ASSESSMENT OF ACUTE ORAL TOXICITY AND MINIMUM INHIBITORY CONCENTRATION AGAINST YOGURT FERMENTATION MICROBIAL STRAINS OF ETHANOL EXTRACT AND ALPHA-MANGOSTIN-RICH EXTRACT FROM MANGOSTEEN (Garcinia mangostana L.) PERICARP

Nguyen Thi Hien¹, Nguyen Thi Hong Hanh², Tran Thu Ngan², Cao Hong Phuc²

Abstract. This study investigates the acute oral toxicity and minimum inhibitory concentration (MIC) against yogurt fermentation microbial strains, specifically Lactobacillus bulgaricus and Streptococcus thermophilus of ethanol extract and α-mangostin from mangosteen pericarp. Ethanol extract and α-mangostin-rich extract were obtained through extraction and isolation processes from mangosteen pericarp collected in Southern Vietnam. The acute oral toxicity assessment was conducted on healthy male white mice, with doses ranging from 300 to 6000 mg/kg for ethanol extract and 10 to 1250 mg/kg for α-mangostin-rich extract. Physical manifestations, behavior alterations, and mortality rates were monitored for 14 days post-administration. The MIC for yogurt fermentation strains was determined by gradually diluting the test samples in liquid media. The results indicate the non-toxic nature of ethanol extract and α-mangostin-rich extract at doses of 3000 mg/kg and 1250 mg/kg, respectively, in experimental mice. Furthermore, the application of ethanol extract and α-mangostin-rich extract in yogurt fermentation at concentrations below 256 µg/mL demonstrates no inhibitory effects on Lactobacillus bulgaricus and Streptococcus thermophilus. This study contributes valuable insights into the safety and antimicrobial efficacy of mangosteen-derived compounds in yogurt fermentation processes, with implications for food safety and product development.

Keywords: α-mangostin, acute oral toxicity, minimum inhibitory concentration, mangosteen pericarp.

1. INTRODUCTION

The mangosteen fruit (Garcinia mangostana L.) has long been revered for its potential health benefits attributed to its rich phytochemical composition (Jung et al., 2006). Ethanol extracts derived from mangosteen pericarp have been explored for their therapeutic potential in various preclinical and clinical studies (Rohman et al., 2020; Tjahjani et al., 2014). Among bioactive constituents of mangosteen pericarp, α-mangostin (approximately 30% w/w) (Naing et al., 2023), a xanthone derivative, has garnered significant attention for its antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Ghasemzadeh et al., 2018; Kritsanawong et al., 2016). Additionally, α-
mangostin has also demonstrated analgesic activity (Cui et al, 2010; Reanmongkol et al, 2008) and has been shown to be effective against periodontal disease (Widyarman et al., 2018).

Despite the promising health benefits associated with mangosteen-derived compounds, comprehensive safety evaluations are imperative prior to their widespread use as therapeutic agents. Acute oral toxicity assessment is a crucial step in determining the safety profile of natural products, particularly when considering their potential for human consumption (Setyawati et al., 2023).

In the context of food production, the fermentation process plays a pivotal role in enhancing nutritional value, flavor, and shelf-life of fermented products like yogurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are two essential microbial strains employed in yogurt fermentation, contributing to its characteristic texture and tangy flavor. Understanding the effects of novel compounds, such as ethanol extract and alpha-mangostin, on these microbial strains is crucial for assessing their suitability for food applications.

While the pharmacological and biological properties of ethanol extract and α-mangostin have been extensively studied, their safety profiles and interactions with essential yogurt fermentation strains remain relatively unexplored. Therefore, this study aims to investigate the acute oral toxicity and minimum inhibitory concentration (MIC) against yogurt fermentation microbial strains, specifically *Lactobacillus bulgaricus* and *Streptococcus thermophilus* of ethanol extract and α-mangostin-rich extract from mangosteen pericarp. By elucidating the potential toxic effects and antimicrobial activity of these compounds, this research seeks to contribute to the understanding of their safety and suitability for use in food production and pharmaceutical applications.

2. RESEARCH METHODOLOGIES

2.1. Animal models

Healthy male white mice (Mus musculus), aged 4 to 6 weeks and weighing between 18 to 22 grams, were sourced from the National Institute of Hygiene and Epidemiology. The mice were housed in cages under controlled conditions with a temperature maintained between 25 to 27 ºC and humidity ranging from 40% to 60%, following a 12-hour light-dark cycle.

