MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Fornal *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 20 (2024) 1317-1330

RESEARCH ARTICLE

# Antibacterial Properties of Garcinia mangostana Linn. Ethanolic and Methanolic Extracts Against Selected Gram-Positive and Gram-Negative Bacteria: A Meta-Analysis

**Sienna Karen Fornal[a], Sophia Nicole Subaldo[a], Romeliza Saquido[a], Dulce Marie Nisperos[a,b], Anthony Ian Pag-ong[a,b*]**

[a]Animal Research Laboratory, Department of Biological Sciences, Institute of Arts and Sciences, Far Eastern University, 1008, Manila, Philipines; [b]Taylors University, Jin Taylors, 47500 Subang Jaya, Selangor Malaysia

Abstract *Garcinia mangostana* Linn., has been studied for its antibacterial properties to augment commercial antibiotics and in the hope of easing reliance on these chemical medications in the future, however, the comparison of the fruit's bactericidal capabilities relative to different bacterial species requires further analyses. This systematic review and meta-analysis compared the antibacterial activity of ethanolic and methanolic mangosteen extracts against three species that commonly cause Healthcare-Associated Infections (HAIs)—*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The results revealed no significant difference [mean difference: 1.42 (CI: -3.53 to 6.37, $I^2$ = 99%, Z = 0.56 ($P$ = 0.57))] between the effectiveness of the extracts against *S. aureus* and *E. coli.* But it was contrary when *P. aeruginosa* was compared with *S. aureus* [mean difference: 5.00 (CI: 4.48 to 5.52, $I^2$= 0%, Z = 18.97 ($P$ < 0.00001))] and *E. coli* [mean difference: 3.96 (CI: 2.01 to 5.92, $I^2$ = 94%, Z = 3.97 ($P$ < 0.0001))]. Literature search and screening were done following the PRISMA guidelines. Quality assessments utilized the JBI Critical Appraisal Tool and a remodified Newcastle-Ottawa Scale. A total of 13 studies were included in the review, only 7 of which were eligible for meta-analyses. In conclusion, *G. mangostana* extracts are indeed effective against multiple microbes, however, relative to the selected bacterial species, inhibition varied. Moreover, this study sheds light on further practical or *in vivo* applications of mangosteen as a treatment for bacterial infections.

**Keywords**: Antibacterial, Mangosteen, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa.*

***For correspondence:**
apag-ong@feu.edu.ph

**Received:** 04 March 2024
**Accepted:** 16 Oct. 2024



## Introduction

*Garcinia mangostana* Linn (common names purple mangosteen and mangostin) mainly grows in Southeast Asia, southwest India, and parts of Colombia and Puerto Rico [38]. The fruit is highly valued for its juicy, delectable white flesh with delicate texture, and slightly sweet and sour taste [35]. Although without reliable clinical evidence, various parts of the plant have a history of being used as traditional medicine for skin infections, dysentery, urinary tract infections, and gastrointestinal problems [44]. Mangosteen peel contains xanthonoids, such as mangostin, and other phytochemicals while fruits, leaves, and heartwood contain polysaccharides and xanthone compounds [44] which might have been the compounds working for these traditional Asian medicines mentioned. These bioactive compounds such as xanthones, particularly α, β, γ – mangostins found in *Garcinia* fruits have antibacterial properties (Iiunuma *et al* 1996). Different sources of α-mangostin coming from various plant species other than *G.*

*magostana* and their potency have been discussed by other authors such as [41] and [57]. As alternatives, certain chemicals have been employed as topical antimicrobial agents [54], however, these caused side effects such as irritations caused by allergic reactions [51].

Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) are three of the most common bacterial strains identified as leading community settings infections and Healthcare-Associated Infections (HAI) [37, 42]. The latter (HAIs) are infections that patients get while or soon after receiving healthcare and are a serious threat to safety. However, a significant proportion of these HAIs are considered preventable [2, 13]. The three selected bacteria are linked to the prevalence of nosocomial infections (NIs) responsible for nosocomial pneumonia, bloodstream infections, urinary tract infections, and surgical site infections [58]. It has also been established that these strains can acquire and express multiple mechanisms for drug resistance [37]. Severe infection stages may differ from country to country, as well as the exposure and incubation period [37, 42]. Further worsening matters is that *S. aureus*, *P. aeruginosa*, and *E. coli* have life-threatening capabilities, and treatment options are limited due to the wide range of infections resulting from extensive virulence factors [58]. Economic burdens include lacking healthcare facilities, uncertain treatment, and antibacterial medication costs [5, 14, 24, 50, 62].

As of the present time, there are hardly any recent reviews bringing together the action of mangostin in overcoming the specific microbial species mentioned above. As it appears, [55] and the work of [54] when they evaluated the antimicrobial activity of α-mangostin versus different microbes seem to be the studies that shed light on this premise but the former was 41 years ago while the latter dealt on a broader scheme. In addition, literature has been inconsistent with this over the years, due to heterogeneous methods of preparation and testing. The existing knowledge we have today does not paint a clear picture of the action of α-mangostin [16]. Thus, a thorough investigation into the benefits of α-mangostin as a potent antibacterial agent is essential for the creation of novel antimicrobial agents. The objective of this study was to systematically review primary literature that investigated the bactericidal properties of ethanolic and methanolic G. mangostana extracts against selected gram-positive and gram-negative bacteria and to inferentially assess the fruit extract's efficacy based on the extracted effect sizes measures from various studies grounded on pre-determined criteria. Using results from experimental research, this systematic review closes the gap regarding the idea that today's antibiotics are not only derived from soil bacteria and fungi but also from natural products. This comprehensive analysis will increase our understanding of the therapeutic use of α-mangostin extracts for the creation of antimicrobial agents soon.

# Materials and Methods

## Study Design

This is a systematic review and a meta-analysis of first-hand publications that evaluated the antibacterial properties of *Garcinia mangostana,* actualized following the Preferred Reporting Items for Systematic Review and Meta-Analysis Guidelines 2020 [45].

## PEO

The construction of the review question was guided by the Population, Exposure, and Outcome format (PEO) (Levine *et al*, 2015) and is accomplished as follows:
Population: Selected gram-positive and gram-negative bacteria—*S. aureus, E. coli* and *P. aeruginosa*
Exposure: The use of *G. mangostana* extracts
Outcome: Antibacterial activity of the extracts against the selected bacterial species
Based on this information, the free-form research question was "Are *G. mangostana* extracts effective antibacterial agents against selected gram-positive and gram-negative bacteria—*S. aureus, E. coli* and *P. aeruginosa*?"

### Information Sources and Search Strategy

Systematic searches for articles published from January 2000 to March 31, 2022, using electronic databases such as Google Scholar, Jstor, PubMed, Academic Search Complete, Applied Science & Technology Source, and CINAHL with Full Text through EBSCO Host. The researchers used combinations of Boolean logic operators AND and OR, Medical Subject Headings (MeSH), keywords, index terms, and truncations in building a search strategy; Table 1 contains the concepts as well as a specific search strategy for each repository. Additionally, manual searching using the keywords "*Garcinia mangostana*", "mangosteen", "extracts", "antibacterial", "antimicrobial", "*Staphylococcus aureus"*, "*Escherichia coli*" and "*Pseudomonas aeruginosa*" and other synonymous terms were employed to ensure that no relevant article was missed.

