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## A PRELIMINARY EVALUATION OF ANTIBACTERIAL AND TOXIC POTENTIALS OF *P. GUINEENSE* SWARTZ. FRUIT PULP FROM DIFFERENT STAGES OF RIPENESS

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### ABSTRACT

*Psidium guineense* Swartz is a shrub native to Brazil. It is consumed *in natura* or in the form of pulp, which presents a high content of C vitamin and proteins. The chemical prospection of the aqueous extracts from unripe and mature pulp revealed the presence of tannins and flavonoids. The extracts demonstrated moderate antibacterial activity against the most of strains tested, and the best result was obtained with the unripe pulp extract against *Staphylococcus aureus* (MR358; MIC 256 µg/mL). The extracts were further tested for *in vivo* brine shrimp lethality test. The mature pulp extract exhibited significant toxicity (LC<sub>50</sub>=125 µg/mL).

**INTRODUCTION:** *Psidium* is a genus of about 100 species of tropical shrubs and small trees<sup>1</sup>, of the genus *Psidium*, the most important species is classified as *Psidium guajava* L. (goiabeira). The genus comprises many species that produce comestible fruits, and woody and ornamental plants with potential commercial value. Among these species, the “aracazeiros” deserve more attention, especially because some characteristics of the fruits, like exotic flavor, high content of C vitamin and good consumer acceptance<sup>2</sup>.

*Psidium guineense* Swartz, a shrub known popularly as “araca verdadeiro”, belongs to the Myrtaceae family<sup>3</sup>. This species is native to Brazil and is cultivated mainly for fruits production<sup>2, 4</sup> that are described to possess high content of C vitamin in comparison to citrus fruits and to present more proteins than guava<sup>3</sup>. The study of antimicrobial activity, mode of action and potential use of medicinal plants has been increasing in importance, mainly the evaluation of antimicrobial properties comprising a great variety of microorganisms<sup>5</sup>. There is any report in literature regarding the antimicrobial and toxicity activities of *P. guineense*. The absence of data stimulates the emerging of studies in these areas.

Given the need of chemical analysis of natural products<sup>6</sup> and the fact that fruits are a source of chemical diversity and the widely domestic and industrial consumption *in nature* or in the pulp form<sup>7</sup>, this work aimed to realize the phytochemical prospection of aqueous extracts from *P. guineense* unripened and mature fruits pulps in order to identify the secondary metabolites and to evaluate the antibacterial and toxic potentials.

## MATERIAL AND METHODS:

**Plant material and extract collection:** Fruits from *P. guineense* Swartz from different stages of ripeness were collected from Grangeiro Municipality, Ceara State (Brazil) and transported to the Natural Products Research Laboratory of

Regional University of Cariri. The selected fruits were cleaned and processed in order to obtain the pulp. The pulp extracts were obtained by cold extraction with distilled water<sup>8</sup> followed by lyophilization until complete dehydration.

**Phytochemical prospection:** The phytochemical prospection of aqueous extracts from unripened (AEUP) and mature (AEMP) fruits to detect the presence of secondary metabolites was performed following the method described by Matos<sup>8</sup>.

**Antibacterial activity evaluation:** The antibacterial activities of the extracts were investigated by employing a microdilution method, recommended by NCCCLS M7-A6<sup>9</sup>. Brain Heart Infusion Broth (BHI 3.8%) was used for bacterial growth (24 h, 35±2°C). The inoculum was an overnight culture of each bacterial species in BHI broth diluted in the same media to a final concentration of approximately 1 x 10<sup>8</sup> UFC/mL (0.5 nephelometric turbidity units - McFarland scale). After this, the suspension was diluted to 1 x 10<sup>6</sup> UFC/mL in 10% BHI. 100 µL of each dilution were distributed in 96-well plates plus extracts in different concentrations, achieving 5x10<sup>5</sup> UFC/mL as the final concentration of the inoculum. Six standard strains were used: two gram-positive (multiresistant *Staphylococcus aureus* MR 358 and *Staphylococcus aureus* MR 6538) and four gram-negative (*Bacillus cereus* ATCC 33018, *Pseudomonas aeruginosa* ATCC 5442, *Escherichia coli* MR 27 e *Escherichia coli* ATCC 2992).