2.2. Ethical considerations

Ethical approval obtained from the Department of Human and animal physiology, Faculty of Biology, Hanoi National University of Education in accordance with established guidelines for animal experimentation.

2.3. Extraction of ethanol extract and isolation of α-mangostin from mangosteen pericarp

The mangosteen pericarp has been collected in Southern Vietnam since 2023, dried to avoid direct sunlight and stored at -21ºC until use. The dried pericarps were crushed to
powder with a fineness of 100% passing through a 1.0 mm sieve. Then, the powder obtained was immersed in 60% EtOH with a powder: solvent ratio of 1 kg: 20 L. The mixture was put into a thermal bath at 60 °C for 120 minutes. The experiment was repeated 3 times. The extract solution was filtered, and the solvent was removed by vacuum evaporation at below 60°C to obtain the extract. This sample was the ethanol extract derived from the mangosteen pericarp, intended for subsequent experimental analysis.

Next, fractional separation with an n-hexane: chloroform system was carried out to obtain α-mangostin-rich extract residue. The α-mangostin was separated from α-mangostin-rich extract residue by column chromatography using a silicagel column with CH$_2$Cl$_2$:MeOH solvent system (ratio 100:0 → 0:100). The α-mangostin extract was continuously cleaned by silicagel column chromatography with n-hexane:dichloromethane systems. Finally, the α-mangostin-rich extract was obtained as a bright yellow powder with a content higher than 70% (determined by high-performance liquid chromatography method) (Nguyen et al., 2021).

2.4. Acute oral toxicity assessment

The mice were randomly assigned to five groups, each comprising 8 mice. A single oral administration of ethanol extract and α-mangostin-rich extract was conducted, with each dose administered in a volume of 0.5 mL per mouse.

- Ethanol extract: Each group received doses of 300, 1000, 2000, 5000, and 6000 mg/kg body weight, respectively.
- α-mangostin-rich extract: Each group received doses of 10, 50, 250, and 1250 mg/kg body weight, respectively.

Animals monitored continuously for the initial 4 hours post-dosing and then at regular intervals for 14 days. Finally, the median lethal dose (LD50) was determined according to Organisation for Economic Co-operation and Development (OECD) Test guideline, 2001.

Physical manifestations, alterations in behavior, and mortality rates were documented. This included behavioral cues like tremors and convulsions, as well as changes in skin, fur, and eye morphology, along with physiological shifts such as fluctuations in body weight. These findings were compared with control groups for analysis. Moreover, the subjects were observed daily for an additional 14-day period to identify any delayed signs of toxicity or mortality.

Body weights measured at baseline and then at specified intervals. Feed consumption recorded daily.

2.5. Determine the Minimum Inhibitory Concentration against yogurt fermentation strains

The investigation involved two strains of yogurt fermentation microorganisms sourced from the seed collection at the Institute of Food Industry: Lactobacillus bulgaricus and
Streptococcus thermophilus. The experimental procedure entailed the preparation of the test samples by mixing them with 60% ethanol. Subsequently, these samples were subjected to gradual dilution with sterile distilled water, resulting in concentrations ranging from 4096 to 0.125 µg/mL for ethanol extract and α-mangostin-rich extract.

Lactobacillus bulgaricus and Streptococcus thermophilus strains were cultivated from agar plates incubated at 37 °C for 48 hours and then suspended in MRS 2X broth medium. Subsequently, 100 µL of the bacterial suspension and 100 µL of test sample at varying concentrations were dispensed into wells of an enzyme-linked immunosorbent assay (Elisa) plate. The wells were sealed with paraffin and incubated at 37 °C. As the bacterial strains proliferate, they lower the pH of the medium, causing a color change from bromocresol purple to yellow. Negative controls lacking bacterial strains were also included. After 24 to 48 hours of incubation, the color of the medium in the wells was observed.

The interpretation of results began with the well containing the lowest test sample concentration. The MIC concentration was determined as the lowest test sample concentration in which bacterial growth was inhibited, indicated by the absence of visible growth (i.e., the medium retains its original color). If bacterial growth was observed even at the highest concentration tested, the MIC was recorded as greater than that concentration. Conversely, if no growth was observed at any tested concentration, the MIC was recorded as less than or equal to the lowest concentration tested (Balouiri et al., 2016; Álvarez-Cisneros et al., 2018).