**Table 1.** Search terms used in PubMed according to concept

| Study Concept | Search Strategy |
|---|---|
| Concept 1: *Garcinia mangostana* | 1. "Garcinia mangostana"[Mesh] OR "garcinia mangostana"[tw] OR mangosteen[tw]<br>2. "Garcinia mangostana/drug effects"[Mesh] OR "Garcinia mangostana/microbiology"[Mesh] OR "Garcinia mangostana/pharmacology"[Mesh] OR "Garcinia mangostana/physiology"[Mesh] OR "Garcinia mangostana/therapeutic use"[Mesh] ) OR "garcinia mangostana"[tw] OR mangosteen[tw] |
| Concept 2: Antibacterial Properties | "Anti-Bacterial Agents"[Mesh] OR bactericidal[tw] OR bacteriostatic[tw] OR antimicrobial[tw] |
| Concept 3: Selected bacterial species | 1. "Staphylococcus aureus"[Mesh] OR gram-positive*[tw] OR cocci[tw] OR coccus[tw]<br>2. "Escherichia coli"[Mesh] OR gram-negative*[tw] OR bacill*[tw]<br>3. "Pseudomonas aeruginosa"[Mesh] OR gram-negative*[tw] OR bacill*[tw] |

## Eligibility Criteria

### Inclusion Criteria

The studies included were reviewed in full text. Those that investigated the antibacterial properties of *G. mangostana* extracts in vitro were mainly looked up and included. The language was restricted to English and only those published from the year 2000 to March of 2022 were included. Furthermore, studies that specifically utilized ethanolic and methanolic extract via disk diffusion method or Kirby-Bauer disk diffusion method against at least two of the bacterial species of interest *S. aureus*, *E. coli*, and *P. aeruginosa* together with their quantitative data were included in the meta-analysis.

### Exclusion Criteria

Studies that used different plant extracts other than ethanolic and methanolic *G. mangostana*, those that used a different method in the evaluation of the antibacterial properties, those that fractionated their extracts to specific pure compounds or used the plant extract in synergy with other antimicrobial agents, and those that did not test on the three bacterial species of interest were excluded in the meta-analysis. In addition, studies that used a different language other than English as a medium, studies published before or after the scope of publication year as well as those that have a vague year of publication, and reports other than journal articles (i.e., book chapters/sections, dissertations) were altogether excluded in the review. A summary of inclusion and exclusion criteria is in Table 2.

**Table 2.** Summary of the inclusion and exclusion criteria used in this study

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| 1. Investigated the ethanolic and methanolic extracts of *G. mangostana in vitro* | 1. Studies that used a different plant other than *G. mangostana* |
| 2. Utilized the disk diffusion technique | 2. Studies that used other solvent/menstruum other than ethanol and methanol |
| 3. Tested against at least two of the bacterial species of interest | 3. Studies that did not tested against at least two of the bacterial species of interest |
| 4. Written in the English language | 4. Written in a different language |
| 5. Published not earlier than 2000 and not later than March 2022 | 5. Published before or beyond the 2000 – March 2022 |
| | 6. Reports other than journal articles |

## Study Selection and Quality Assessment

The selection and quality assessment of the studies included in the review and meta-analysis were aided by third-party applications Zotero 6 and Rayyan, as well as appraisal tools Joanna Briggs Institute (JBI)'s Appraisal Checklist for Quasi-experimental Studies and a remodified Newcastle-Ottawa Scale. The removal of duplicate studies initially utilized the detection and merging feature of Zotero 6.

### Initial Screening of Studies for Systematic Review

Zotero, an open-source reference management software was used in data collection, organization, annotation, and dissemination [25, 59]. Zotero was used for data input and re-visiting data when necessary. Rayyan (rayyan.ai), a semi-automated web-based application, with offline app compatibility was used by the systematic reviewers to collaboratively work on the initial screening of abstracts and titles. Compared to the conventional practice of manually reading through each publication, Rayyan expedited the process by

allowing the researchers to utilize visual cues such as highlighting keywords, which made it easier to assess a paper's relevance.

### Risk of Bias Assessment

The JBI Critical Appraisal Tool, a tool for risk bias assessment was used. The instrument used thirteen (13) different checklists, each one made for a particular study design. Every checklist came with an extensive explanation for each question it contained. In the present study, the appraisal checklist for quasi-experimental studies was used. This consists of nine (9) questions, answerable by yes, no, unclear, and not applicable, directed towards assessing the methodological quality of the publications to determine possible sources of bias in its study framework, the conduct of methods, and the analysis of results. For ease of assessment, a point system adopted from (Sultan, *et al*., 2022) was employed. For each question answered by "yes", one point was given while zero point constitutes any answer otherwise. Total points for each study were computed and interpreted according to the following scheme: 1-3 points = Low methodological quality, 4-6 points = Moderate methodological quality, and 7-9 points = High methodological quality.

### Eligibility Assessment for Meta-Analysis

To evaluate the quality of non-randomized studies and their eligibility to be included in a meta-analysis according to three broad parameters namely selection, comparability, and outcome, the Newcastle-Ottawa Scale (NOS) [34] was used. It is divided into eight items that are subjected to a merit or "star system" with the highest possible score of 9 but any score greater than 3 is acceptable [34].

### Data Extraction and Synthesis

The type of data extracted for this study is shown in Table 3. The meta-analysis of the quantitative data was done using the statistical method of inverse variance of random effect with a 95% confidence interval. The heterogenicity was evaluated with Tau2, chi2, and I2 and tested for overall effect with Z using the Cochrane Review Manager 5.4.1.

**Table 3.** Data extracted from the studies included in the systematic review and meta-analysis

| Category/Variable | Definition |
|---|---|
| 1. Nature of Publication | |
| Year of publication | Year of publication as it appears in the final print |
| Journal | Name of the journal where the study was published |
| Discipline/Study area | Research areas of the journal (e.g., Pharmacology, Microbiology, Epidemiology, etc.) |
| 2. Sample Preparation | |
| Country origin of the plant used | The geographical region where the *G. mangostana* were collected |
| Menstruum | The solvent of extraction used and the amount in milliliter (mL) (e.g., 100 mL, 500 mL) |
| Portion used | The exact portion of the *G. mangostana* used (e.g., pericarp, pulp, seed, leaves, bark, etc.) |
| Method of Extraction | The extraction procedures utilized to isolate active compounds on interest (e.g., maceration, evaporation, centrifugation, etc.) |
| Length of solvent exposure | The estimated time frame for solvent exposure in hours |
| 3. Susceptibility Testing | |
| Method of antimicrobial susceptibility testing | Used to identify which antimicrobials will hinder the growth of the microbes. These include disk diffusion, agar well, broth and agar microdilution, etc. (Bayot & Bragg, 2022) |
| Incubation period | The length of time of microbial plate incubation in hours |
| Tested Microorganisms | The selected strains of microorganisms subjected to testing |
| Control groups | Both positive and negative controls are used to evaluate the validity of the experimental treatment by producing known and expected outcomes (Moser, 2020). |
| 4. Quantitative Data | |
| Extract dose | The amount of extract concentration inoculated on the paper disks (e.g., 10µL, 40µL, 80µL) |
| Sample size | The number of replicates per intervention used in the susceptibility testing. |
| Outcome measures | The results of the outcome depending on the test used; may be in measures of zone of inhibition (ZOI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). |

# Results and Discussion

## Search Results

An initial hit of 2834, 46, and 50 potentially suitable papers were found using the selected search words "*Garcinia mangostana*", "mangosteen", "extracts", "antibacterial", "antimicrobial"," "*Staphylococcus aureus*", "*Escherichia coli*" and "*Pseudomonas aeruginosa*" in Google Scholar, EBSCOhost, and PubMed databases, respectively (Table 4). There were no search results found from JSTOR. Initial screening was done in Zotero which resulted in the elimination of 1647 studies citing the reasons: (1) duplicated records, (2) non-journal articles, and (3) non-English articles. Manual screening of titles as well as available abstracts was done in Rayyan utilizing its tagging feature keeping the inclusion and exclusion criteria in mind. The reasons for the exclusion of studies during this screening procedure were as follows: Tested at least two bacterial species of interest (n = 484), a different plant used other than mangosteen (n = 389), different method used—not in vitro, the different extract used, and a different measure of inhibition (n = 767), studies that were found irrelevant to the subject matter (n = 662), studies with conflicted publication dates such as those before the year 2000 or no publication dates mentioned (n = 6), and duplicated studies (n = 13).

