintained the same Minimum Inhibitory Concentration (MIC) with and without the use of EEDA, while the combination with norfloxacin resulted in an antagonistic effect.

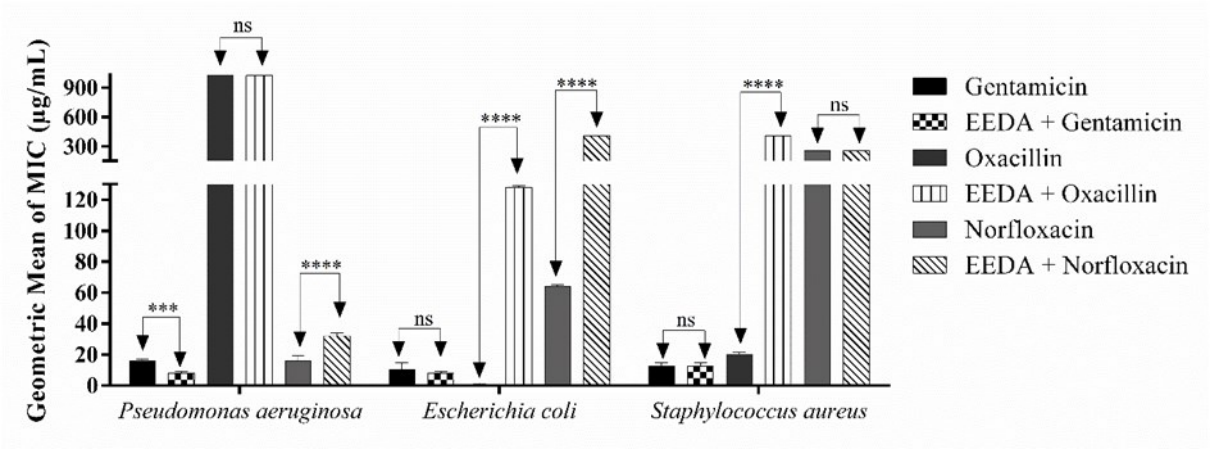

Figure 1: *Combined effect of ethanolic extract of Dysphania ambrosioides (EEDA) with gentamicin, oxacillin and norfloxacin against Pseudomonas aeruginosa 24, Staphylococcus aureus 10 and Escherichia coli 06. Control refers to only the antibiotic: gentamicin, oxacillin and norfloxacin. **** statistically significant when p < 0.0001; *** p < 0.001; not statistically significant (ns) when p > 0.05.*

For *Escherichia coli* strains, the combination of gentamicin with EEDA also demonstrated a potentiating effect, leading to a reduction in MIC, although this reduction was not statistically significant (p > 0.05). On the other hand, the combination of EEDA with oxacillin and norfloxacin exhibited an antagonistic effect. For *Staphylococcus aureus* strains, both gentamicin and norfloxacin, when combined with EEDA, maintained the same MIC as the antibiotics alone. However, the combination of EEDA with oxacillin resulted in an antagonistic effect. These results suggest that EEDA may have a specific modifying role depending on the antibiotic and the bacterial strain in question, highlighting the complexity of interactions between natural compounds and conventional antimicrobials.

According to Quaresma [12], the phytochemistry of the ethanolic extract, as observed through thin-layer chromatography, reveals the presence of secondary metabolites such as triterpenes and steroids, flavonoid aglycones, flavonoid glycosides, coumarins, saponins, polyphenols, alkaloids, tannins, and anthraquinone glycosides. These metabolites are essential for the plant's physiological processes, providing protection against insects, fungi, and ultraviolet rays. In humans, they can act as agonists or antagonists in biochemical processes, influencing mechanisms that affect bacterial cell walls [13].

Flavonoids are metabolites found in some medicinal plants, and they exhibit significant therapeutic activity through various mechanisms that can inhibit the growth of Gram-negative and Gram-positive bacteria [14]. Preparations containing flavonoids have been used historically to treat various human diseases, such as using propolis for treating wounds and ulcers [15]. According to Silva [16], when flavonoids are combined with antibiotics, they can enhance activity against several bacteria. They can act in various ways, such as altering membrane permeability, inhibiting nucleic acid synthesis, and neutralizing virulence factors.