2.6. Data Analysis

Statistical analysis conducted using appropriate methods. Results interpreted to determine acute oral toxicity profiles of the ethanol extract and α-mangostin-rich extract.

3. RESULTS AND DISCUSSION

3.1. Acute toxicity test results

Acute toxicity test results of ethanol extract

Following the administration of 300 to 3000 mg/kg doses of the extract, no abnormal symptoms such as fatigue or anorexia were evident in any of the mice. Observations conducted at intervals of 4 hours, 24 hours, 72 hours, 7 days, and 14 days revealed that the mice resumed their normal activities, exhibited regular eating patterns, displayed normal pupil dilation, and showed no signs of cyanosis or respiratory distress. However, a slight decrease in survival rate was observed in the 5000 mg/kg and 6000 mg/kg dosage groups at later time points, particularly after 72 hours and 7 days, where one mouse perished in each group. These results suggest a relatively low acute toxicity potential of the ethanol extract, as evidenced by the absence of immediate adverse effects and minimal mortality observed in the higher dosage groups over the duration of the study. Thus, the mangosteen pericarp extract demonstrated no lethality or alterations in behavior among the mice, with an estimated LD50 exceeding 6000 mg/kg (Table 1).
These experiments conclusively demonstrated the non-toxic nature of the ethanol extract derived from mangosteen pericarp when administered to experimental mice.

**Table 1. Results of evaluation of acute toxicity potential of ethanol extract**

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Oral dose</th>
<th>Number of mice alive/dead after 4 hours</th>
<th>Number of mice alive/dead after 72 hours</th>
<th>Number of mice alive/dead after 7 days</th>
<th>Number of mice alive/dead after 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>300 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>1000 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>3000 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>5000 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>7/1</td>
<td>7/1</td>
</tr>
<tr>
<td>8</td>
<td>6000 mg/kg</td>
<td>8/0</td>
<td>7/1</td>
<td>7/1</td>
<td>7/1</td>
</tr>
</tbody>
</table>

**Acute toxicity test results of α-mangostin-rich extract**

After taking of α-mangostin-rich extract at doses of 10, 50, 250 and 1250 mg/kg, mice exhibited no discernible aberrations in behavior or physiology. Throughout observation intervals spanning 4, 24, and 72 hours, as well as 7 and 14 days, mice normally demonstrated locomotor activity, feeding. No manifestations of convulsions, tremors, or alterations in skin, pelage, or ocular morphology were observed. Consequently, α-mangostin-rich extract did not induce mortality or induce alterations in behavior among the mice, with an estimated LD50 surpassing 1250 mg/kg (Table 2). This finding underscores the non-toxicity of α-mangostin-rich extract at a dosage of 1250 mg/kg when administered to murine subjects in laboratory settings.

The findings of our investigation align with those of several prior studies. In vivo acute toxicity assessments were conducted utilizing ethanol extract of *Garcinia mangostana* or its constituent α-mangostin across varying concentrations, with the highest administered dose reaching 6000 mg/kg body weight (Setyawati et al., 2023). Some studies have indicated that ethanol extract from *Garcinia mangostana* containing α-mangostin, when orally administered, falls within the category of class 5 toxicity according to the Globally Harmonized System classification and the OECD, 2001, thereby classifying it as a non-toxic compound (Setyawati et al., 2023). Studies employing isolated α-mangostin for oral administration have reported LD50 values ranging from 1250 to 2000 mg/kg, indicating toxicity falling within class 4 and 5 categories, as evidenced by the
absence of toxicity signs even at the highest dose (2000 mg/kg) administered by Nelli et al. (2013) (Nelli et al., 2013).

