Antibacterial activity of jackfruit leaves extracts and the interference on antimicrobial susceptibility of enteropathogen

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Abstract

The objective of the present study was to evaluate the antimicrobial potential of Artocarpus heterophyllus dry leaves extracts on S. enterica and E. coli and their interaction with conventional antimicrobials. Dried powdered leaves were used to produce hexane (Hex), methanolic (MeOH) and ethanolic residue (EtOHr) extracts. The antimicrobial test was performed against Escherichia coli, ATCC 25922, E. coli EPEC, CDC 086H35, and Salmonella enterica serotype Enteritidis phagotype 4 (SE PT4) through minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC). The agar diffusion technique, well and disc-variants were used to measure the antimicrobial effect of the plant extract combination with antimicrobial of clinical usage. We highlight the bactericidal effect of jackfruit tree leaves on E. coli and S. enterica SE PT4 at 7.2 mg/mL and the effect of the extracts on antimicrobial activity. The interaction between chloramphenicol and dry leaves extracts was characterized by both synergism and antagonism depending on extract type and bacteria used. The interaction between antimicrobial of clinical usage and jackfruit tree leaves extracts demonstrated changes in susceptibility profile of antimicrobials tending to an antagonist effect. As the jackfruit tree leaves may interfere on antimicrobials action, special attention should be given to its usage as traditional medicine in the treatment of food borne diseases.

Keywords: drug antagonism; drug synergism; medicinal plants; Escherichia coli; Salmonella; Artocarpus heterophyllus.

Practical Application: Antimicrobial activity and interaction of plant metabolites with antimicrobials.

1 Introduction

Foodborne diseases cause morbidity and mortality worldwide being a growing public health problem. According to the World Health Organization (WHO), the global burden of foodborne diseases is considerable, affecting individuals of all ages (World Health Organization, 2015). This report reveals that among hazards that contribute significantly to the burden of foodborne diseases there are 18 enteric pathogens. Among bacteria associated diseases, Salmonella spp. and Escherichia coli are the most frequent agents (Casburn-Jones & Farthing, 2004). For example, in 2015, 59,000 cases of registered death were caused by Salmonella enterica; 37,000 by enteropathogenic E. coli (EPEC); and 26,000 by enterotoxigenic E. coli (ETEC) (World Health Organization, 2015).

The food related pathogens have become an ever-increasing threat to humans. The treatment of these agents is centered in the management of electrolyte disturbances, although, in some cases, antimicrobial therapy is required (Centers for Disease Control and Prevention, 2004). In addition, the emergency of resistant microorganisms due to the indiscriminate use of antibiotics in animal food (Angulo et al., 2004; Mathew et al., 2007) and the pharmacological interaction between substances present in natural products (van Vuuren & Viljoen, 2011) may be a constraint to the resolution of foodborne diseases.

Artocarpus heterophyllus Lam, Moraceae, is popularly known as jaqueira (Portuguese), jackfruit tree (English); jacquer (French); kapiak (Papua New Guinea); ‘uluinitia (Samoa) (Elevitch & Manner, 2006). It is native of Western India, Malaysia, East Africa, Southeast Asia, Caribbean, Florida, Australia, Puerto Rico, and Pacific islands (Siqueira, 2006). The fruits are big, sweet flavored, and have a strong and characteristic scent (Prakash et al., 2009). They are widely used as food and as traditional medicine (Jagtap & Bapat, 2010), ingested in natura or as sweets and homemade jelly (Fonseca, 2016). The seeds are used as ingredients of “multimixtures” in order to prepare cookies, desserts, and bread, as an alternative source of carbohydrate, protein, and iron (Landim et al., 2012).

All parts of jackfruit tree are used as traditional medicine. They are recommended for the treatment of inflammation, malarial fever, kidney stones (Araújo & Lima, 2010), ulcers, infected wounds, diarrhea, fever, asthma, anemia, and dermatitis (Jagtap & Bapat, 2010), as well as soothing (Madaleno, 2011). The seeds are used to heal sexual disorders due to its aphrodisiac properties (Fonseca, 2016).

