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Aqueous extract of *Luffa cylindrica*: modulation of antibiotic action and antioxidant and cytoprotective evaluation

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Abstract. This study evaluated the antibacterial, antioxidant, and cytoprotective activities of the decoction from *Luffa cylindrica* fruits (DFLC). The aqueous extract was obtained by boiling the dried fruits, followed by lyophilization, and subjected to phytochemical screening and biological assays. Antibacterial and modulatory activities were investigated by broth microdilution against *Staphylococcus aureus* 10 and *Escherichia coli* 06, while cytotoxicity was assessed by hemolysis in erythrocytes and antioxidant activity by the DPPH method. DFLC did not exhibit direct antibacterial activity (MIC > 512 µg/mL), but reduced the MIC of norfloxacin against *S. aureus*, indicating a potential modulatory effect. The extract showed low cytotoxicity at lower concentrations and a progressive increase in hemolysis at higher doses. Antioxidant activity was modest compared to ascorbic acid. These results suggest that, although not inherently antimicrobial, DFLC may act as a potential adjuvant to antimicrobials and exhibits concentration-dependent biological effects.
Keywords: Antibacterial modulation, Antioxidant activity, Cytoprotective activity, Secondary metabolites.

Introduction

Luffa cylindrica (L.) M. Roem., commonly known as loofah or vegetable sponge, is a climbing plant that belongs to the Cucurbitaceae family, widely cultivated in tropical and subtropical regions. When the fruits are mature and dried, their internal fibers become rigid and are widely used as natural sponges (Wu *et al.*, 2016). The fruit is edible in the early stages of growth, but when it matures, its peel is removed to be used as bath sponges, filters, kitchen utensils, decorations, or stuffing material (Chen *et al.*, 2019).

In addition to being used as a vegetable sponge, the species shows potential for applications in biotechnology, in the production of biomaterials, and in sustainable agriculture practices. Due to its adaptability to different soil types, *L. cylindrica*stands out for its ease of cultivation and for the economic and environmental relevance associated with its use. (Chen *et al.*, 2022).

L. cylindrica exhibits several pharmacological effects described in the literature, highlighting its therapeutic potential. Studies indicate that extracts from this plant possess anti-inflammatory properties (Muthumani *et al.*, 2010), antioxidant (Bulbul *et al.*, 2011), antibacterial (Mahdi, Bahrami & Kakaei, 2022), antifungal (Aboh *et al.*, 2017) and cytotoxic properties (Bulbul *et al.*, 2011), attributed to the presence of bioactive compounds, such as saponins, flavonoids, and alkaloids (Akinwumi; Eleyowo; Oladipo, 2021). Furthermore, there are reports of antitumor and hepatoprotective activity, suggesting its use as an adjuvant in treatments related to chronic and infectious diseases (Abdel-Salam; Awadein; Ashour, 2019; Sharma *et al.*, 2016). The seed extract has also been explored for its laxative and antiparasitic properties (Stright *et al.*, 2024). Despite its vast potential, it is important to emphasize the need for further clinical studies to validate its efficacy, safety, and feasibility as a therapeutic resource.

Therefore, the importance of studying the antibacterial, antioxidant, and cytotoxic effects of *L. cylindrica* was recognized, as it is a species of great economic and medicinal importance. Its antioxidant properties are relevant for preventing and investigating damage caused by free radicals, which are associated with chronic diseases such as cancer, diabetes, and cardiovascular diseases (Bulbul *et al.*, 2011). Furthermore, investigating the antibacterial potential of plants may contribute to the development of new agents against antibiotic-resistant microorganisms, a global public health problem (Coutinho *et al.*, 2010). Cytoprotective activity, in turn, stands out for its ability to protect cells against toxic agents, which can be explored in therapies for inflammatory and degenerative conditions. (AZEEZ, 2019).

Understanding these effects may contribute to expanding the pharmacological potential of *L. cylindrica*, as well as enabling the development of more sustainable and economically viable therapeutic alternatives in the field of healthcare. In this context, the present study aims to evaluate the antibacterial, antioxidant, and cytoprotective activities of the decoction obtained from the fruits of *L. cylindrica*.