The ability of flavonoids to interact with the bacterial membrane is due to their lipophilicity and the presence of hydroxyl groups, which confer slight acidity and polarity to the molecule. Their interaction with the lipid bilayer depends on the pH; a lower pH causes deprotonation of polar groups, leading to deeper penetration of flavonoids into the lipid layer. The capacity to reduce DNA and RNA synthesis through topoisomerase inhibition has been reported, with fluorinated chalcone-1,2,3-triazole conjugates showing promising antimicrobial activity by interacting with topoisomerase through various non-covalent interactions [16].

Among the antibiotics used for testing was gentamicin, an aminoglycoside with bactericidal action. It works by inhibiting protein synthesis; once inside the cell, it irreversibly binds to the bacterial 30S ribosomal subunit, leading to the production of faulty proteins, altering cell membrane function, and causing the organism's death [17]. Norfloxacin inhibits DNA gyrase and topoisomerase IV, enzymes involved in bacterial DNA synthesis [18]. Lastly, oxacillin inhibits bacterial growth by blocking cell wall synthesis through its binding to penicillin-binding proteins (PBP) in the cell wall of these microorganisms [19].

As shown in Figure 1, EEDA had a potentiating effect when combined with gentamicin in *Pseudomonas aeruginosa* and *E. coli* strains. This may be due to the ability of flavonoids to inhibit protein synthesis, thereby enhancing gentamicin's effect and reducing the MIC against these strains. However, since norfloxacin is an antibiotic that inhibits bacterial DNA synthesis and flavonoids exhibit similar effects, no potentiating effect was observed when the drug was combined with EEDA. The same applies to oxacillin, which inhibits bacterial cell wall synthesis, a function that metabolites also possess, thus not providing clinical interest when the drug is combined with EEDA. According to Kuzhuppillymyal, Martínez, and Cruz [20], it is possible to discover new compounds with antimicrobial activity from the use of plant secondary metabolites, enabling the direct use of these metabolites as therapeutic agents.

4. Conclusion

The results indicate that EEDA has a potentiating effect when combined with gentamicin on *P. aeruginosa* and *E. coli*, while it presents antagonistic effects with other antibiotics and strains. These findings highlight the importance of investigating the specific interactions between natural compounds and antibiotics to develop more effective therapeutic strategies.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

None

References

1. Oliveira, M., Pereira, K. D. S. P. S., Zamberlam, C. R. Resistência bacteriana pelo uso indiscriminado de antibióticos: Uma questão de saúde pública. *Revista Ibero-Americana de Humanidades, Ciências e Educação*, **2020**, *6*, 183-201. https://doi.org/10.29327/4426668
2. Silveira, G. P., Mendes, L. R., Costa, J. A., Martins, D. C., Lima, T. M., Fernandes, P. L. Estratégias utilizadas no combate à resistência bacteriana. *Química Nova*, **2006**, *29*, 844-855. https://doi.org/10.1590/S0100-40422006000400037

