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Research Article

Antibacterial, Antitumoral and Cytotoxic Potential of the Ethanolic Extract of *Dysphania ambrosioides* (Amaranthaceae) Leaves

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Abstract

The indiscriminate use of antibiotics is currently the main factor driving the development of microbial resistance, making the search for new therapies based on effective natural agents to combat resistant infections crucial. In this context, the genus *Dysphania* stands out for its species with medicinal properties, and *Dysphania ambrosioides* L. is particularly relevant, being widely used in traditional medicine for its antimicrobial, antioxidant, and anticancer activities. This study investigated its potential, revealing that *D. ambrosioides* extracts showed significant inhibitory activity against *Escherichia coli* (87.46% at 1000 µg/mL) and *Staphylococcus aureus* (66.49% at 1000 µg/mL). Regarding cytotoxicity in fibroblasts, a dose-dependent effect was observed, with 1000 µg/mL being the most toxic (80.82% reduction in cell viability), while lower doses were less cytotoxic (20.54% at 250 µg/mL). In terms of antitumor activity in breast cancer cells, the extract demonstrated a non-linear pattern, with the 250 µg/mL dose showing an inhibitory effect of 43.01%, which increased to 71.19% at a concentration of 1000 µg/mL. Thus, the *D. ambrosioides* extract demonstrated promising potential against bacterial strains, lower cytotoxicity at reduced doses, and variable antitumor activity, justifying further investigations into its therapeutic profile.

Keywords: *Mastruz, Medicinal Plants, Botanical Extracts*

Introduction

Antibiotic resistance is currently classified as a serious public health problem. Its global spread occurs in an uncontrolled manner and is hindered by the development of effective therapies [1]. The misuse of antibiotics is one of the main factors contributing to the significant increase in resistance, especially in clinical practice, resulting in the failure of antibacterial treatments [2]. Analyses indicate that by the year 2050, an estimated 50 million deaths will be caused by resistant infections [3]. In this context, the need to seek new approaches to develop solutions that can combat this crisis becomes imperative [4].

The development of antimicrobials focusing on the use of natural products can be considered an effective therapy for treating infections caused by resistant strains [5]. These plant-derived products are known to be a rich source of chemical constituents with potential therapeutic applicability due to their biological activities [6]. Based on this, several studies have highlighted the evaluation of these compounds for use in modern medicine, aiming to combat bacterial resistance [7,8].

The use of medicinal plants is popularly known for therapeutic purposes, which promotes the development of new drugs derived from this natural raw material [9]. The *Dysphania* genus is recognized for the medicinal activities present in its species. Among them, *Dysphania ambrosioides* L. stands out, commonly used in traditional medicine [10]. *D. ambrosioides* belongs to the Amaranthaceae family and is popularly known as "mastruz" or "erva-de-santa-maria" [11]. It is a herbaceous plant widely distributed in several parts of the world, including Africa, North America, Europe, and Asian countries [12].

Dysphania ambrosioides is widely applied in ethnopharmacological studies and is frequently used by traditional communities [13]. In these contexts, the use of its leaves through maceration and infusion has shown potential for treating ailments ranging from stomach pain to infections caused by intestinal parasites in humans [14]. The biological constituents present in the plant can also be employed against diseases such as tuberculosis, rheumatism, uterine hemorrhage, and respiratory disorders [15].

Various parts of the plant contain a large number of phytochemical compounds, such as phenolics, including flavonoids, terpenoids, and phenolic acids [16]. Authors have reported several biological activities in the plant, such as antifungal [17], antioxidant [18], and antiviral [19]. Furthermore, *D. ambrosioides* demonstrated *in vitro* anticancer activity when tested against the proliferation of hepatic cancer cells [20].

Despite the reports on the medicinal use of *Dysphania ambrosioides*, few studies have systematically evaluated the ethanolic extract of its leaves regarding antibacterial, antitumor, and cytotoxic activities. Therefore, this study aims to assess its antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 strains, as well as its cytotoxic activity on fibroblasts and antitumor activity on breast cancer cells. All tests will be conducted using in vitro models, contributing to the understanding of the plant's bioactive properties and its potential therapeutic application.