Methodology

Preparation of the aqueous extract

The dried fruits were collected in the municipality of Farias Brito, Ceará. Subsequently, the fruits were mechanically cut to remove the seeds. After that, the fruits were boiled in water for 10 minutes at 100 °C. The decoction obtained from the fruits of *L. cylindrica* (DFLC) was then filtered, frozen, and lyophilized to obtain the extract.

Phytochemical screening

Phytochemical tests were performed to detect the presence of heterosides, saponins, tannins, flavonoids, steroids, coumarins, quinones, organic acids, and alkaloids, according to the method described by Matos (1997). The tests were based on the visual observation of a color change or precipitate formation after the addition of specific reagents.

Evaluation of antibacterial activity

Minimum Inhibitory Concentration (MIC)

The evaluation of antibacterial activity was carried out using the Minimum Inhibitory Concentration (MIC) test. The bacterial strains used, *Staphylococcus aureus* 10 and *Escherichia coli* 06, were previously cultured on Brain Heart Infusion (BHI) agar medium in a bacteriological incubator at 37 °C for 24 hours. Subsequently, Eppendorf® tubes were prepared containing 900 µL of 10% BHI and 100 µL of the bacterial suspension, previously diluted in saline solution to reach a concentration of approximately 10^5 CFU/mL.

The 96-well microplates were filled sequentially, adding 100 µL of this bacterial suspension to each well. Subsequently, a serial microdilution was performed using a decoction solution of *L. cylindrica* fruits (DFLC), at concentrations ranging from 512 to 8 µg/mL. The plates were incubated at 35 °C for 24 hours, and MIC determination was carried out using resazurin as an indicator. The MIC was defined as the lowest concentration of the extract at which no visible bacterial growth was observed, as described by (Coutinho *et al.*, 2008; Javadpour *et al.*, 1996). All assays were performed in triplicate (n = 3).

Antibiotic-modifying activity

The evaluation of the antibiotic-potentiating activity was conducted by combining DFLC, sulbactam, and chlorpromazine (CPZ) with the antibiotics ampicillin, gentamicin, and norfloxacin, which were also tested individually. For this assay, subinhibitory concentrations (MIC/8) were used for all tested products. The microorganisms employed were *S. aureus* 10 and *E. coli* 06.

Initially, 100 µL of the drug solutions, prepared at a concentration of 1024 µg/mL, were added to the first well of the microplate, followed by serial microdilution at a 1:1 ratio up to the penultimate well. The final concentrations of the antimicrobials ranged from 512 to 0.5 µg/mL, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2018). All assays were performed in triplicate (n = 3) to ensure the reliability of the results.

Cytotoxic activity

The cytotoxic effect of the extract was evaluated using erythrocyte samples previously washed with saline solution and incubated with the *L. cylindrica* extract in DMSO (1%) at six different concentrations (5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL). For each extract concentration, the erythrocytes were incubated in seven NaCl concentrations (0.12%, 0.24%, 0.36%, 0.48%, 0.60%, 0.72%, and 0.90%) at 37 °C for 1 or 2 hours. Subsequently, the samples were centrifuged (1500 rpm for 15 minutes), and the supernatants were collected and analyzed using a spectrophotometer at 540 nm (Diniz, 2006).

Antioxidant activity

The antioxidant assay was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical method. Initially, 100 µL of a 0.3 mM DPPH solution were added to 96-well plates along with 50 µL of ethanol and 50 µL of DFLC, resulting in final concentrations ranging from 5 to 1,000 mg/mL. The plate was incubated at room temperature for 30 minutes, and absorbance was measured at 517 nm. Ascorbic acid was used as a positive control, and the antioxidant effect was calculated as a percentage (Choi *et al.*, 2002).