Scientific evidence of the healing properties of different parts of the jackfruit tree has already been presented. Particular emphasis has been given in the literature to the antioxidant activity from...
leaf, bark, and fruit extracts (Loizzo et al., 2010; Omar et al., 2011), jacalin and artocarpin antiviral activity (Tamma et al., 2006), anticancer activity of artocarpin (Sun et al., 2017), anti-inflammatory activity of flavonoids isolated from the bark (Wei et al., 2005), and antibacterial (Khan et al., 2003a; Loizzo et al., 2010) and antifungal potential (Trindade et al., 2006) of several extracts and fractions. However, there is a lack of information on the antimicrobial potential of dry leaf extracts on S. enterica and E. coli and about the interaction of extracts and fractions with conventional antimicrobials, which is the object of this study.

2 Material and Methods

2.1 Botanical field collection

Leaves of jackfruit tree (A. heterophyllus) were collected from an Atlantic Forest fragment characterized by riparian secondary vegetation, in a dense ombrophilous and semideciduous seasonal forest on the roadside of the BR 415 Jorge Amado highway, in Ilhéus, Bahia, Brazil (14°47’33” S and 39°11’0” W). The Botanical collection of Santa Cruz herbarium. Leaves of jackfruit tree (Artocarpus heterophyllus L.) from the cEtOH (10 g) was resuspended in 200 mL of EtOH/H2O (1:2), homogenized and, after 12 h, the insoluble residue was filtered. The hydroalcoholic solution was fractionated with hexane (1:2), chloroform (1:2), ethyl acetate (1:2), and methanol (1:2) until constant weight. Thus, 500 g of dried and powered material was submitted to ethanolic extraction for 72 h, protected from light (1.0 L; w/v), being sonicated for 1 h with the Ultra-Sonic equipment (Unique Ultrasonic Cleaner: USC - 3380) every 24 h. This procedure was repeated three times with the same dried material resulting in a final volume of 3 L, which was filtered and the ethanol was evaporated under reduced pressure at 50 °C (SOLAB: SL-126).

2.2 Plant extraction

Leaves were dried under forced air ventilation at 50 °C in a drying oven (Quimis’ Q317M-12, Diadema, Brazil) until constant weight. Thus, 500 g of dried and powered material was submitted to ethanolic extraction for 72 h, protected from light (1.0 L; w/v), being sonicated for 1 h with the Ultra-Sonic equipment (Unique Ultrasonic Cleaner: USC - 3380) every 24 h. This procedure was repeated three times with the same dried material resulting in a final volume of 3 L, which was filtered and the ethanol was evaporated under reduced pressure at 50 °C (SOLAB: SL-126).

2.3 Ethanolic extract partition

The liquid-liquid partition of ethanolic extract (100 g), called here the cEtOH, was done using the technique proposed by Goulart et al. (2008) and described in Sousa et al. (2019). Briefly, the cEtOH (10 g) was resuspended in 200 mL of EtOH/H2O (1:2), homogenized and, after 12 h, the insoluble residue was filtered. The hydroalcoholic solution was fractionated with hexane (QHEMIS, Jundiaí, Brazil), chloroform (QHEMIS, Jundiaí, Brazil), ethyl acetate (QHEMIS, Jundiaí, Brazil) and methanol (QHEMIS, Jundiaí, Brazil). This experimental step was performed three times. After assembling each solvent residue together and evaporated, fractions yielded 3.05 g (fHex), 3.87 g (fICHCl3), 3.53 g (fEtAc), and 2.02 g (fMeOH). For each extract solvent was evaporated under reduced pressure at 50 °C (SOLAB: SL-126).

A stock solution of 10 mg/mL of each plant extract using 0.15% dimethylsulfoxide (DMSO, SYNTH, Jundiaí, Brazil) in water solution as solvent was filtered using a 0.22 μm pore membrane (Kasvi, São José do Pinhais, Brazil) and stored at -8°C in aliquots of 1 mL.

2.4 Microorganisms

The microorganisms used in this study were Escherichia coli ATCC 25922, E. coli EPEC, CDC 086H35, and Salmonella enterica serotype Enteritidis phagotype 4 (SE P74), obtained from the Collection of Reference Microorganisms on Health Surveillance (CMRVS, FIOCRUZ-INCQ, Rio de Janeiro-RJ, Brazil).