However, it is important to note that the sum of these figures will not coincide with the total number of studies removed since multiple exclusion tags per study were applied in Rayyan. Following the shortlisted included articles, the overall records sought for retrieval were n = 83 having to remove 8 studies whose full-text papers were not retrieved as these papers had access charges to download, hence the total number of studies assessed for eligibility was n = 75. The remaining articles were examined thoroughly for full-text screening and the reasons for the removal of the studies throughout this screening process include: Tested at least two bacterial species of interest (n = 39), the different plants used other than mangosteen (n = 7), different method used—in vitro, the different extract used, and a different measure of inhibition (n = 47), studies that were non-journal (n = 2), studies with conflicted publication dates before 2000 (n = 3), and non-English articles (n = 3). Therefore, a total of 13 articles were included in the review and were also evaluated using the JBI Critical Appraisal Tool and Newcastle-Ottawa Scale (Table 6) for the credibility of the underlying research. Almost 77% of these 13 articles were mostly extracted from the Google Scholar database (10 of 13) while PubMed and EBSCOhost accounted for 15% (2 of 13) and 7% (1 of 13) respectively. The PRISMA 2020 flowchart used is shown in Figure 1.

**Table 4.** Summary of the screening process

| Database | Initial Hits | After Screening 1 | After Screening 2* | After full-text Retrieval* | After full-text Screening* |
|---|---|---|---|---|---|
| Google Scholar | 2834 | 1236 | 83 | 75 | 13 |
| EBSCOHost | 46 | 26 | | | |
| PubMed | 50 | 21 | | | |
| JSTOR | 0 | 0 | | | |
| Total | 2930 | 1283 | 83 | 75 | 13 |

*The studies from different databases were screened collectively

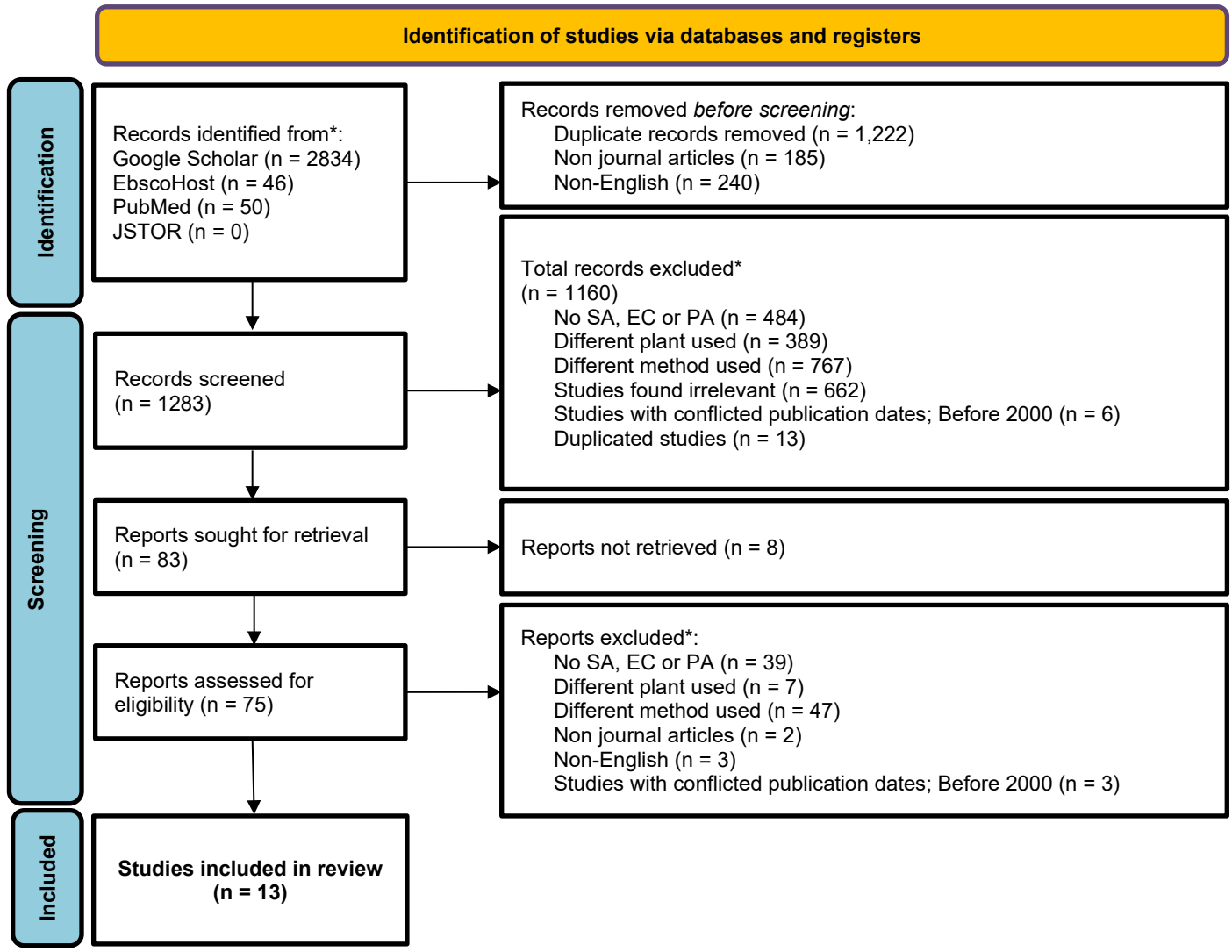
### Characterization Of Included Studies

The majority of the studies were from India and Indonesia which both comprised 23.1%, while the other countries include Saudi Arabia [7, 13), Thailand [56], China [31], Malaysia [32], and Sri Lanka [19]. In terms of the publication year, the studies included in this review were found between the years 2006 to 2021. Most of the journals were pharmacologic in origin, however, the following areas of discipline were also observed: biotechnology, food science, and horticulture.

**Table 5.** Risk of bias of included studies

| No. | Authors, Year | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Total score | Interpretation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | [7] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 0 | 6 | M |
| 2 | [8] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 0 | 6 | M |
| 3 | [13] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 1 | 7 | H |
| 4 | [15] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 1 | 7 | H |
| 5 | [17] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 0 | 6 | M |
| 6 | [19] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 0 | 6 | M |
| 7 | [28] | 1 | 1 | 1 | 0 | 0 | NA | 1 | 1 | 0 | 5 | M |
| 8 | [29] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 0 | 6 | M |
| 9 | [31] | 1 | 1 | 1 | 0 | 0 | NA | 1 | 1 | 1 | 6 | M |
| 10 | [32] | 1 | 1 | 1 | 1 | 0 | NA | 1 | 1 | 1 | 7 | H |
| 11 | [43] | 1 | 1 | 1 | 1 | 1 | NA | 1 | 1 | 0 | 7 | H |
| 12 | [47] | 1 | 1 | 0 | 1 | 0 | NA | 1 | 1 | 1 | 6 | M |
| 13 | [56] | 1 | 1 | 1 | 0 | 0 | NA | 0 | 1 | 1 | 5 | M |

*L =Low methodological quality, M = moderate methodological quality, and H = high methodological quality




*Multiple tags/reasons for exclusion were applied to some studies

**Figure 1.** PRISMA Flowchart for this study

## Risk of Bias Assessment

The JBI Critical Appraisal Tool was used to assess the risk of bias. Most of the included studies (69.23%) have moderate methodological quality obtaining a total score of 5 or 6 while 4 out of 13 (30.7%) were of high methodological quality which scored a total of 7 (Table 5). The differences among the studies were the lack of use of a control group as well as the use of appropriate statistical tools; three studies had no control group, while statistical analysis was missing from 6 studies. In Q5, due to the nature of the studies, the outcome was not measured pre- and post-intervention/exposure, hence, the results were also unclear. With regards to the sixth question, it did not apply to the studies as there were no follow-up measures or interventions done. A stacked graph of the risk of bias scores is presented in Figure 2.