Methodology

Collection of plant material

Leaves of *Dysphania ambrosioides* were collected from the Medicinal Plants Garden of the Regional University of Cariri – URCA, located in the municipality of Crato-CE, Brazil. The collection was carried out in March 2015 between 9:00 and 9:30 AM at coordinates 07º14´19.2º S and 39º24´52.8´´ longitude of Greenwich. A specimen was pressed and identified by the Anchieta Herbarium - PACA-AGP under voucher number 116226. An ethanolic extract of the plant was prepared from the collected material for subsequent analysis.

Preparation of the ethanolic extract (EE)

The collected leaves were selected, naturally dried, and ground to increase the surface area, making them suitable for the production of the ethanolic extract of *D. ambrosioides* (EEDA). The ground material was subjected to a cold extraction process by immersion in analytical grade ethanol (P.A.) for 96 hours. Then, the solution was filtered and the ethanol was removed using a rotary evaporator operating under reduced pressure, with the temperature maintained between 30 °C and 40 °C to preserve the active compounds.

In vitro antibacterial activity

To evaluate the antibacterial activity of the extracts, a reference strain of Gram-negative bacteria (*Escherichia coli* ATCC 25922) and a strain of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) were used. The bacterial strains were cultured in Mueller-Hinton broth (MHB). The screening assay was performed following the CLSI M7-A113 methodology with modifications. Bacteria were seeded in a 96-well plate at a concentration of 5 x 10^5 (*E. coli*) and 2.5 x 10^5 (*S. aureus*) CFU/mL (200 µL). The bacteria were incubated at 35 °C for 6 hours with different concentrations of the extract. Then, 20 µL of resazurin solution was added, reaching a final concentration of 30 µM.

Non-specific *in vitro* cytotoxicity

For the experiment, NCTC 929 clone fibroblasts were cultured in a 96-well plate with 180 µL of RPMI-1640 supplemented with 10% FBS and the antibiotics penicillin and streptomycin. Approximately 2x104 cells per well were cultured and incubated at 37 °C for 48 h in a 5% CO2 environment with different concentrations of the samples. Then, 20 µL of a resazurin solution were added, reaching a final concentration of 2 mM. The plate was incubated again, this time at 37 °C for 4 h, and finally, the absorbance was monitored at 570 and 600 nm using a Synergy H1 multimode spectrophotometer (BIOTEK). The cytotoxicity of the samples was estimated by calculating the percentage of cytotoxicity activity.

In vitro antitumor activity

In these experiments, J774 macrophages and MCF-7 breast cancer cells were cultured in a 96-well plate with 180 µL of RPMI-1640 supplemented with 10% FBS and the antibiotics penicillin and streptomycin. Approximately $2x10^4$ cells per well were cultured and incubated at 37°C for 48 h in a 5% CO2 environment with different concentrations of the samples. Then, 20 µL of a resazurin solution were added, reaching a final concentration of 2 mM. The plate was incubated again, this time at 37°C for 4 h, and finally, the absorbance was monitored at 570 and 600 nm using a Synergy H1 multimode spectrophotometer (BIOTEK). The antitumor activity of the samples was estimated by calculating the percentage of antitumor activity.

Statistical Analysis

The tests were performed in triplicate and expressed using a linear regression model with a one-way ANOVA followed by the Bonferroni and/or Tukey test, using GraphPad Prism 8.0 software. Results with $p < 0.05$ were considered statistically significant.

Results

Antibacterial Activity

Figure 1 shows the evaluation of the inhibitory effect of the ethanolic extract of *Dysphania ambrosioides* (EEDA) against two standard bacterial strains: *Escherichia coli* ATCC 25922 (A), a Gram-negative bacterium, and *Staphylococcus aureus* ATCC 25923 (B), a Gram-positive bacterium. Figure 1A shows that against the E. coli strain, the concentration of 1000 µg/mL exhibited the greatest inhibitory effect, reaching 87.46% inhibition of bacterial growth. This indicates that the antibacterial activity of the extract increases significantly at higher concentrations.