All strains were cultivated and maintained in nutrient agar (NA) (Acumedia, João Narezi Indaiatuba, Brazil) containing tubes from which the bacterial inoculum was solubilized in a NaCl 0.9% solution and standardized to 0.5 McFarland (1.5x10^8 UFC/mL) scale.

2.5 Antibacterial essay

The minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) were obtained through the broth microdilution technique described by CLSI-M100-S22 (Clinical and Laboratory Standards Institute, 2012). For MIC determination, 96 well plates were filled with serial dilutions (9.0; 8.1; 7.2; 6.3; 5.4; 4.5; 2.2; 1.2 mg/mL) of the extract diluted in the brain heart infusion (BHI) broth (Kasvi, São José do Pinhais, Brazil) and 10 μL of the bacterial inoculum, resulting in a final volume of 100 μL per well. Plates were incubated at 37 °C for 24 h. Chloramphenicol (NeoQuimica Anápolis, Brazil) at 50 μg/mL, BHI, and extracts without bacteria were used as controls. After the incubation period, 20 μL of resazurin (Sigma-Aldrich, Darmstadt, Alemanha) (0.01%) were added to each well to determine the bacterial growth (pink color) or inhibition (blue color). For MBC determination, 10 μL of each well was transferred to a plate containing 20 mL NA and incubated for 24 h at 37 °C. Experiments were done in triplicates and repeated three times. The MBC was determined by lack of visible bacterial growth in NA after 24 h.

2.6 Effect of antimicrobial and plant extract combination evaluation

The antimicrobial and plant extract interaction was evaluated through both the well-variant and disc-variant the agar diffusion technique using the methods. Protein, cell wall synthesis, and DNA synthesis inhibitors were used: ampicillin, ampicillin + sulbactam, cefepime, amoxicillin + clavulanate, cephalixin, penicillin G, chloramphenicol, gentamicin, ciprofloxacin, and amikacin (CECON, São Paulo, Brazil).

For agar diffusion tests, the technique described in CLSI-M100-S15 (Clinical and Laboratory Standards Institute, 2005) with adaptations was used. Briefly, a final concentration of 8.2 mg/mL of the extracts was poured on the agar at 45 °C. After solidification, microorganisms were inoculated by spreading over the entire agar surface. Then, antimicrobial paper discs (about 6 mm in diameter) were added at determined distance so as not to have interference between the halos. The diameters (mm) of inhibitory zones were recorded after incubation.
time (24 h) at 37 °C. Results were expressed as a mean of two independent experiments.

Synergism, antagonism, or indifferent interaction was determined as described by Canton & Onofre (2009). Synergy was considered when the difference between the initial antimicrobial diameter zone and the inhibition zone formed when the extracts were combined with antimicrobials was ≥ 2 mm. When the combined zone was less than the diameter formed by the antimicrobial alone, the result was interpreted as antagonism; and when the zone diameter did not change, the result was expressed as indifferent. Tests were repeated twice.

3 Results

The potential antimicrobial activity and antimicrobial interaction of some jackfruit dry leaf extracts are presented in this study. First, the antimicrobial activity showed a bactericidal effect of MeOH, Hex and EtOH extracts against E. coli and S. enterica SE PT4 at ≥7.2 mg/mL. Between the three extracts used, the MeOH results are highlighted since it was active with less concentration against all bacterial strains used (Table 1).

Next, when the interaction between antimicrobials of clinical use and jackfruit tree leaf extracts was investigated (Table 2) there was not a specific relationship between the extract interaction and the mechanism of action of the antimicrobial. However, the changes in the susceptibility profile of some specific strains were remarkable, tending to an antagonist effect.

Interestingly, the MeOH extract showed synergic effect with β-lactamic ring (Ampicillin) and ribosomes 50S (chloramphenicol) acting antimicrobials against S. enterica SE PT4 and antagonism to the great majority of antimicrobial tested, with exception to cefepime, ciprofloxacin and gentamicin that showed an indifferent effect against E. coli EPEC. For this extract, only gentamycin had a severe antagonism resulting in conversion from susceptible to intermediate state for E. coli ATCC 25922 (Table 2).