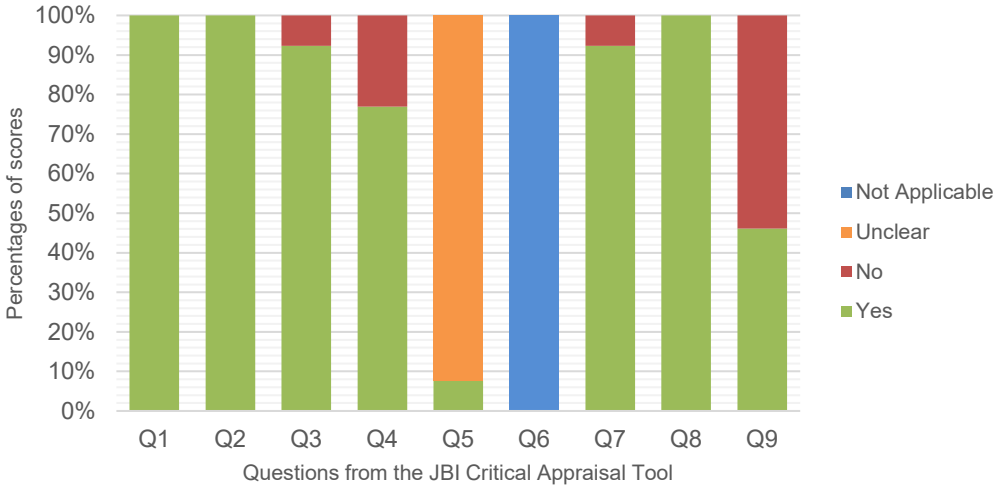



**Figure 2.** Stacked graph of the risk of bias scores

**Table 6.** Modified Newcastle-Ottawa scale

| Score | Criteria |
|---|---|
| **0** | a. Inadequate description of interventions<br>b. Studies that did not clearly define their sample sizes along with their means |
| **1** | a. Studies that did not used *G. mangostana* extracts as source of antibacterial agent.<br>b. Studies that tested on different species of bacteria other than *S. aureus, E. coli* and *P. aeruginosa* |
| **2** | a. Studies that used *G. mangostana* extracts neither ethanolic nor methanolic in origin.<br>b. Studies that used other antibacterial assays other than the disk diffusion method |
| **3** | Studies included in this meta-analysis:<br>a. Studies that provided a proper description of the interventions<br>b. Studies that provided their sample sizes along with the mean and the standard deviations<br>c. Studies that tested the antibacterial properties of *G. mangostana* ethanolic and methanolic extracts against *S. aureus*, *E. coli* and *P. aeruginosa* utilizing the disk diffusion technique resulting to measures of zone of inhibitions. |

**Table 7.** Modified NOS scores

| No. | Author, Year of publication | Title of the Study | NOS Score |
|---|---|---|---|
| 1 | [7] | Phytochemical, antimicrobial, and antiprotozoal evaluation of *Garcinia mangostana* pericarp and α-mangostin, its major xanthone derivative | 2 |
| 2 | [8] | The isolation of xanthones from trunk latex of *Garcinia mangostana* Linn. and their antimicrobial activities | 1 |
| 3 | [13] | Antimicrobial activity of *Garcinia mangostana* using different solvent extracts | 3 |
| 4 | [15] | Extraction of tropical fruit peelsand development of HPMC film containing the extracts as an active antibacterial packaging material | 3 |
| 5 | [17] | Antibacterial (*Staphylococcus aureus* and *Escherichia coli*) and Antifungal (*Saccharomyces cerevisiae*) Activity Assay on Nanoemulsion Formulation of Ethanol Extract of Mangosteen Leaves (*Garcinia mangostana L.*) as Fruit Preservative | 3 |
| 6 | [19] | Antibacterial activity of extracts of pericarp of *Garcinia mangostana* | 3 |
| 7 | [28] | Antibacterial activity of mangosteen (*Garcinia Mangostana*) Pericarp | 1 |
| 8 | [29] | Preparation of silver nanoparticles by *Garcinia mangostana* stem extract and investigation of the antimicrobial properties | 1 |
| 9 | [31] | Effects of Extraction Solvents on Phytochemicals and Antibacterial Activities of *Garcinia mangostana* Pericarp | 3 |
| 10 | [32] | Antioxidant capacity and antibacterial activity of different parts of mangosteen (*Garcinia mangostana Linn.*) extracts | 3 |
| 11 | [43] | In-vitro analysis of antioxidant and antimicrobial properties of *Garcinia mangostana* L. (pericarp) and *Clitoria ternatea* (flower) | 1 |
| 12 | [47] | The effect of mangosteen pericarp (*Garcinia mangostana* Linn) extract on inhibits the growth of bacteria *Escherichia Coli* ATCC 25922 and bacteria *Staphylococcus Aureus* ATCC 25923 | 3 |
| 13 | [56] | Antityrosinase and Antibacterial activities of mangosteen pericarp extract | 0 |

## Meta-Analysis Results

From the assessments of studies to be included in the meta-analysis, a total of 7 out of the 13 (53.8%) articles were found to have satisfied the pre-defined criteria in the NOS and scored 3 while the rest score < 3 and were, therefore, excluded. These, together with the reason for exclusion were tabulated in Table 7 for reference. Of the 7 included studies, [13] tested the efficacy of ethanolic and methanolic extracts against all three bacterial species. On the other hand, [31] used both extracts against *S. aureus* and *E. coli* only. The same two microbes were subjected to ethanolic extracts alone by three of the studies [15, 17, 47] and against methanolic extract alone by [32] which reported no observable measure of inhibition for *E. coli*. Lastly, [19] used only methanolic extracts against all three bacteria but recorded no zone of inhibition for *E. coli* and *P. aeruginosa*. With the data available to the researchers and in the pursuit of addressing the objectives of this study, the results of the meta-analyses were divided into three parts: Inhibition against *S. aureus* compared to *E. coli* (Figures 3-5), inhibition against *E. coli* compared to *P. aeruginosa* (Figure 6) and inhibition against *P. aeruginosa* compared to *S. aureus* (Figure 7).