Statistical analysis

The experimental results were expressed as geometric mean ± standard error of the mean (± SEM) and statistically evaluated using two-way analysis of variance (two-way ANOVA) followed by Bonferroni's post hoc test, using GraphPad Prism 8.3 software. Differences were considered significant when $p < 0.05$, expressed as: * = $p < 0.05$, *** = $p < 0.01$, and **** = $p < 0.0001$.

Results

Phytochemical screening

The phytochemical screening of the extract revealed the presence of secondary metabolites, including flavones, flavonols, and xanthones, as shown in Table 1.

Table 1: Phytochemical screening of the aqueous extract from the fruits of *L. cylindrica*

Metabolites															
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-

1: phenols; 2: pyrogallol tannins; 3: phlobaphene tannins; 4: anthocyanins; 5: anthocyanidins; 6: flavones; 7: flavonols; 8: xanthones; 9: chalcones; 10: aurones; 11: flavanonols; 12: leucoanthocyanidins; 13: catechins; 14: flavanones; 15: alkaloids; 16: terpenes; +: presence; −: absence.

Regarding the pharmacological aspects of the constituents identified in the phytochemical screening, it is important to highlight the presence of flavonoids, which exhibit significant antioxidant activity but may also present high toxicity(Silva *et al.*, 2015).

Antibacterial activity

According to the MIC results, DFLC did not exhibit significant antibacterial activity, showing MIC values higher than 512 μg/mL. However, the analysis of the combination of DFLC with antibiotics allowed the observation of possible modulatory effects on the efficacy of the tested drugs.

As illustrated in Figure 1, the interaction of DFLC with ampicillin, gentamicin, and norfloxacin against the *S. aureus* 10 strain revealed distinct behaviors. It was observed that the combination with norfloxacin resulted in a significant reduction in MIC values when compared to the combinations with sulbactam and CPZ, indicating a potentiating effect. In the case of ampicillin, a decrease in MIC was observed exclusively in the ampicillin + sulbactam association, whereas for gentamicin, the association with DFLC led to an increase in MIC values, suggesting an antagonistic effect on antibiotic activity.

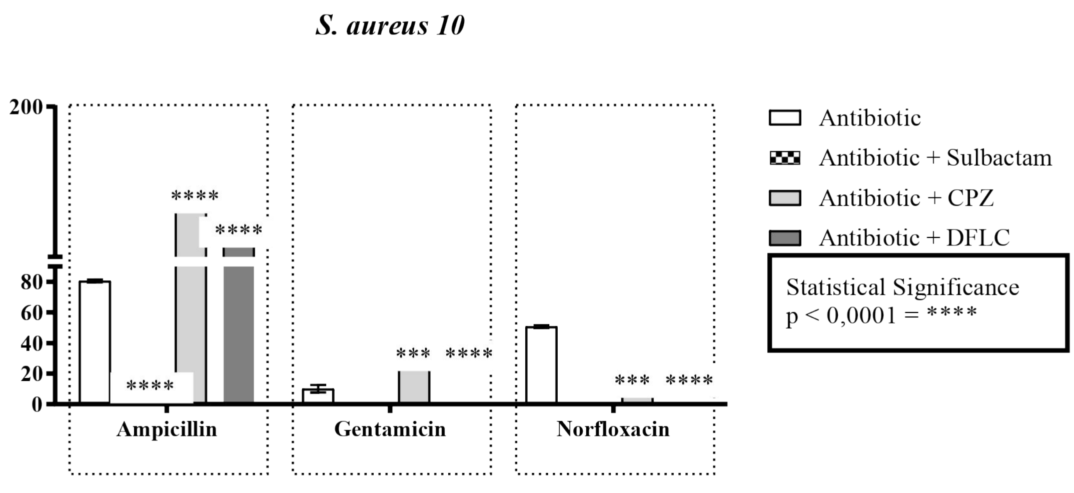

Fig. 1. Modulatory effect of the aqueous extract of *L. cylindrica* (DFLC), sulbactam, and chlorpromazine (CPZ) in combination with ampicillin, norfloxacin, and gentamicin against *S. aureus* 10.