Regarding the Hex extract, results showed that for the majority of antimicrobials an antagonist effect was visualized,

### Table 1. Jackfruit leaves extracts Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MeOH Extracts (mg/mL)</th>
<th>Hex Extracts (mg/mL)</th>
<th>EtOH Extracts (mg/mL)</th>
<th>CHLO</th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25922 E. coli</td>
<td>≥7.2</td>
<td>≥8.1</td>
<td>≥8.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 086H35 E. coli EPEC</td>
<td>≥8.1</td>
<td>≥9</td>
<td>≥8.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. enterica SE PT4</td>
<td>≥7.2</td>
<td>≥9</td>
<td>≥9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MeOH: methanolic extract; Hex: hexanic extract; EtOHr: ethanolic extract; CHLO: Chloramphenicol 50 μg/mL. *Bactericidal action.

### Table 2. Effect of jackfruit leaf extracts and their interaction with antimicrobials by the agar diffusion technique.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Ref.</th>
<th>E. coli ATCC 25922</th>
<th>E. coli EPEC CDC 086H35</th>
<th>S. enteritis SE PT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP 10 ug</td>
<td>≥17</td>
<td>19.0 (S) 18.5 (S)</td>
<td>23.7 (S) 21.0 (S)</td>
<td>11.0 (R) 10.5 (R)</td>
</tr>
<tr>
<td>CPM 30 ug</td>
<td>≥18</td>
<td>36.0 (S) 36.5 (S)</td>
<td>27.0 (S) 29.0 (S)</td>
<td>40.0 (S) 40.0 (S)</td>
</tr>
<tr>
<td>ASB 20 ug</td>
<td>≥15</td>
<td>26.3 (S) 26.5 (S)</td>
<td>23.0 (S) 24.3 (S)</td>
<td>14.0 (I) 13.0 (I)</td>
</tr>
<tr>
<td>CHLO 30 ug</td>
<td>≥18</td>
<td>28.7 (S) 29.0 (S)</td>
<td>23.7 (S) 27.5 (S)</td>
<td>32.0 (S) 31.5 (S)</td>
</tr>
<tr>
<td>CYP 5 ug</td>
<td>≥18</td>
<td>28.7 (S) 29.0 (S)</td>
<td>23.0 (S) 24.3 (S)</td>
<td>35.0 (S) 35.0 (S)</td>
</tr>
<tr>
<td>GEN 10 ug</td>
<td>≥15</td>
<td>15.3 (S) 13.5 (S)</td>
<td>14.3 (S) 14.3 (S)</td>
<td>15.0 (I) 15.0 (I)</td>
</tr>
<tr>
<td>AMC 30 ug</td>
<td>≥18</td>
<td>28.7 (S) 29.0 (S)</td>
<td>25.3 (S) 25.3 (S)</td>
<td>11.3 (S) 11.0 (S)</td>
</tr>
<tr>
<td>AMI 30 ug</td>
<td>≥17</td>
<td>22.3 (S) 22.5 (S)</td>
<td>18.0 (S) 20.3 (S)</td>
<td>22.3 (S) 22.0 (S)</td>
</tr>
<tr>
<td>CFM 30 ug</td>
<td>≥19</td>
<td>23.7 (S) 23.5 (S)</td>
<td>11.3 (S) 17.5 (S)</td>
<td>10.7 (R) 10.5 (R)</td>
</tr>
<tr>
<td>PEN 10 ug</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Ref: Minimal values in millimeters determined by M10021 (Clinical and Laboratory Standards Institute, 2011) to consider a bacteria sensible to the antimicrobial. AE: antimicrobial efficacy on bacteria tested; (S): Susceptible; (I): Intermediate; (R): Resistant; (-) no inhibition zone; S/A: synergism/antagonism effect (�) Synergistic; (ג) Antagonistic; (פ) Indifferent; NT: Not tested; AMP: Ampicillin; CPM: Cefepime; ASB: Ampicillin+Sulbactam; CHLO: Chloramphenicol; CYP: Ciprofloxacin; GEN: Gentamycin; AMC: Amoxicillin+Clavulanic acid; AMI: Amicacyn; CFM: Ceftxime; PEN: Penicillin. The results are the mean of two independent experiments.
being more pronounced for gentamicin and cefixime, which turned from the sensitive to the resistant state against *E. coli* ATCC 25922 and *S. enterica* SE PT4. This extract had a promising result only for ampicillin against *E. coli* ATCC 25922, and amoxicillin+clavulanate against *E. coli* EPEC and *S. enterica* SE PT4a (Table 2).