3. Silveira, Z. P., Oliveira, M. C., Fernandes, A. S., Santos, J. A., Ribeiro, D. F., Souza, L. M. A automedicação com antibióticos e as repercussões na resistência bacteriana. *Revista Ibero-Americana de Humanidades, Ciências e Educação*, 2023, *9*, 545-556. https://doi.org/10.51891/rease.v9i7.10653
4. Vieira, P. N., Vieira, S. L. V. Uso irracional e resistência a antimicrobianos em hospitais. *Arq*. *ciências saúde UNIPAR*, 2017, 209-212. https://doi.org/10.25110/arqsaude.v21i3.2017.6130
5. Dantas, H. P., Silva, C. F., Andrade, L. N., Gomes, F. A., Almeida, M. P. Atividade antibacteriana de imburana-de-espinho sobre bactérias do meio ambiente bucal. *Research, Society and Development,* 2022, *11*, 1-11. https://doi.org/10.33448/rsd-v11i7.29666
6. Souza, Z. N., Córdula, C. R., Cavalcanti, I. M. F. The potential usage of Caatinga natural products against multi-drug-resistant bacteria. *Fitoterapia*, 2023, *172*. https://doi.org/10.1016/j.fitote.2023.105752
7. Ferreira, S. B., Dantas, I. C., Catão, R. M. R. Avaliação da atividade antimicrobiana do óleo essencial de sucupira (*Pterodon emarginatus* Vogel). *Revista Brasileira de Plantas Medicinais*, 2014, *16*, 225-230. https://doi.org/10.1590/S1516-05722014000200009
8. Gomes, L. N., Rodrigues, R. A., Ferreira, J. S., Alves, A. S., Martins, M. S. Efeito do extrato de *Dysphania ambrosioides* (L) na resposta de neutrófilos humanos. 2018.
9. Almeida-Bezerra, J. W., Silva, L. M., Almeida, A. M., Pereira, M. T., Lopes, P. S., Oliveira, C. R. Chemical composition, antimicrobial, modulator and antioxidant activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants. *Comparative immunology, microbiology and infectious diseases,* 2019, *65*, 58-64. https://doi.org/10.1016/j.cimid.2019.04.010
10. Gomes, F. M. S., Silva, A. A., Rodrigues, T. F., Santos, A. L., Costa, R. F., Oliveira, M. P. Evaluation of antibacterial and modifying action of catechin antibiotics in resistant strains. *Microbial Pathogenesis,* 2018, *115*, 175-178. https://doi.org/10.1016/j.micpath.2017.12.058
11. Coutinho, H. D. M., Costa, J. G. M., Lima, E. O., Falcão-Silva, V. S., Siqueira-Júnior, J. P. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. *Chemotherapy*, 2008, *54*, 328-330. https://doi.org/10.1159/000151267
12. Quaresma, A. C. S., Carvalho, R. S., Lima, C. F., Oliveira, J. S., Oliveira, F. V. S. Caracterização farmacognóstica, fitoquímica e avaliação *in silico* da atividade de monoterpenos isolados da espécie *Dysphania ambrosioides* (L.) Mosyakin & Clemants. *Research, Society and Development*, 2021, *10*, 1-16. https://doi.org/10.33448/rsd-v10i8.16715
13. Cunha, L. E. B., Ferreira, M. R. S., Santos, A. P. F., Nascimento, A. M., Martins, J. C. Perfil químico e atividade antibacteriana e moduladora do extrato etanólico de *Chenopodium ambrosioides* L. contra *Streptococcus pneumoniae* e *Klebsiella pneumoniae*. *Contribuciones a las Ciencias Sociales*, 2024, *17*, 1-17. https://doi.org/10.55905/revconv.17n.7-331
14. Song, M., Liu, Y., Huang, X., Ding, S., Wang, Y., Shen, J. Plant natural flavonoids against multidrug resistant pathogens. *Advanced Science*, 2021, *8*, 1-11. https://doi.org/10.1002/advs.202100749
15. Flambó, D. F. A. L. P. Atividades biológicas dos flavonoides: Atividade antimicrobiana. 2013.
16. Silva, A. D., Pereira, J. P., Costa, L. S., Almeida, R. S. Atividade Antimicrobiana de Flavonoides. *Revista Interdisciplinar em Ciências da Saúde e Biológicas*, 2020, *4*, 51-65.
17. Siqueira, N. S., Almeida, T. A., Reis, R. M. O uso da gentamicina e sua monitorização terapêutica. 2013.
18. Reis, F. C. C. Investigação química de complexos de coordenação dos antibióticos enrofloxacina e norfloxacina combinados ao íon Ru (III) e suas interações com biomoléculas alvo. 2014.
19. Terasawa, L. B. Caracterização da resistência à oxacilina em estafilococos coagulase negativa isolados no hospital de clínicas de Curitiba–Paraná. Curitiba, 2006.
20. Kuzhuppillymyal-Prabhakarankutty, L., Martínez-Meléndez, A., Cruz-López, F. Los metabolitos secundarios como agentes antimicrobianos. *Biología y Sociedad*, 2023, *6*, 33-40. https://orcid.org/0000-0002-5180-4178

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