In the evaluation of the modulatory potential against *E. coli* 06 (Figure 2), it was observed that sulbactam promoted a significant reduction in MIC values when combined with all analyzed antibiotics (ampicillin, gentamicin, and norfloxacin), indicating a consistent synergistic effect. However, the association of DFLC with the antibiotics did not show statistically significant differences, suggesting that the decoction did not exert a relevant influence on the antibacterial activity of these drugs against the tested strain.

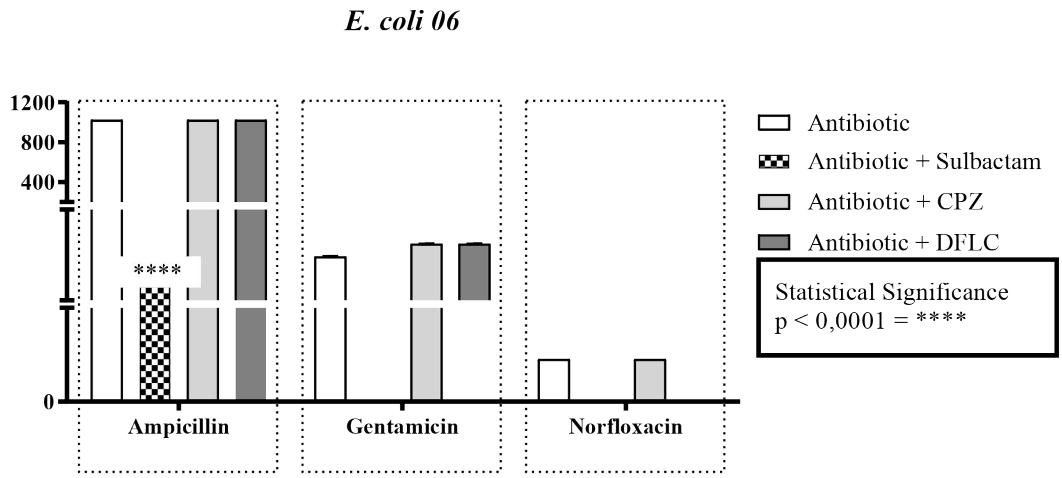

Fig. 2. Modulatory effect of the aqueous extract of *L. cylindrica* (DFLC), sulbactam, and chlorpromazine (CPZ) in combination with ampicillin, norfloxacin, and gentamicin against *E. coli* 06.

Cytotoxic activity

Figure 3 presents the evaluation of the cytotoxic activity of DFLC on erythrocytes, compared with the positive control (distilled water) and negative control (0.9% NaCl solution). No hemolysis was observed in the negative control, while the positive control maintained high and constant levels of cytotoxicity. DFLC showed low cytotoxic activity at concentrations of 10 and 50 µg/mL; however, at 1000 µg/mL, a marked increase in cytotoxicity was observed, approaching the pattern seen in the positive control. These results indicate a dose-dependent behavior, highlighting the importance of further investigations to identify the responsible compounds and determine safe concentrations for use.