Finally, the EtOH extract interacted with antimicrobials as an antagonist. It is worth noting that the interaction of this extract with gentamycin and cefixime for *E. coli* ATCC 25922, ampicillin+sublactam and amikacin for *E. coli* EPEC, and gentamycin for *S. enterica* SE PT4 resulted in antagonism, with conversion of the susceptibility profile. For this extract, the only beneficial interaction observed took place when amoxicillin+clavulanate was tested, which presented a synergistic effect (Table 1).

### 4 Discussion

The antimicrobial activity of jackfruit tree against foodborne pathogens has already been reported in the literature. Specifically from leaves, crude methanolic (Khan et al., 2003b) and aqueous (Loizzo et al., 2010) extracts has been shown to be effective against *E. coli* (ATCC 35150), *Salmonella typhimurium* (ATCC 14028), and *Salmonella enterica* (ATCC 10708). Moreover, methanolic and ethanolic fractions showed to be active against antibiotic resistant bacteria such as methicillin resistant *S. aureus* (Karthly et al., 2009). In the present study we corroborate the bactericidal effect of jackfruit leaf extracts on foodborne pathogens (Cavalcante et al., 2013; Jagtap & Bapat, 2010) adding results from two other strains, the *E. coli* ATCC 25922 and *S. enterica* SE PT4. In our study, the best extract regarding inhibitory concentration was the MeOH extract, which presented bactericidal effect at ≥7.2 mg/mL against the three bacterial strains used. In the literature, although with a smaller bacterial spectrum, the best result was found using the ethanolic extract compared to extract concentration (Barbosa, 2017). It is important to note that the solvent plays a role in the selection of natural compounds class with biological activity (Do et al., 2014) reinforcing the need to perform antibacterial screening of certain plants with different solvents before discarding their biological activity.

In addition, geographic, and phenological aspects about the collection time, part of the plant, and especially the processing of plant parts before solvent extraction played an important role in qualitative and quantitative composition of secondary metabolites (Yang et al., 2018) and might affect the biological activity of some plants. From the jackfruit tree leaves found specifically in the Atlantic forest fragment in the climate conditions described here, it was possible to obtain an anti- *E. coli* ATCC25922 and *S. enterica* SE PT4 extract with more polar molecules by ethanolic and methanolic extraction.

Concerning the interaction between conventional antimicrobials and extracts, to our knowledge, this is the first report of the interference of *A. heterophyllus* leaves extract with these substances. Results showed that the three extracts tended to have an antagonistic effect on antimicrobials. Changes in the susceptibility status were observed, highlighting the synergistic effect of the EtOH extract combined with amoxicillin+clavulanate for *E. coli* EPEC. The non-specific effect regarding bacteria and antimicrobials revealed that the interference of plant compounds may have more than one mechanism of action. This was specially noted for the Hex extract with gentamycin, which inhibits the 30S ribosome, or cefixime, which acts via competitive inhibition of the transpeptidase enzyme.

### 5 Conclusion

In summary, in this work, we did not only demonstrate the antimicrobial potential of jackfruit tree leaf extracts but also the singular interaction of these extracts with clinically used antimicrobials. Regarding this interaction, we emphasize the importance that must be given to the administration of phytotherapics or homemade teas during the oral treatment of bacterial borne disease. However, in order to provide further conclusions on the consumption of plant derivatives in support of disease treatment, further studies about the interaction between plant products and antimicrobials should be conducted in *in vivo* models.

### References


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