In vitro Antibacterial and Antioxidant Activity of Muntingia calabura Fruits Extract

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Abstract

The study aim was to evaluate the antioxidant and antimicrobial activity of Muntingia calabura fruits extracts, cultivated in Colombia. The antioxidant activities of the extracts were also examined using diphenylpicrylhydrazyl (DPPH) method. The antibacterial activity of the extracts was evaluated through disc diffusion method. M. calabura fruits extracts were tested against foodborne pathogens such as Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, and Escherichia coli. Based on the minimal inhibitory concentration (MIC), the highest antimicrobial activity was found against S. aureus and B. cereus in comparison with P. aeruginosa and E. coli. It means that the inhibition degrees are different between Gram positive and Gram negative bacteria. With respect to the antioxidant activity, high values \( IC_{50} = 83.17 \pm 1.8 \) were obtained. These findings suggest the possibility of use of M. calabura for the active films development for the
food matrixes conservation. This study reports firstly a high degree of antibacterial and antioxidant activities of *M. calabura* fruit cultivated in Colombia against foodborne pathogens.

**Keywords:** antimicrobial, antioxidant, Minimum inhibitory concentration, Muntingia calabura

## 1 Introduction

Traditional medicine has been supported by indigenous traditions around the world for primary healthcare due to several products used in medicine include vegetal extracts. Herbal medicine is considered as an essential source of natural products and useful for treating some diseases in developing countries [1], representing more than 25% of new drugs tested for clinical use [2]. In recent years, plants have enticed great interest of researchers, becoming an attractive alternative in complementary medicine [3].

Some of the compounds with a high interest at pharmaceutical and industrial level are: phenolics, tannins, quinones, alkaloids, glycosides, terpenes and other volatile compounds utilized as plant defense compounds [4]. Antioxidants have gained relevance due to their ability to neutralize free radicals which are involved in the development of diseases such as Cancer, neurodegeneration and inflammatory process [5][6]. Antimicrobial agents derived from plant metabolites have gained attention lately as a result of the resistant bacteria emergency to antimicrobial agents. These factors highlight the importance of search alternative sources on antimicrobial and antioxidant agents from plants [7].

*Muntingia calabura* is a small tree that belongs to the Elaeocarpaceae family and it grows in tropical areas. Its leaves are lanceolate with margins irregularly serrate. The plant flowers throughout the year; its fruits are berries which turn purple when mature. A report from *M. calabura* stands out that *Muntingia calabura* has been used as antiseptics in the swelling treatment in the lower extremities. In addition, it has another uses such as the gastric ulcers reduction, headache and for the relief of incipient colds [1][8][9]. Moreover, it has antioxidant, antinociceptive, cardioprotective and antipyretic properties [10][11]. It is important to clarify, that different parts of *M. calabura* such as flowers, leaves, roots and barks, since they have been employed in different studies.

Studies on *M. calabura* have been carried out mainly on leaf ethanolic and methanolic extracts. For example, determine the phytochemical and antimicrobial properties of various parts of *M. calabura* such as leaf, bark and fruits [12]. The reference [4] studied the antimicrobial activity of ethanolic extracts from *M. calabura* leaves and stems. Likewise, antimicrobial properties of *M.*
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calabura from different areas have been determined [11][13]. Nevertheless, the antioxidant and antimicrobial properties of *M. calabura* extract cultivated in Colombia have not been determined yet. It is known that variations in the growing conditions are major contributors to the differences in the plants composition [4]. There is almost no information respect to antioxidant and antibacterial activity of *M. calabura* fruits cultivated in Colombia. Therefore, this study was conducted to evaluate the antibacterial and antioxidant activities of the extract from *M. calabura* fruits cultivated in Colombia.

2 Experiments

2.1 Plant material and chemicals

Muntingia calabura fruits were collected from the Department of Bolivar (Colombia) during the spring season. The microorganisms employed during the analysis were obtained from the Microbiological Analysis Laboratory (University of Cartagena, Colombia). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2 Extraction methodology

Initially, the *M. calabura* fruits were dried for 72 hours at 37°C, also it was ground into powder and stored at 25°C in dark bottles. Then, an extraction process took place by mixing 1g of powder dried with water and methanol for 72 hours. Afterward, the extract was filtered by using filter and concentrated cellulose to dryness under reduced pressure by rotary evaporation. Finally, the extract was stored under refrigerated condition until further analysis.