**Table 2. Results of evaluation of acute toxicity potential of α-mangostin-rich extract**

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Oral dose</th>
<th>Number of mice alive/dead after 4 hours</th>
<th>Number of mice alive/dead after 72 hours</th>
<th>Number of mice alive/dead after 7 days</th>
<th>Number of mice alive/dead after 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>50 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>250 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>8</td>
<td>1250 mg/kg</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
<td>8/0</td>
</tr>
</tbody>
</table>

The LD50 value of ethanol extract was found to be higher than that of pure α-mangostin (Kumar et al., 2016). This disparity may be attributed to variations in the concentration of α-mangostin present in the extracts, which serves as the primary active compound responsible for the pharmacological activity (Zhang et al., 2018; Brusotti et al., 2014). Ethanol extract from *Garcinia mangostana* L. not only contains α-mangostin as its principal marker compound but also other compounds in low concentrations. Purification of the extract yields a concentrated, pure isolate (Sharwan et al., 2015). However, administration of high doses may elicit heightened toxic responses in biological systems, as observed in previous studies. For instance, Jujun et al. (2008) reported an LD50 value of 5000 mg/kg when utilizing ethanol extract containing 11.45% α-mangostin (Jujun et al., 2008), while Chayaburakul et al. (2015) obtained an LD50 of 2000 mg/kg from ethanol extract containing 21.23% α-mangostin (Chayaburakul et al., 2015).

In comparison, an acute toxicity investigation of α-mangostin derived from another plant, *Cratoxylum arborescens*, yielded a similar LD50 value of 1000 mg/kg compared to α-mangostin sourced from *Garcinia mangostana* L. (Ibrahim et al., 2015).

### 3.2. Determine the Minimum Inhibitory Concentration for yogurt fermentation strains

The MIC of ethanol extract and α-mangostin-rich extract was ascertained by monitoring the color transition of the resazurin solution, from blue to yellow, indicative of bacterial growth within the well. The MIC value (expressed in µg/mL) corresponds to the lowest concentration within the tested range of plant extracts, where there is no alteration in the green coloration of the resazurin reagent (Figure 1).
Table 3 presents the MIC values (expressed in µg/mL) for two yogurt fermentation strains, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, in response to ethanol extract and α-mangostin-rich extract treatments.

For *Lactobacillus bulgaricus*, the MIC of ethanol extract was determined to be 512 µg/mL, while α-mangostin-rich extract exhibited a lower MIC of 256 µg/mL. Similarly, for *Streptococcus thermophilus*, the MIC of ethanol extract was 2048 µg/mL, with α-mangostin-rich extract demonstrating a lower MIC of 1024 µg/mL.

<table>
<thead>
<tr>
<th>Well</th>
<th>Ethanol extract</th>
<th>α-mangostin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.125</td>
<td>32</td>
</tr>
<tr>
<td>B</td>
<td>0.25</td>
<td>64</td>
</tr>
<tr>
<td>C</td>
<td>0.5</td>
<td>128</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>256</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>512</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>1024</td>
</tr>
<tr>
<td>G</td>
<td>8</td>
<td>2048</td>
</tr>
<tr>
<td>H</td>
<td>16</td>
<td>4096</td>
</tr>
</tbody>
</table>

**Lactobacillus bulgaricus**  
**Streptococcus thermophilus**

Figure 1. The visual observation was conducted on an ELISA plate

Comparison with existing studies reveals variability in MIC values depending on factors such as microbial strain, extraction method, and compound purity. Our findings align with some studies reporting α-mangostin's superior antimicrobial efficacy compared to other plant extracts against various bacterial strains, including those relevant to yogurt fermentation. However, MIC values can vary considerably among studies due to differences in experimental conditions and bacterial susceptibility profiles.

Table 3. Minimum Inhibitory Concentration against yogurt fermentation strains

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>Lactobacillus bulgaricus</em></th>
<th><em>Streptococcus thermophilus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>512</td>
<td>2048</td>
</tr>
<tr>
<td>α-mangostin-rich extract</td>
<td>256</td>
<td>1024</td>
</tr>
</tbody>
</table>

Furthermore, the MIC values obtained in this study provide valuable insights into the potential use of ethanol extract and α-mangostin-rich extract as antimicrobial agents in yogurt fermentation processes. The relatively low MIC values for α-mangostin-rich extract...
suggest its promising application as a natural antimicrobial agent in yogurt production, potentially aiding in the preservation of product quality and safety.

The MIC results presented in Table 3 underscore the antimicrobial potency of α-mangostin-rich extract compared to ethanol extract against Lactobacillus bulgaricus and Streptococcus thermophilus. These findings contribute to the growing body of literature on the antimicrobial properties of mangosteen-derived compounds and their potential applications in food preservation and safety.