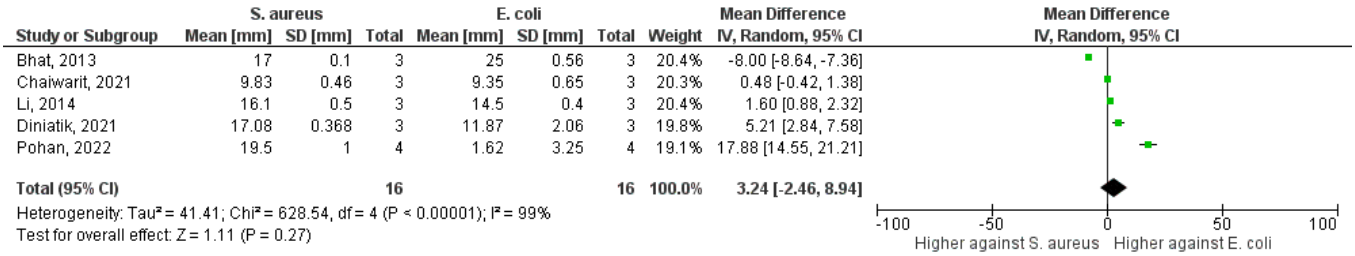



**Figure 3**. Ethanolic *G. mangostana* extracts against *S. aureus* and *E. coli*

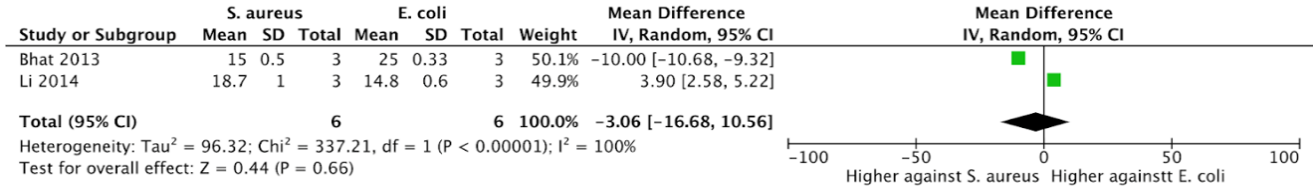
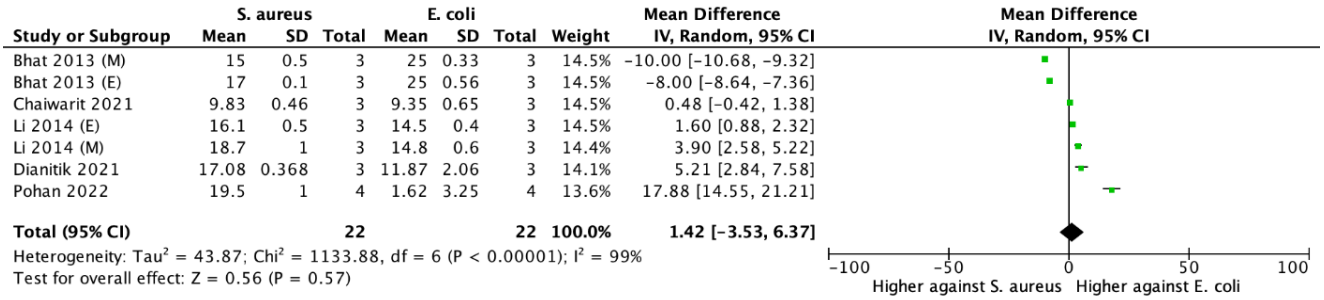


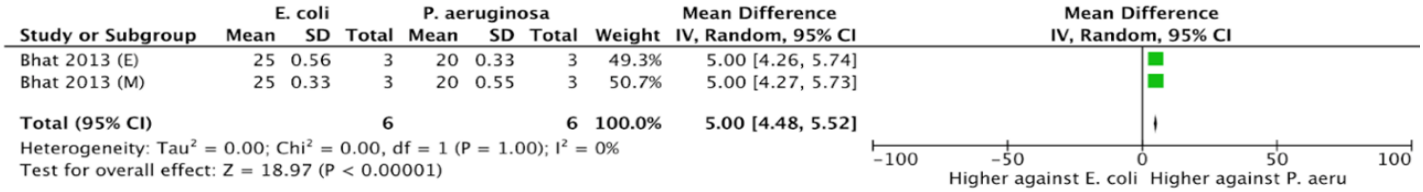

**Figure 4.** Methanolic *G. mangostana* extracts against *S. aureus* and *E. coli*




*E = ethanolic extracts, M = methanolic extracts

**Figure 5.** Overall inhibitory capacities *G. mangostana* extracts against *S. aureus* and *E. coli*

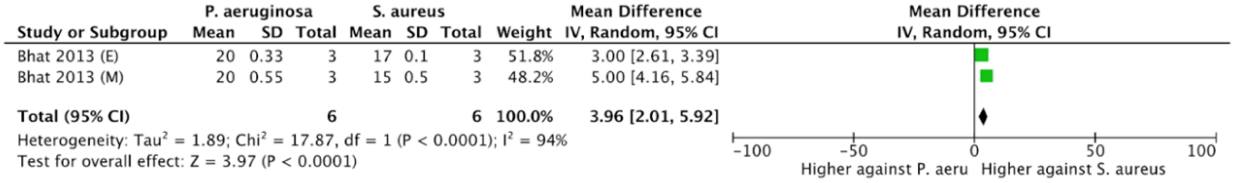
Based on the results of the series of meta-analyses, in terms of the antibacterial activity of ethanolic and methanolic extracts of *G. mangostana*, there was no significant difference between its ability to inhibit the growth of *S. aureus* compared to *E. coli* as evidenced by the overall point estimates (black diamond) inclining towards the line of null effect (vertical line) in all of the forest plots. However, as can be seen in Figure 5, only one study by [15] individually exhibited the same case where its point estimate (green square) aligned with the line of null effect, while two reports, [13] ethanolic and methanolic results were oriented far towards the left favoring greater inhibition against *S. aureus* and 3 studies [31 ethanolic and methanolic results; 17 and 47) were oriented far towards the right favoring greater inhibition against *E. coli* at a confidence interval (CI) of 95%, $I^2$ = 99%, heterogeneity with Tau$^2$ = 43.87, Chi$^2$ = 1133.88, df = 6 ($P$ < 0.00001) and a test for overall effect Z of 0.56 ($P$ = 0.57).




*E = ethanolic extracts, M = methanolic extracts

**Figure 6.** Overall inhibitory capacities *G. mangostana* extracts against *E. coli* and *P. aeruginosa.*

On the other hand, the opposite was observed with *E. coli* and *P. aeruginosa*, and *P. aeruginosa* and *S. aureus*. There was a significant difference between the inhibition of *G. mangostana* extracts in these two comparisons. In close inspection, Figure 6 shows both records by [13] inclined in favor of greater inhibition against *P. aeruginosa* in contrast to *E. coli* with a 95% CI, $I^2$ = 0%, heterogeneity with $Tau^2$ = 0.00, $Chi^2$ = 0.00, df = 1 (*P* = 1.00) and test for overall effect Z = 18.97 (*P* < 0.00001). Moreover, in Figure 7, despite the results of the [13] ethanolic extracts slightly touching over the vertical line of null effect both the black diamond and the outcome of methanolic counterpart were situated towards the right which is biased towards greater effect against *S. aureus* as compared to *P. aeruginosa* at a CI of 95%, $I^2$ = 94%, heterogeneity with $Tau^2$ = 1.89, $Chi^2$ = 17.87, df = 1 (*P* = 1.00) and test for overall effect Z = 3.97 (*P* < 0.0001).




*E = ethanolic extracts, M = methanolic extracts

**Figure 7.** Overall inhibitory capacities *G. mangostana* extracts against *P. aeruginosa* and *S. aureus*

## Implications of the Meta-Analyses Heterogeneity Among Studies

Among the three overall comparisons (Figures 3-7), heterogeneity was recorded to be the highest with the comparison between *E. coli* and *S. aureus* (Figure 5) at $I^2$ statistic = 99% followed by the comparison between *P. aeruginosa* and *S. aureus* (Figure 7) at 94%. Both yields imply substantial heterogeneity, while an $I^2$ statistic of 0% was recorded between the *P. aeruginosa* and *E coli* (Figure 6). According to [11], it has been a longstanding difficulty to assess antimicrobial effects of natural products based only on the comparisons of their results due to non-standardized approaches across studies. Heterogeneity cannot be eliminated as variability may range from declared parameters to those that might not be accessible to the reviewers, and may even be out of the control of primary literature's authors. The resulting zone of inhibition measures are widely acknowledged to be influenced by the growth rate of the bacteria and the diffusion rate of the antimicrobial agent, some less discussed parameters, however, involve pre-treatment preparations and during treatment conditions [21, 48]. Disk diffusion assays, in particular, are prepared with agar, the bacterial inoculum, filter paper disks, and the antimicrobial agent to be tested, and quite certainly, they also affect the outcome of the test. [23] aforementioned that ZOI is dependent on the size of the paper disk, the amount of the compound that was impregnated therein, the type of agar, the thickness and pH of the medium, the microbe tested, and lastly, the temperature at which the cultures are incubated.