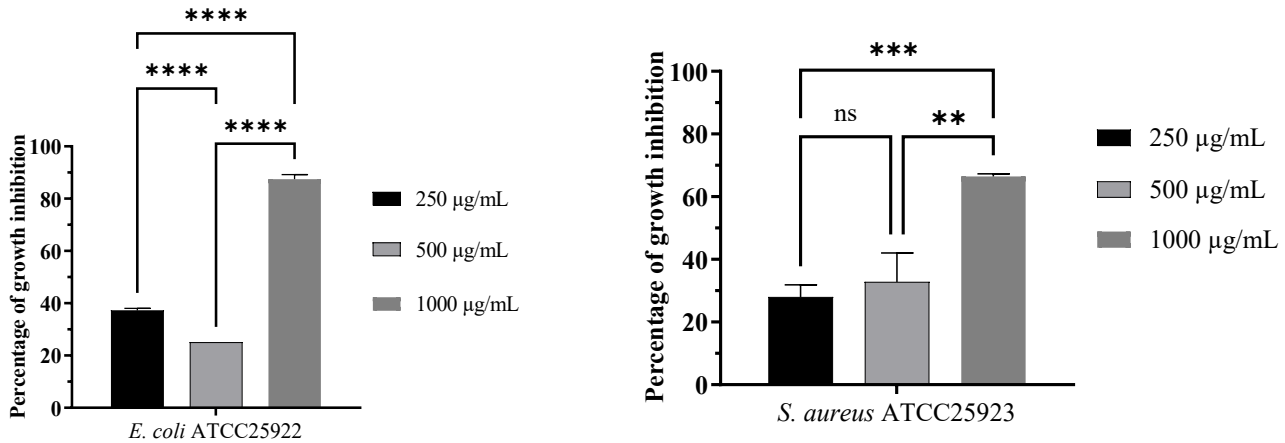

Figure 1. *Evaluation of the inhibitory effect of the ethanolic extract of Dysphania ambrosioides (EEDA) against Escherichia coli ATCC 25922 (A) and Staphylococcus aureus ATCC 25923 (B). Values are presented as mean ± standard deviation.*

On the other hand, unexpected behavior occurs when comparing the concentrations of 250 µg/mL and 500 µg/mL. The inhibition at 500 µg/mL (25%) was lower than that observed for 250 µg/mL (37.2%). This data may indicate a possible interference at intermediate doses, such as the presence of compounds in equilibrium that may have antagonistic effects at certain concentrations.

In Figure 1B, the data demonstrate a behavior where increasing the extract concentration resulted in greater inhibition of S. aureus growth. The concentration of 1000 µg/mL was the most effective, reducing bacterial growth by 66.49%, while 500 µg/mL showed 32.83% inhibition and 250 µg/mL, 27.97%. Unlike what was observed for *E. coli*, the response to the extract was more linear, with a progressive increase in inhibition as the extract concentration increased. This suggests that the bioactive compounds present in the extract are more effective against *S. aureus* or Gram-positive strains at higher concentrations, possibly due to a direct dose-dependent mechanism of action on the cell wall structure or bacterial metabolism.

Figure 2 illustrates the evaluation of the cytotoxic effect of EEDA on NCTC 929 clone fibroblasts. This cell type is frequently used in toxicity assays due to its sensitivity to chemical compounds and natural extracts. A significant reduction in cell viability as a function of increasing extract concentration may indicate a toxic effect on the fibroblasts, which is relevant data for assessing the safety of the extract for therapeutic applications.