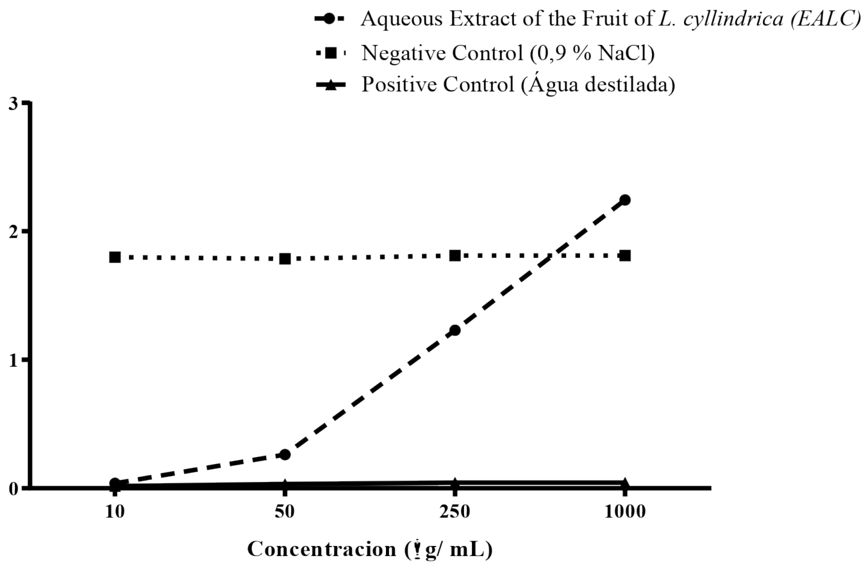

Fig. 3. Cytotoxic activity of the aqueous extract from the fruits of *Luffa cylindrica* (DFLC) on erythrocytes at different concentrations (10, 50, 250, and 1000 µg/mL), compared with the negative control (0.9% NaCl) and positive control (distilled water).

Antioxidant activity

The results shown in Figure 4 demonstrate that ascorbic acid exhibits high antioxidant activity, reaching nearly 100% DPPH inhibition at low concentrations, with consistent efficiency. In contrast, DFLC showed significantly lower and irregular antioxidant activity, with a peak at 100 mg/mL followed by a decrease in inhibition values. The difference between the two treatments is due to their distinct chemical composition: ascorbic acid is a pure compound, whereas the extract is a complex mixture with possible interference from other components. Although DFLC presents some antioxidant potential, it is much less effective.

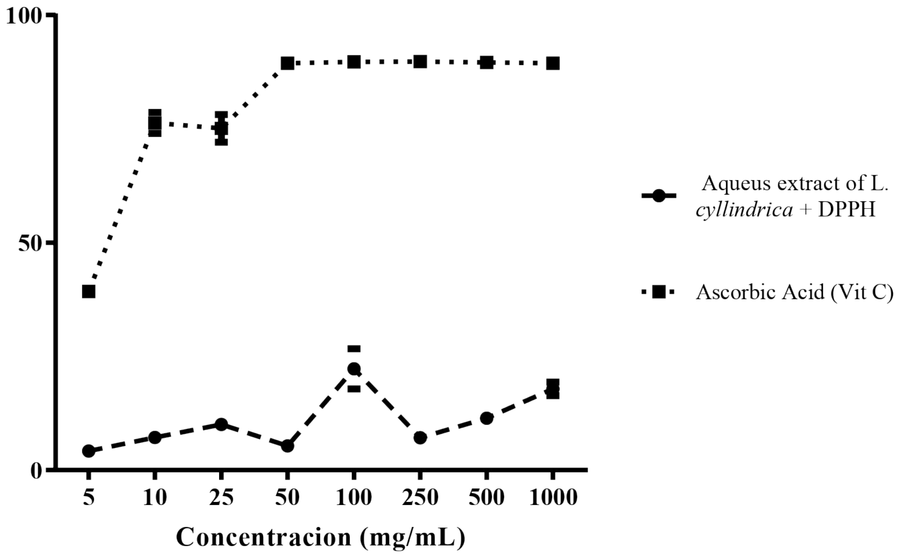

Fig. 4. Graph of the free radical scavenging activity of the aqueous extract from the fruits of *L. cylindrica* (DFLC), measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (0.20 mM), with ascorbic acid (AA -■-) as the positive control.

Discussion

The phytochemical screening revealed the presence of metabolites belonging to the class of phenolic compounds, highlighting the groups of flavonols, flavones, and xanthones. Studies conducted with species of the *Luffa* genus have reported the occurrence of alkaloids, diterpenes, phenols, tannins, phytosterols, and cucurbitacins(Ahmed; Alam; Khan, 2001). The characterization of the aqueous extract from the peel of *L. cylindrica* demonstrated a high content of total phenolic compounds, while the alcoholic extract showed a higher concentration of total flavonoids (Kao; Huang; Chen, 2012). Similarly, the analysis of metabolites from *Luffa operculata*Cogn. revealed the presence of phenolic compounds, such as flavonoids (Bulka *et al.*, 2020).