2.3 Antimicrobial assay

Initially, the antimicrobial activity was established employing the disc diffusion method. The microorganisms used for the antimicrobial activity of the plant extract were two Gram-positive bacteria: *Staphylococcus aureus* isolated from cheeses and *Bacillus cereus* isolated from rice; and two Gram-negative bacteria: *Escherichia coli* isolated from food handlers and *Pseudomonas aeruginosa*, a strain isolated from residual water. Petri dishes containing Nutritive agar were inoculated with the respective bacterial suspensions. Later, cellulose discs impregnated with plant extract were located into the Petri dishes. Distillate water was utilized as a negative control. The plates inoculated with bacterial samples and extracts were incubated at 37°C during 24 hours. The average diameters of the extracts inhibition zones against the tested organisms were measured in order to evaluate the antimicrobial activity.
2.4 Determination of minimal inhibitory concentration (MIC)

Antibacterial tests were performed against those bacteria that were inhibited by plant extract. Each bacterium was cultivated on Mueller Hinton agar. Then, they were suspended on Mueller Hinton broth. Serial dilutions of the fruit extract containing broth medium were prepared. Subsequently, microbial suspensions were incorporated at concentration of $10^6$ UFC/mL. The bacterial cell number was adjusted to approximately $10^6$ CFU (colony forming unit)/mL (0.4 on the McFarland scale). Lastly, it was carried out an incubation at 37°C for 48 hours. The growth or no-growth was considered by observation, and the MIC value was determined as the lowest extract concentration that avoids the bacterial growth. Distillate water as negative control. Each assay was repeated three times.

2.5 DPPH assay

80µl of each extract concentrations (25, 50, 100, 200, 400, 800 mg/mL) were mixed with 150µL of ethanol solution of DPPH and then, it was incubated in the dark at 30°C for 1 hour. Thereafter, each preparation absorbance was measured at 550 nm. Ascorbic acid was employed as positive control. Finally, the scavenging ability of each extract was determined according to the following equation:

$$\text{Scavenging effect} = \frac{\text{ControlOD} - \text{SampleOD}}{\text{ControlOD}} \times 100$$

2.6 Statistical analysis

All data were expressed as Mean ± SD. Statistical analysis was performed by SPSS 17.0. One-way analysis of variance (ANOVA) was utilized to evaluate differences.

3 Results and Discussion

3.1 Antimicrobial assay

It needs to be mentioned that the potential antibacterial of M. calabura fruits extracts was initially determined using the disc diffusion assay. The in vitro antibacterial properties of M. calabura extracts fruits are presented in Table 1. Where, it can be observed significant differences ($p<0.05$) between Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli. The most sensible bacterium was S. aureus with inhibition zones of 42.03 ±
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followed by *B. cereus* with 38.07 ± 0.39, while *P. aeruginosa* and *E. coli* showed inhibition zones of 31.05 ± 1.0 and 19.15 ± 0.72 respectively. It means that tested extracts of *M. calabura* fruits had antibacterial activity against both Gram-positive and Gram-negative bacteria. However, it was noticeable that Gram-positive bacteria were more sensible than Gram-negative bacteria. These results disagree with those reported by [14], who evaluate the antioxidant, antibacterial, and chemical properties of Ardisia elliptica (A. elliptica) methanolic extracts found better antibacterial activities against Gram-negative than Gram-positive bacteria. On the other hand, the reference [15] reported the antibacterial activity of crude methanolic extract from Larrea tridentata leaves founding that Gram-positive bacteria are more sensible than Gram-negative bacteria. In fact Gram-negative bacteria sensibility could be explained by the presence of a cellular membrane, which limits access of the antimicrobial agents to their targets in the bacterial cell [16][17].