In terms of the polarity of the target solute, solvents are primarily selected to extract biomolecules from plants, thus the solute will dissolve precisely in a solvent with a polarity that is similar to the solute [6]. A study by [30] mentioned that out of the seven solvents (ethanol, acetone, ethyl acetate, methanol, hexane, acetic acid, and water) used in extraction, ethanol yielded the best results in extracting xanthones and antioxidants. Additionally, the extracted xanthones showed considerable differences between all of the solvents. Based on the comprehensive study conducted by Lima *et al*. (2019), fruit extracts obtained from methanol and ethanol were more effective in inhibiting harmful microbes in contrast with extracts acquired with water as a solvent. Similarly, [46] reported that aqueous extracts acquired from *G. mangostana* by-products did not show antibacterial potential against Gram-positive bacteria due to their extracts which have a low concentration of bioactive substances and had lesser ability to harm the Gram-positive bacteria's cell membrane. In the selected 7 studies for the meta-analysis, the pericarp of mangosteen was used by [31, 19, 47]. Arils, peel and leaves were utilized by [13], [15], and [17] in that particular order while [32] made use of multiple portions such as pulp, pericarp, and seed. [1] and [36] noted that the most frequently used portion of the plant in Antimicrobial Susceptibility Testing (AST) s is the pericarp due to the abundance of xanthone in it. According to [3], the appropriate way of extracting from a raw plant source is very vital and this could be dependent on the intended use of the extracts. Among the seven studies, the method of extraction followed that of the procedure of maceration which utilized grounded or powdered plant material, immersed with the solvent for a certain period, later filtered or separated from the marc, and then subsequently evaporated to attain the organic material of interest. The rationale of this method involved the softening of the plant's cell wall to allow the soluble constituents to be released [22]. Multiple studies showed that this method is a convenient and widely used option, in addition to its applicability to thermolabile components which explains its prominence in literature [3, 22, 61]

In the studies included in the meta-analyses, [13, 15, 32] exhibited different amounts of extracts impregnated on the filter paper disks, whereas some studies did not report anything regarding this. Prior literature found that the amount of antimicrobial agent in the filter disk affects ZOI. For instance, [60] observed that the higher the amount of antimicrobial agent, the larger the resulting measure of ZOI as compared to the corresponding lowest concentration. Similarly, a study that tested the antibiotics against microbes showed that the highly concentrated disks translated to a greater inhibition zone [2]. Furthermore, the size of the filter paper disk can also influence the size of the inhibition zone [21], and disks of about 6 mm in diameter are to be used according to standards [11, 40, 49]. Three of the seven studies did not specify this, while the rest except [13], utilized 6 mm disks.

Depending on the species, bacteria have different requirements that would enable them to grow and thrive. In ASTs, the culture media are the primary means for these requirements to be met, hence, they also vary in composition depending on the use and specific microbes they are designed to cultivate. Standard guidelines recommend the use of Mueller-Hinton agar (MHA) in ASTs, but this has not been strictly observed [40, 49]. Three agar media were used in the studies, and NA, was used in three of the seven studies. Brain heart infusion agar (BHIA) and Mueller-Hinton agar (MHA) were used by [15 and [47], respectively. One study by [31], did not declare the type of agar in their AST while [17] was inconsistent in reporting the media they utilized. NA and BHIA belong to the general-purpose media that contain sources of carbon, energy, mineral salts, amino acids, and vitamins that permit the growth of a wide range of microorganisms. On the other hand, MHA, according [18], is known as one of those “assay media” frequently used to assess certain concentrations of substances such as that in an AST. Despite the possibility of contributing to the heterogeneity among the studies, the literature further suggests that NA and MHA when compared in an antibiotic sensitivity test against clinical bacterial pathogens concluded that the resulting ZOI in MHA was not significantly greater than that in NA [20]. [9] claimed BHIA to be a favorable nutritious media in the cultivation of fastidious and non-fastidious microorganisms as opposed to NA as an alternative to blood agar which is not easily accessible to small-scale laboratories. In terms of the thickness of the agar media, only [17] clearly stated the amount of agar plated in petri dishes, which was 12 mL. The pH of the media used was not present across all of the studies, on the contrary, the temperature at which the cultures were incubated was uniform across all reports at 37°C.

### Efficacy of *G. Magostana* Extracts on Selected Bacteria

Based on the meta-analyses results, the differences between *S. aureus* and *E. coli* showed no significant difference denoting equal efficacy of the extracts on them, unlike the comparisons with *P. aeruginosa*, that showed significant differences implying efficacy on a lesser degree on this bacterium. The variability of efficacy depends on different morphological characteristics of the bacteria as well as the mechanism of inhibition by the antibacterial agent. In terms of morphology, the bacterial cell envelope is of utmost importance as it serves as a protection [61] that also influences the way they adapt to changes in the environment which is essential to their survival since it enables them to endure stress as well as to establish complex communities [52].
Studies, as early as 1983, showed that *G. mangostana* extracts are capable of inhibiting the three selected bacterial species. Specifically, this study by [53] showed that at optimum concentrations of 100 µg/mL, *S. aureus* and *P. aeruginosa* are highly susceptible to ethanolic extracts of the fruit while *E. coli* was moderately susceptible to it. However, literature has been inconsistent with this over the years, most probably due to heterogeneous methods of preparation and testing as discussed earlier. [46], noted that *G. mangostana* extracts were effective only against Gram-positive bacteria at 500 µg/mL and not against Gram-negative counterparts even at concentrations higher than this. Similarly, a more recent study by [4] was not able to report ZOI for *P. aeruginosa* but concluded the antibacterial properties of mangosteen against *S. aureus* and *E. coli* tested under the same parameters. The same inconsistency was also observed among the seven studies included in the meta-analyses. To re-iterate, two studies by [32] and [19] failed to produce ZOI measures for both the Gram-negative bacteria *E. coli* and *P. aeruginosa* despite having to record viable outcomes for *S. aureus*. Nevertheless, there are still studies that report successful inhibition of the fruit extracts against these species such as [53] for *P. aeruginosa*, [27] for *E. coli* and one study included in the meta-analyses by [13] for both species.

## Conclusions

From the results of the systematic review and meta-analysis, it can be determined that *G. mangostana* extracts have the potential against multiple Gram-positive (S. aureus) and Gram-negative (E. coli and P. aeruginosa) that are known prevalent causes of community and Healthcare-Associated Infections (HAIs). This is a comprehensive analysis of the said plant that delved into its effect relative to bacterial species instead of comparisons against available antibiotics. Comparing the inhibitory capacity of the ethanolic and methanolic extracts among three bacterial species that commonly cause HAIs—*S. aureus, E. coli,* and *P. aeruginosa*, the meta-analysis revealed that mangosteen extracts are equally effective against *S. aureus* and *E. coli* in contrast with each other, however, when compared with *P. aeruginosa, S. aureus* was more readily inhibited.

## Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

## Acknowledgement

We wish to thank the senior author, Dr. Anthony Ian Pag-ong, for his guidance in helping put up a significant study even if fieldwork and specimen collections were unavailable due to the pandemic. We are also indebted to our alma mater – Far Eastern University. We wish to thank our parents, professor, and special ones who stood and believed with us during the conceptualization and formations of this study.