The interaction between antibacterial, antioxidant, and cytotoxic activities plays a crucial role in combating infections and protecting cells. During infections, oxidative stress is often intensified due to the production of reactive oxygen species (ROS), either as part of the host inflammatory response or as a result of bacterial metabolism (Nagar, Piao & Kim, 2018). In this context, antioxidant activity contributes by neutralizing these ROS, minimizing cellular damage (Li *et al.*, 2021).

Cytotoxicity analysis, in turn, is essential to determine the biological safety of extracts, evaluating potential deleterious effects on healthy cells and ensuring their viability for therapeutic use (Bhamidipati; Fabris, 2017). The association between antibacterial, antioxidant, and cytotoxic properties indicates a potential synergistic effect, capable of acting in infection control, protection of healthy tissues, and elimination of damaged cells, constituting a promising basis for the development of new bioactive agents.

Studies on the toxicity of *L. cylindrica* have indicated a median lethal dose of 48.4 µg/mL in toxicity assays with *Artemia salina*(Fernández-Calienes Valdés *et al.*, 2009). Similar results were observed by Alves *et al.*, (2018), who investigated the neurotoxic and reproductive effects of the aqueous extract of *Luffa operculata*Cogn. administered orally in adult rats. The animals exhibited locomotor and behavioral alterations associated with anxiety, as well as histological changes in the parenchyma, lumen, and diameter of seminiferous tubules, indicating potential adverse effects resulting from exposure to the extract.

In the study conducted byDe la Paz Lorente *et al.*, (2017), it was observed that aqueous extracts obtained from the leaves, fruits, and stems of *L. cylindrica* did not show antibacterial activity against *S. aureus* and *E. coli*. Regarding xanthones, it has been reported that the presence of prenyl groups tends to increase the lipophilicity of these molecules, influencing their three-dimensional properties and favoring interactions with different biological targets. Additionally, the prenyl group acts as a structural moiety responsible for hydrophobic interactions, contributing to the wide range of biological activities associated with these substances, especially when located at strategic positions in the molecular structure(Castanheiro *et al.*, 2009; Pinto; Castanheiro, 2009).

These characteristics are commonly observed in compounds that act as efflux pump inhibitors(Castanheiro *et al.*, 2009; Pinto; Castanheiro, 2009). In the modulation assay, it was observed that the extract potentiated the action of norfloxacin in a manner similar to the positive control

(CPZ). In antibacterial activity assays, chlorpromazine is widely recognized for its role as an efflux pump inhibitor, reinforcing the importance of this mechanism in modulating bacterial resistance (George et al., 2019).

Topoisomerase enzymes have their activity inhibited by flavonoids, which play a fundamental role in DNA transcription and replication(Bulka *et al.*, 2020). Several metabolites act as inhibitors of bacterial resistance factors; for example, glycosylated flavones reduce the activity of topoisomerase IV(Bernard *et al.*, 1997). The possible interference of these metabolites present in the extract on topoisomerase suggests that blocking its activity may enhance the effects of norfloxacin.

Conclusion

The results indicate that the extract obtained from the fruits of Luffa cylindrica (DFLC) did not exhibit relevant antibacterial activity against S. aureus and *E. coli* strains. However, a significant reduction in the minimum inhibitory concentration (MIC) of norfloxacin in *S. aureus* strains was observed, suggesting a modulatory effect. Cytotoxicity evaluation demonstrated that DFLC exhibits low toxicity at concentrations of 10 and 50 µg/mL, whereas at 1000 µg/mL a significant increase in cytotoxicity was observed, indicating a dose-dependent behavior.

Regarding antioxidant activity, the results show that ascorbic acid (AA) maintains high and consistent antioxidant capacity, whereas DFLC presents a lower and more variable antioxidant effect, likely associated with the complexity of its chemical composition.

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