Table 1: Inhibition zone diameter for *M. calabura* fruits extracts against Gram positive and Gram negative bacterial strains

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Average inhibition zone diameter (mm)</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>42.03 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>974 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>38.07 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>875 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>31.05 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1140 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>19.15 ± 0.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5754 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rows with no common letter showed statistically difference at 95%.

The MIC value of the fruit extract ranged from 974 to 5754 µg/mL for tested bacteria. These findings are completely different to those published by the reference [18] in a Letter to the Editor. These authors found MIC values in the range of 10–40 µg/mL for *Bacillus cereus* and B. subtilis which can be a consequence of the microorganism’s sensitivity to acetone employed during the extracts elaboration. Following the MIC results of this study, it is interesting to note that the highest antibacterial activity was exhibited against *S. aureus* (974 ± 0.04 µg/mL) followed by *B. cereus* (875 ± 0.27) and *P. aeruginosa* (1140 ± 0.37), while the lowest activity was observed against *E. coli* (5754 ± 0.01), which mean that there were significant differences (p<0.05) between all analyzed microorganisms. The antibacterial activity of the extracts could be occasioned by the high phenolic content which has been described to be involved in the nucleic acid biosynthesis inhibition [19]. This premise raised due to [4][20], who found a high content (75.7 ± 5.4 mg GAE/g) of total phenolic in leaf extract of *M. calabura*.

The tested extracts of *L. calabura* fruits had greater antibacterial activity against Gram-positive than Gram-negative bacteria. This is likely by a higher...
sensitivity of the Gram-positive bacteria than Gram-negative bacteria, which could be attributed to the differences in cell membrane constituents. Gram-positive microorganisms have a peptidoglycan layer, which is not an effective permeability barrier. These results are in accordance with those reported by the reference [21] who stated that extracts of G. kurroo roots and leaves display a high antibacterial activity against Gram positive bacteria. According to [22], plant extracts with MIC values lower than 1000 µg/mL are considered notable. Therefore, taking into account this criterion *M. calabura* extract was active against Gram-positive bacteria. The study results could likely contribute to the utilization of *M. calabura* as an alternative source of antibacterial agents against Gram-positive bacteria especially *B. cereus* and *S. aureus*. Moreover, these extract can be included into the edible film in order to produce active films with antimicrobial properties.

### 3.2 Antioxidant activity

DPPH is a method which has been commonly used as a measure for evaluating free radical-scavenging activities for antioxidant analysis. The free-radical scavenging activities of *M. calabura* fruit extract are shown in Figure 1. The *M. calabura* fruit extract exhibited DPPH percentage inhibition activity with (75 ± 0.7%) at a concentration of 500 mg/mL with IC50 = 83.17 ± 1.8. However, there was a significant difference (*p* < 0.05) in IC50 value in comparison to standard Ascorbic acid (72.3 ± 2.1). These antioxidant values are higher than other fruit as Ardisia elliptica that have IC50 = 45.0 ± 2.3 [14][23]. Raw fruits extracts, herbs, vegetables and other plant materials with high content in phenolics compounds have been employed in the food industry due to their antioxidant properties and health benefits [21][24].

To summarize, the antioxidant and antibacterial activities of *M. calabura* fruits extract may be attributed to its high phenolic and flavonoid contents due to these compounds have been commonly reported in plants and they are known to exert antioxidant and antibacterial activities [25][26][27]. It is interesting to mention that further research is required in order to use *M. calabura* fruit extract as a preservative agent in system foods. However, these findings are promising and encourage the use of *M. calabura* fruits for developing functional food due to antioxidant and antimicrobial activities founded[28][29][30].

### 4 Conclusions

The results provide evidence that *M. calabura* fruits can serve as a new and alternative source of antioxidant molecules and antibacterial agents against *S. aureus* and *B. cereus*. Therefore, the extracts can be used in pharmaceutical and food industries. Nevertheless, a further phytochemical analysis is needed.
This paper is the first report on antimicrobial and antioxidant properties of *M. calabura* from Colombia.

**Conflict of interests.** The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**


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