The extracts were dissolved in distilled water and dimethyl sulfoxide (DMSO) to a concentration of 1024µg/mL. Further serial dilutions were performed by addition of BHI broth to reach a final concentration in the range of 512 a 8µg/mL. All experiments were performed in triplicate, and the microdilution trays were incubated at 35±2°C for 24h. Antibacterial activity was detected using a colorimetric method by adding 25µL of resazurin staining (0.01%) aqueous solution in each well at the end of the

incubation period. The minimal inhibitory concentration (MIC) was defined as the lowest the extracts able to inhibit the bacteria growth, as indicated by resazurin staining (bacteria died cells are not able to change the staining color by visual observation-blue to red).

Both extracts were tested for brine shrimp lethality. The assay was performed basically according to the simplified Meyer's *et al*<sup>10</sup>. Briefly, brine shrimp, *Artemia salina* L. encysted eggs were incubated in artificial seawater at 28 °C. Samples were dissolved in DMSO plus artificial seawater. Serial dilutions (triplicate) were prepared in the same solution. Metanauplii (10 units) was added to each set of tubes containing samples and the cultures further incubated for 24h. Controls containing DMSO were included on each set of experiments. Potassium dichromate was used as reference standard. LD<sub>50</sub> (after 24h) were calculated by Probit analysis. When found value of LC<sub>50</sub><1000 µg/mL the assayed product was regarded as toxic bioactive compound<sup>11</sup>.

**RESULTS AND DISCUSSION:** Phytochemical prospection of *P. guineense* fruits aqueous extracts indicated the presence of the same secondary metabolites classes as tannins and flavonoids. These chemical constituents present a wide range of biological activities as antimicrobial<sup>12, 13, 14</sup>, antitumoral<sup>15</sup> and antiofidic<sup>16</sup>. Especially the antioxidant potential of phenolic compounds, described to be significantly found in fruits from *Psidium* genus<sup>17</sup>.

The extracts here studied presented moderate activity against gram-positive and gram-negative strains and the best result was obtained with the unripe pulp extract against *Staphylococcus aureus* (MR358), MIC 256 µg/mL (**Table 1**). According to the literature, extracts from roots and leaves from *Psidium* species showed antimicrobial properties<sup>18, 19, 20, 21, 22</sup> and only the pulps fruits of *Psidium guajava*<sup>23</sup> and *Psidium sartorianum*<sup>24</sup> are antifungal.

**TABLE 1: ANTIMICROBIAL ACTIVITY OF THE AQUEOUS EXTRACTS FROM P. GUINEENSE PULPS FRUITS**

Plant material ( <i>P. guineense</i> )	MINIMAL INHIBITORY CONCENTRATION – MIC (µg/mL)					
	<i>S. aureus</i> (MR 358)	<i>S. aureus</i> (MR 6538)	<i>B.cereus</i>	<i>P. aeruginosa</i>	<i>E. coli</i> (MR 27)	<i>E. coli</i> (ATCC 5922)
AEUP	256	≥1024	512	512	≥1024	512
AEMP	≥1024	512	512	512	≥1024	512

The larval mortality index obtained on toxicity bioassay ranged from 27 to 100%. The LC<sub>50</sub> found was 125 µg/mL to AEMP, indicating significant cytotoxic activity against *Artemia salina* and 1000 µg/mL to AEUP.

**CONCLUSION:** The chemical prospection of *P. guineense* extracts detected the presence of tannins and flavonoids. The antibacterial activity might be related to the presence of tannins as these metabolites have proved antibacterial properties. Concerning the antibacterial evaluation, the extracts presented moderate activity against the tested bacteria and the best result was obtained with the unripe pulp extract against *Staphylococcus aureus* (MR358), MIC 256 µg/mL. The extract from mature fruits was

classified as toxic to *Artemia salina* as the observed LC<sub>50</sub> was minor than standard limit (1000 µg/mL). According to the preliminary bioassays, the extracts presented significant differences in relation to the toxicity and a moderate biological potential against pathogenic bacteria. The results can propel new researches with *P. guineense* isolated substances in the search of therapeutic alternatives from Brazilian natural products.

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