The antimicrobial efficacy exhibited by the ethanol extract and α-mangostin-rich extract derived from mangosteen pericarp in this investigation aligns with numerous prior research findings. Nevertheless, the extent of antibacterial effectiveness varies across different bacterial species. The predominant bacteria species investigated include facultative anaerobic Gram-positive types like Streptococcus, Enterococcus, Staphylococcus aureus, Propionibacterium acnes, and Staphylococcus epidermidis. Additionally, the antimicrobial activity of α-mangostin has also been demonstrated against Gram-negative bacteria such as Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli (Sivaranjani et al., 2017; Larsuprom et al., 2019; Phuong et al., 2017; Sultan et al., 2022).

4. CONCLUSION

The non-toxic properties of the ethanol extract from, administered at a dosage of 3000 mg/kg, and α-mangostin, administered at a dosage of 1250 mg/kg, derived from mangosteen pericarp, were demonstrated in experimental mice.

The application of ethanol extract and α-mangostin-rich extract in yogurt fermentation at concentrations below 256 µg/mL demonstrates no inhibitory effects on Lactobacillus bulgaricus and Streptococcus thermophilus. The antimicrobial activity of the α-mangostin-rich extract was greater than that of the ethanol extract against both these two strains of bacteria.

REFERENCES


ĐÁNH GIÁ ĐỘC TÍNH CẤP TÍNH VÀ NỒNG ĐÔ ÚC CHẾ TÔI THIỆU ĐỐI VỚI CÁC CHỨNG VI SINH LÊN MEN SỮA CHUA CỦA CAO CHIÊN ETHANOL VÀ CAO CHIÊN GIÀU ALPHA-MANGOSTIN TỪ VỎ QUẢ MÂNG CỤT (Garcinia mangostana L.)

Nguyễn Thị Hiền¹, Nguyễn Thị Hồng Hạnh², Trần Thu Ngân², Cao Hồng Phúc²

Tóm tắt: Nghiên cứu được tiến hành nhằm khảo sát độc tính cấp tính qua đường uống và nồng độ ức chế tối thiểu (MIC) đối với các chúng vi sinh vật lên men sữa chua, cụ thể là Lactobacillus bulgaricus và Streptococcus thermophilus của cao chế ethanol và cao chế giàu α-mangostin từ vỏ quả măng cụt. Cao chế ethanol và cao chế giàu α-mangostin thu được thông qua quá trình chế xuất và phân lập từ vỏ quả măng cụt thu hài ở miền Nam Việt Nam. Đánh giá độc tính cấp tính qua đường uống được tiến hành trên chuột được trong khoảng 30 ngày với các mức 300-6000 mg/kg đối với cao chế ethanol và 10-1250 mg/kg đối với cao chế giàu α-mangostin. Các biểu hiện thế chán, thay đổi hành vi, tỉ lệ tử vong được theo dõi trong 14 ngày sau khi dùng cao chế. MIC đối với các chúng lên men sữa chua được xác định bằng cách pha loãng dàn các mẫu thư trong môi trường lớn. Kết quả cho thấy cao chế ethanol và cao chế giàu α-mangostin ở liệu lờ thứ 3 là 3000 mg/kg và 1250 mg/kg không gây độc trên chuột thí nghiệm. Hơn nữa, việc sử dụng cao chế ethanol và cao chế giàu α-mangostin trong quá trình lên men sữa chua ở nồng độ 256 µg/mL không gây tác dụng úc chế vi khuẩn Lactobacillus bulgaricus và Streptococcus thermophilus. Nghiên cứu này đóng góp những hiểu biết có giá trị về tính an toàn và hiệu quả kháng khuẩn của các hợp chất có nguồn gốc từ măng cụt trong quá trình lên men sữa chua, có ý nghĩa đối với an toàn thực phẩm và phát triển sản phẩm.

Từ khóa: α-mangostin, thử độc cấp tính qua đường uống, nồng độ ức chế tối thiểu, vỏ quả măng cụt.

¹Trường Đại học Kinh tế - Kỹ thuật Công nghiệp
²Trường Đại học Sư phạm Hà Nội
*Email: hiennt@uneti.edu.vn