In vitro antimicrobial activity of *Muntingia calabura* fruit extracts against food borne pathogens

Food products are prone to spoilage and are excellent sources for many pathogens to colonize in a new host. Foodborne outbreaks from microbial contamination, chemicals and toxins are common in many countries. Trading of contaminated food between countries increases the potential for outbreaks and health risks. The burden of foodborne disease remains substantial and safety of food is an important health, social and economical issue which has become the global topic of increasing research efforts. An increasing number of customers prefer foods with mild processing and without chemical preservatives. Food borne illnesses caused by microbial contamination is due to the growth of pathogenic bacteria and their toxins in food. Concerns also arise from emergence or recognition of the importance of certain microbial food pathogens or spoilage organisms. Natural products which inhibit the growth of pathogenic bacteria in food have been developed and used since ancient times.

*Muntingia calabura* (Elaeocarpaceae) is native to American continent and is widely grown in warm regions of Asia. The plant has been reported to possess antinociceptive, antiproliferative, antioxidant and antipyretic effects. Various parts (bark, roots and leaves) contain flavonones, flavones, flavans and biflavans which exhibited cytotoxic effects. Recently, gastroprotective activity of the leaf extracts was also reported. Antimicrobial activity of *M. calabura* was documented by previous studies. In our previous study, we have found out the significant antibacterial activities of various parts of *M. calabura* against human and plant pathogens. Those findings encouraged us to evaluate the antimicrobial activity of *M. calabura* fruit extracts against food borne pathogens.

Ripened fruits of *M. calabura* were collected, homogenized and extracted at room temperature with petroleum ether, chloroform, ethyl acetate, acetone, methanol and distilled water in the ratio of 1:25 (w/v). Each solvent was collected and filtered using Whatman No.1 filter paper to obtain the respective solvent extracts. The filtrates were concentrated under reduced pressure and dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions. Varying polar solvent extracts of *M. calabura* fruits were screened for their phytoconstituents and antibacterial activities.

Preliminary in vitro antimicrobial activity was performed against *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Salmonella typhi* MTCC 3224, *Shigella flexneri* ATCC 12022 and *Candida albicans* ATCC 10231 using well diffusion assay. In brief, 100 μl of the appropriate bacterial suspension was inoculated on Mueller Hinton agar (bacteria) and Sabouraud’s dextrose agar (yeast) using sterile swabs. 20 μl of the extract was added into the 5 mm wells and the plates were allowed for pre-diffusion of the extract before incubation. The diameter of zone of inhibition mean of two replicates ± SD as indicated by clear area which was devoid of growth of microbes was measured to determine antibacterial activity. The experiment was replicated twice to confirm the reproducible results.

The minimum inhibitory concentration (MIC) assay was performed in both well diffusion and broth dilution method. Briefly, different concentrations (160, 80, 40, 20 and 10 μg ml⁻¹) were prepared in DMSO and the zone diameter of inhibition was determined for the well diffusion assay. Both dilution assay was performed by dissolving the extracts in DMSO and added into Luria–Bertani (LB) broth to obtain a concentration of 320 μg ml⁻¹ and serially diluted to achieve concentrations of 160, 80, 40, 20 and 10 μg ml⁻¹. A 10 μl standardized suspension of each tested organism (10⁷ CFU/ml) was transferred to each tube. The control tubes containing only bacterial suspension were incubated at 37 °C for 24 h. The lowest concentration of the extract which did not show any growth of tested organism was determined as the MIC.

The data obtained were statistically analyzed and the results were expressed as means along with standard deviation of three parallel measurements.

Phytochemical analysis of various solvent extracts of *M. calabura* fruits revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, tannins, steroids and terpenoids. Results obtained from preliminary antimicrobial screening of *M. calabura* fruit extracts showed its effectiveness against all the Gram positive organisms tested with zone diameter of inhibition in the range of 5–21 mm. *S. flexneri* ATCC 12022 was found sensitive to acetone and methanol extracts whereas, *E. coli* ATCC 8739 and *S. typhi* MTCC 3224 were found to be resistant against all the extracts tested. The Gram negative bacterium, *S. flexneri* ATCC 12022 was inhibited by the acetone and methanol fruit extracts of the plant and highest inhibition was observed in *C. albicans* ATCC 10231 (21 mm) against petroleum ether extract.

Minimum inhibitory concentration (MIC) values (Table 1) in the range of 20–40 μg ml⁻¹ were recorded against *B. cereus* ATCC 10876 with zone diameter of 7.4–12.0 mm, whereas it was 10–40 μg ml⁻¹ in case of *B. subtilis* ATCC 6633 (5.5–10.1 mm). For *S. aureus* ATCC 6538, the range was between 20 and 160 μg ml⁻¹ (6.7–16.1 mm) and a moderate inhibitory action was recorded with *S. flexneri* ATCC 12022 with the zone diameter of 5.9–9.3 mm. Highest inhibitory action was recorded with *C. albicans* ATCC 1023 (19.6 mm) with the MIC value of <40 μg ml⁻¹. Various degrees of inhibitory action were observed with varying solvents. In general, more inhibition was seen with increasing concentrations. None of the extracts were able to inhibit the growth of *E. coli* ATCC 8739 and *S. typhi* MTCC 3224 throughout the study. Both petroleum ether and chloroform were ineffective against most of the pathogens tested and...
the acetone extract of *M. calabura* fruits seemed to be the most promising with the MIC values of 10–40 \(\mu\)g ml\(^{-1}\) which exerted strong inhibition against *B. cereus* ATCC 10876, *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. flexneri* ATCC 12022. Significant inhibitory activity was observed with aqueous extracts against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538 with a considerably low MIC.

### Table 1

MIC of *M. calabura* fruit extracts against food borne pathogens (\(\mu\)g ml\(^{-1}\)).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pet</th>
<th>Chl</th>
<th>Eta</th>
<th>Ace</th>
<th>Met</th>
<th>Aqu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. cereus</em> ATCC 10876</td>
<td>–</td>
<td>–</td>
<td>(\leq 20)</td>
<td>(\leq 40)</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td><em>B. subtilis</em> ATCC 6633</td>
<td>–</td>
<td>–</td>
<td>40</td>
<td>(\leq 10)</td>
<td>20</td>
<td>(\leq 10)</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 6538</td>
<td>(\geq 160)</td>
<td>–</td>
<td>20</td>
<td>(\leq 10)</td>
<td>40</td>
<td>(\leq 20)</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 8739</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>S. typhi</em> MTCC 2224</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>S. flexneri</em> ATCC 12022</td>
<td>–</td>
<td>–</td>
<td>20</td>
<td>(\geq 160)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>C. albicans</em> ATCC 10231</td>
<td>(\leq 40)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Pet = petroleum ether, Chl = chloroform, Eta = ethyl acetate, Ace = acetone, Met = methanol, Aqu = aqueous.


### References


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