## References

[1] Abate, M., Pagano, C., Masullo, M., Citro, M., Pisanti, S., Piacente, S., & Bifulco, M. (2022). Mangostanin, a xanthone derived from *Garcinia mangostana* fruit, exerts protective and reparative effects on oxidative damage in human keratinocytes. *Pharmaceuticals (Basel), 5*(1). https://doi.org/10.3390/ph15010084

[2] Abdel-Azim, A., & Abdel-Azim, G. (2016). The impact of antibiotic exposure and concentration on resistance in bacteria. *Journal of Emerging Investigators*. Retrieved from https://emerginginvestigators.org/articles/the-impact-of-antibiotic-exposure-and-concentration-on-resistance-in-bacteria/pdf

[3] Abubakar, A., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Science, 2*(1), 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

[4] Albuquerque, B., Dias, M., Pinela, J., Calhelha, R., Pires, T., Alves, M., . . . Barros, L. (2023). Insights into the chemical composition and in vitro bioactive properties of mangosteen (*Garcinia mangostana* L.) pericarp. https://doi.org/10.3390/foods12050994

[5] Allel, K., Garcia, P., Labarca, J., Manuta, J. M., Rendic, M., Grupo Colaborativi de Resistencia Bacteriana, & Undurraga, E. A. (2020). Socioeconomic factors associated with antimicrobial resistance. *Revista Panamericana De Salud Pública (Impresa), 44*(1). https://doi.org/10.26633/rpsp.2020.30

[6] Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts—A review. *Plants (Basel), 6*(4), 42. https://doi.org/10.3390/plants6040042

[7] Al-Massarani, S., El Gamal, A., Al-Musayeib, N., Mothana, R., Basudan, O., Al-Rehaily, A., & Maes, L. (2013). Phytochemical, antimicrobial and antiprotozoal evaluation of *Garcinia mangostana* pericarp and α-mangostin, its major xanthone derivative. *Molecules, 18*(9), 10599-10608. https://doi.org/10.3390/molecules180910599

[8] Anggia, V., Bakhtiar, A., & Arbain, D. (2015). The isolation of xanthones from trunk latex of *Garcinia mangostana* Linn. and their antimicrobial activities. *Indonesian Journal of Chemistry, 15*(2), 187-193. https://doi.org/10.22146/ijc.21213

[9] Asna, S., Karmaker, M., & Sarker, U. (2018). Brain heart infusion agar: A surrogate of agar blood. *Bangladesh Journal of Medical Microbiology, 12*(1), 24-26. https://doi.org/10.3329/bjmm.v12i1.51688

[10] Bacteriana, & Undurraga, E. A. (2020). Socioeconomic factors associated with antimicrobial resistance. *Revista Panamericana De Salud Pública (Impresa), 44*(1). https://doi.org/10.26633/rpsp.2020.30

[11] Balouiri, M., Sadiki, M., & Ibnsouda, S. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal, 2*, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[12] Bayot, M., & Bragg, B. (2022). Antimicrobial susceptibility testing. *StatPearls Publishing LLC*.

[13] Bhat, R., & Al-Daihan, S. (2013). Antimicrobial activity of *Garcinia mangostana* using different solvent extracts. *International Journal of Biosciences, 3*(10), 267-272. https://doi.org/10.12692/ijb/3.10.267-272

[14] Cannon, J., Zhung, J., Bennett, J., Moreland, N., Baker, M., Geelhoed, E., . . . Jack, S. (2021). The economic and health burdens of diseases caused by group A *Streptococcus* in New Zealand. *International Journal of Infectious Diseases*, 176-181. https://doi.org/10.1016/j.ijid.2020.11.193

[15] Chaiwarit, T., Kantrong, N., Sommano, S. R., Rachtanapun, P., Junmahasathien, T., Kumpugdee-Vollrath, M., & Jantrawut, P. (2021). Extraction of tropical fruit peels and development of HPMC film containing the extracts as an active antibacterial packaging material. *Molecules, 26*, 2265. https://doi.org/10.3390/molecules260822

[16] Chen, G., Li, Y., Wang, W., & Deng, L. (2018). Bioactivity and pharmacological properties of α-mangostin from the mangosteen fruit: A review. *Expert Opinion on Therapeutic Patents, 28*, 415–427.

[17] Diniatik, D., & Anggraeni, R. (2021). Antibacterial (*Staphylococcus aureus* and *Escherichia coli*) and antifungal (*Saccharomyces cerevisiae*) activity assay on nanoemulsion formulation of ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) as fruit preservative. *Journal of Food and Pharmaceutical Sciences, 9*(1), 351-365. https://doi.org/10.22146/jfps.1008

[18] Erkmen, O. (2021). Practice—Preparation of media and sterilization techniques. In *Laboratory Practices in Microbiology* (pp. 3-18). Elsevier. https://doi.org/10.1016/B978-0-323-91017-0.00004-4

[19] Fernando, K., & Dasanayake, P. (2007). Antibacterial activity of extracts of pericarp of *Garcinia mangostana*. *Vidyodaya Journal of Science, 13*, 99-107. Retrieved from http://dr.lib.sjp.ac.lk/handle/123456789/1046

[20] Festus, O., & Emmanuella, O. (2020). Testing the efficacy of Mueller Hinton agar over nutrient agar for optimal antibiotic sensitivity testing response by selected clinical bacterial pathogens. *GSC Advanced Research and Reviews, 5*(2), 61-74. https://doi.org/10.30574/gscarr.2020.5.2.0037

[21] Flanagan, J., & Steck, T. (2017). The relationship between agar thickness and antimicrobial susceptibility testing. *Indian Journal of Microbiology, 57*(4), 503-506. https://doi.org/10.1007/s12088-017-0683-z

[22] Fotsing Yannick Stéphane, F., Kezetas Jean Jules, B., El-Saber Batiha, G., Ali, I., & Ndjakou Bruno, L. (2022). Extraction of bioactive compounds from medicinal plants and herbs. In *Natural Medicinal Plants*. https://doi.org/10.5772/intechopen.91542

[23] Horváth, G., Bencsik, T., Ács, K., & Kocsis, B. (2016). Chapter 12: Sensitivity of ESBL-producing gram-negative bacteria to essential oils, plant extracts, and their isolated compounds. In *Antibiotic Resistance* (pp. 239-269). https://doi.org/10.1016/b978-0-12-803642-6.00012-5

[24] Huong, V., Turner, H., Kinh, N., Thai, P., Hoa, N., Horby, P., . . . Wertheim, H. (2019). Burden of disease and economic impact of human *Streptococcus suis* infection in Viet Nam. *Transactions of The Royal Society of Tropical Medicine and Hygiene, 113*(6), 341-350. https://doi.org/10.1093/trstmh/trz004

[25] Idri, N. (2016). Zotero software: A means of bibliographic research and data organisation; Teaching bibliographic research. *Arab World English Journal (AWEJ)*. https://doi.org/10.2139/ssrn.2843984

[26] Iinuma, M., Tosa, H., Tanaka, T., Asai, F., Kobayashi, Y., Shimano, R., & Miyauchi, K. I. (1996). Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. *Journal of Pharmacy and Pharmacology, 48*, 861–865.

[27] Indrianingsih, A., Rosyida, V., Darsih, C., & Apriyana, W. (2021). Antibacterial activity of *Garcinia mangostana* peel-dyed cotton fabrics using synthetic and natural mordants. *Sustainable Chemistry and Pharmacy, 21*. https://doi.org/10.1016/j.scp.2021.100440

[28] Jacob, D., Vigasini, N., & Iyer, P. (2016). Antibacterial activity of mangosteen (*Garcinia mangostana*) pericarp. *World Journal of Pharmaceutical Research, 5*(9), 1797-1802. https://doi.org/10.20959/wjpr20169-7046

[29] Karthiga, P. (2018). Preparation of silver nanoparticles by *Garcinia mangostana* stem extract and investigation of the antimicrobial properties. *Biotechnology Research and Innovation, 2*(1), 30-36. https://doi.org/10.1016/j.biori.2017.11.001

[30] Kusmayadi, A. (2018). The effect of solvents and extraction time on total xanthone and antioxidant yields of mangosteen peel (*Garcinia mangostana* L.) extract. *Drug Invention Today, 10*(12), 2572-2576.