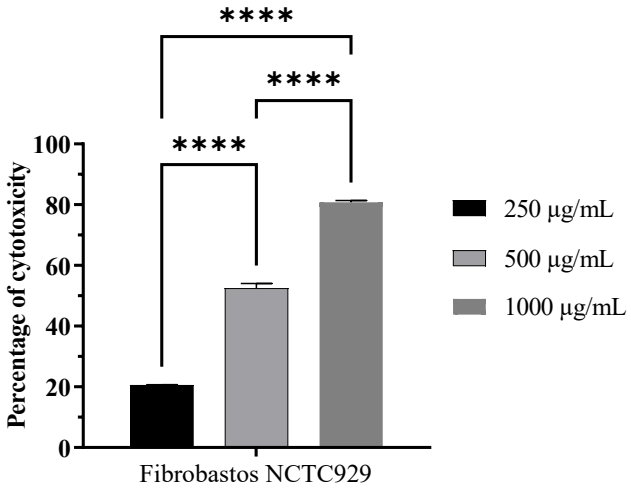

Figure 2. *Evaluation of the cytotoxic effect of the ethanolic extract of Dysphania ambrosioides (EEDA) on NCTC 929 clone fibroblasts. Values are presented as mean ± standard deviation.*

The results in Figure 2 indicate that the extract exhibited a dose-dependent effect, meaning that as the concentration increases, there is a significant increase in cytotoxicity. The concentration of 1000 µg/mL was the most toxic, reducing cell viability by 80.82%, while 500 µg/mL caused an intermediate inhibition of 52.43%, and 250 µg/mL showed a milder effect, with 20.54% cytotoxicity. This pattern suggests that the extract contains active compounds that affect fibroblast viability proportionally to the administered dose.

Figure 3 presents the evaluation of the antitumor effect of EEDA on two cell lines: J774 macrophages and MCF-7 cells. J774 macrophages are widely used in immunological studies and can reflect the extract's ability to modulate the inflammatory and immune response. MCF-7 cells, on the other hand, are a human cancer cell line frequently used in screening assays for potential antitumor agents.

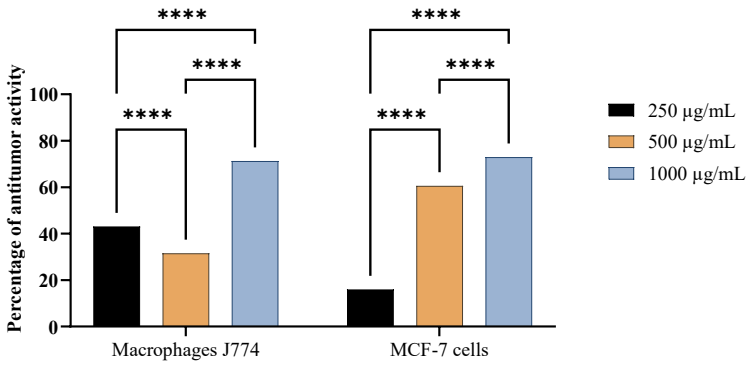

Figure 3. *Evaluation of the antitumor effect of the ethanolic extract of Dysphania ambrosioides (EEDA) on J774 macrophages and MCF-7 cells. Values are presented as mean ± standard deviation.*

In the action against macrophages, we observed a non-linear pattern: the 250 µg/mL dose had a greater inhibitory effect (43.01%) than 500 µg/mL (31.47%), but the inhibition increased significantly at the highest dose (1000 µg/mL, 71.19%). This variation may indicate that, at intermediate concentrations (500 µg/mL), some compounds in the extract may be triggering adaptive responses in macrophages, reducing the cytotoxic effect. Another hypothesis is that the extract has components with biphasic effects, stimulating macrophages at low concentrations and inhibiting their viability at higher doses.

The fact that 1000 µg/mL resulted in the greatest inhibition (71.19%) suggests that the extract has immunomodulatory potential, potentially affecting the function of these cells, which are crucial in the inflammatory response and in defense against tumors. This may be relevant to assess whether the extract compromises or stimulates the immune response.

Unlike macrophages, MCF-7 tumor cells showed a clear dose-dependent response pattern, where increasing the extract concentration resulted in greater cell inhibition. The lowest dose (250 µg/mL) had a discreet effect (15.85%), while the intermediate dose (500 µg/mL) showed a significant increase in inhibition (60.46%). At the highest dose (1000 µg/mL), the effect was even stronger (72.87%), indicating a possible toxic effect of the extract on these cancer cells.

This result suggests that the EEDA may contain compounds with significant antitumor activity, especially at higher doses. This is a promising finding, as it indicates that there are bioactive substances that directly impact the viability of MCF-7 cells, a widely used breast cancer model for testing new anticancer compounds.

Discussion

The use of medicinal plants in traditional medicine currently stands out, a fact that is likely due to their use by Indigenous peoples who live in close contact with these plants in their regions. In this context, research aimed at applying plant materials in the treatment of various diseases is important to ensure safety and to better understand the therapeutic effects that such plants can provide.

The *Dysphania* genus comprises a group of medicinal plants widely studied for their disease-treating properties and chemical composition [21]. *Dysphania ambrosioides* is a condiment and medicinal species, popularly known as “erva-de-santa-maria,” “mastruz,” or “epazote.” It has a significant social impact and can be found in various parts of the world [22].

The results obtained demonstrate that the ethanolic extract of *Dysphania ambrosioides* (EEDA) exhibits significant biological activities, with dose-dependent antimicrobial, cytotoxic, and antitumor effects. The species has already been reported in studies describing its antimicrobial activity, both antifungal and antibacterial [23,24].

In antibacterial assays, the extract showed greater potency against *Escherichia coli* ATCC 25922 compared to *Staphylococcus aureus* ATCC 25923, particularly at the concentration of 1000 µg/mL, with inhibition rates of 87.46% and 66.49%, respectively.

This result indicates a tendency of the extract's active components to act more effectively against Gram-negative bacteria, possibly linked to interactions with elements of the cell wall or outer membrane. However, the response was not entirely consistent for *E. coli*, since the intermediate dose (500 µg/mL) showed lower inhibition than the lowest one (250 µg/mL), suggesting a possible antagonistic effect of certain substances in the extract or experimental variations that require further investigation. In assays conducted by Almeida Bezerra et al. [23], the essential oil of *Dysphania ambrosioides* demonstrated significant antibacterial activity against *S. aureus* and moderate activity against *Pseudomonas aeruginosa*.

Cytotoxicity analysis on NCTC 929 fibroblasts revealed an increasing effect as the extract concentration rose, reaching 80.82% cytotoxicity at 1000 µg/mL. These findings indicate that, despite the extract's bioactive potential, its therapeutic use should be approached with caution, as high doses may compromise the viability of normal cells. Nevertheless, this same effect could represent a promising alternative in antitumor activity. The species' toxicity may be caused by the various compounds it contains or by interactions among them [25].

In the antitumor activity assays, a promising effect was observed against MCF-7 human breast cancer cells, with significant inhibition starting at 500 µg/mL (60.46%) and reaching 72.87% at 1000 µg/mL. These results suggest that the extract contains compounds with selective cytotoxic action against tumor cells.

Regarding J774 macrophages, the results showed a variable pattern, with greater inhibition at the lowest and highest concentrations (43.01% at 250 µg/mL and 71.19% at 1000 µg/mL, respectively), but reduced activity at the intermediate concentration (31.47% at 500 µg/mL). This may indicate a possible immunomodulatory effect of the extract, which should be further explored in future studies. In assays conducted by Nascimento et al. [26], antitumor activity was inhibited after treatment with *D. ambrosioides*, suggesting that the inhibitory effect was related not only to local cytotoxicity but also to systemic effects.

Overall, the findings suggest that *Dysphania ambrosioides* possesses compounds with antimicrobial and antitumor activities; however, the cytotoxicity observed in normal cells highlights the need for further research on its selectivity. Additional assays, including studies of mechanisms of action and in vivo tests, are essential to evaluate its potential as a therapeutic candidate.

Conclusion

This study demonstrated that the ethanolic extract of *Dysphania ambrosioides* possesses antimicrobial, cytotoxic, and antitumor activity. The antibacterial effect was most pronounced against *E. coli*, while the cytotoxicity observed in fibroblasts and macrophages suggests the need for safety evaluation. The promising antitumor activity against MCF-7 tumor cells was noteworthy, showing inhibition greater than 70% at the highest concentration tested. However, the cytotoxic effects also showed interference with immune system cells, which may indicate immunomodulatory effects. Therefore, further studies are needed to investigate the mechanisms of action and confirm the selectivity of the extract for future therapeutic studies.

Conflict of Interest

The authors declare no conflict of interest.

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