[31] Li, Q., & Xu, J.-G. (2014). Effects of extraction solvents on phytochemicals and antibacterial activities of *Garcinia mangostana* pericarp. *Asian Journal of Agriculture and Food Sciences, 2*(4). Retrieved from https://www.ajouronline.com/index.php/AJAFS/article/view/1509

[32] Lim, J. Y., Yoon, J., & Hovde, C. (2013). A brief overview of *Escherichia coli* O157 and its plasmid O157. *Journal of Microbiology and Biotechnology, 20*(1), 5-14. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645889/

[33] Lima, M.C., de Sousa, C., Fernandez-Prada, C., Harel, J., Dubreuil, J.D., & de Souza, E.L. (2019). A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. *Microbial Pathogenesis, 130*, 259–270. https://doi.org/10.1016/j.micpath.2019.03.025

[34] Luchini, C., Stubbs, B., Solmi, M., & Veronese, N. (2017). Assessing the quality of studies in meta-analyses: Advantages and limitations of the Newcastle Ottawa Scale. *World Journal of Meta-Analysis, 5*(4), 80–84. https://doi.org/10.13105/wjma.v5.i4.80

[35] Ma, H., Feng, C., & Mills, J.V. (1970). *Ying-yai Sheng-lan: 'The Overall Survey of the Ocean's Shores'* (1433). p. 92. ISBN 9780521010320.

[36] Marzaimi, N., & Aizat, W.M. (2019). Chapter 17 - Current review on mangosteen usages in antiinflammation and other related disorders. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (2nd ed., pp. 273–289). https://doi.org/10.1016/B978-0-12-813820-5.00017-9

[37] Micek, S.T., Wunderink, R.G., Kollef, M.H., Chen, C., Rello, J., Chastre, J., *et al.* (2015, December 1). An international multicenter retrospective study of *Pseudomonas aeruginosa* nosocomial pneumonia: Impact of multidrug resistance. *Critical Care*. https://doi.org/10.1186/s13054-015-0926-5

[38] Morton, J.F. (1987). *Fruits of warm climates*. Miami, Florida, USA: Center for New Crops & Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, 301–304.

[39] Moser, P. (2020). Out of control? Managing baseline variability in experimental studies with control groups. In A. Bespalov, M. Michel, & T. Steckler (Eds.), *Good Research Practice in Non-Clinical Pharmacology and Biomedicine* (Vol. 257).

[40] Nassar, M., Hazzah, W., & Bakr, W. (2019). Evaluation of antibiotic susceptibility test results: How guilty a laboratory could be? *Journal of Egypt Public Health Association, 94*, 4. https://doi.org/10.1186/s42506-018-0006-1

[41] Negi, P.S., Jayaprakasha, G.K., & Jena, B.S. (2008). Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against foodborne pathogens and spoilage bacteria. *LWT - Food Science and Technology, 41*, 1857–1861. https://doi.org/10.1016/j.lwt.2008.01.015

[42] Nimer, N. (2022). Nosocomial infection and antibiotic-resistant threat in the Middle East. *Infectious Diseases and Therapy, 15*, 631–639. https://doi.org/10.2147/IDR.S351755

[43] Niranjan, M., Vaishnav, V., & Mankar, P. (2020). In-vitro analysis of antioxidant and antimicrobial properties of *Garcinia mangostana* L. (pericarp) and *Clitoria ternatea* (flower). *The Pharma Innovation Journal, 9*(3), 468–472. Retrieved from https://www.researchgate.net/publication/351226781

[44] Obolskiy, D., Pischel, I., Siriwatanametanon, N., & Heinrich, M. (2009, August). *Garcinia mangostana* L.: A phytochemical and pharmacological review. *Phytotherapy Research, 23*(8), 1047–1065. https://doi.org/10.1002/ptr.2730

[45] Page, M., McKenzie, J., Bossuyt, P., Boutron, I., Hoffmann, T., Mulrow, C., Lalu, M. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ, 372*, n71. https://doi.org/10.1136/bmj.n71

[46] Palawakong, C., Sophanodora, P., Toivonen, P., & Delaquis, P. (2013). Optimized extraction and characterization of antimicrobial phenolic compounds from mangosteen (*Garcinia mangostana* L.) cultivation and processing waste. *Journal of the Science of Food and Agriculture, 93*(15). https://doi.org/10.1002/jsfa.6277

[47] Pohan, D., & Rahmawati, F. (2022). The effect of mangosteen pericarp (*Garcinia mangostana* Linn) extract on inhibiting the growth of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. *International Journal*

*of Research in Pharmacy and Pharmaceutical Sciences*, 29–38. Retrieved from http://repository.uki.ac.id/7816/

[48] Russell, A.D. (2004). Chapter 3: Factors influencing the efficacy of antimicrobial agents. In *Russell, Hugo & Ayliffe's Principles and Practice of Disinfection, Preservation & Sterilization* (4th ed., pp. 98–127). Blackwell Publishing Ltd. https://doi.org/10.1002/9780470755884.ch3

[49] Salam, M., Al-Amin, M., Pawar, J., Akhter, N., & Lucy, I. (2023). Conventional methods and future trends in antimicrobial susceptibility testing. *Saudi Journal of Biological Sciences, 30*, 103582. https://doi.org/10.1016/j.sjbs.2023.103582

[50] Schmidt, A., Bénard, S., & Cyr, S. (2015). Hospital cost of Staphylococcal infection after cardiothoracic or orthopedic operations in France: A retrospective database analysis. *Surgical Infections, 16*(4), 428–435. https://doi.org/10.1089/sur.2014.045

[51] Sodata, P., Juntavee, A., Juntavee, N., & Peerapattana, J. (2017). Optimization of adhesive pastes for dental caries prevention. *AAPS PharmSciTech, 18*, 3087–3096. https://doi.org/10.1208/s12249-017-0916-4

[52] Spratt, M., & Lane, K. (2022). Navigating environmental transitions: The role of phenotypic variation in bacterial responses. *mBio, 13*(6), e02212-22. https://doi.org/10.1128/mbio.02212-22

[53] Suhartati, R., Apriyani, F., Khusnul, V., Virgianti, D., & Fathurohman, M. (2019). Antimicrobial activity test of mangosteen leaves ethanol extract (*Garcinia mangostana* Linn) against *Pseudomonas aeruginosa* bacteria. *Journal of Physics: Conference Series, 1179*. https://doi.org/10.1088/1742-6596/1179/1/012167

[54] Sultan, O., Kantilal, H., Khoo, S., Davamani, A., Eusufzai, S., Rashid, F., Alam, M. (2022). The potential of α-mangostin from *Garcinia mangostana* as an effective antimicrobial agent: A systematic review and meta-analysis. *Antibiotics, 11*(6), 717. https://doi.org/10.3390/antibiotics11060717

[55] Sundaram, B., Gopalakrishnan, C., Subramanian, S., Shankaranarayanan, D., & Kameswaran, L. (1983). Antimicrobial activities of *Garcinia mangostana*. *Planta Medica, 48*(5), 59–60. https://doi.org/10.1055/s-2007-969882

[56] Tadtong, S., Vorarat, S., Viriyaroj, A., Nimkulrat, S., & Suksamrarn, S. (2009). Antityrosinase and antibacterial activities of mangosteen pericarp extract. *Journal of Health Research, 23*(2), 99–102. Retrieved from https://www.researchgate.net/publication/242617097

[57] Taher, M., Susanti, D., Rezali, M.F., Zohri, F.S.A., Ichwan, S.J.A., Alkhamaiseh, S.I., & Ahmad, F. (2012). Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk.f. *Asian Pacific Journal of Tropical Medicine, 5*, 136–141. https://doi.org/10.1016/S1995-7645(12)60023-6

[58] Van Spijk, J.N., Schmitt, S., & Schoster, A. (2017). Infections caused by multidrug-resistant bacteria in an equine hospital (2012–2015). *Equine Veterinary Education, 31*(12), 653–658. https://doi.org/10.1111/eve.12837

[59] Winslow, R., Skripsky, S., & Kelly, S. (2016). Not just for citations: Assessing Zotero while reassessing research. In B.J. D'Angelo, S. Jamieson, B. Maid, & J.R. Walker (Eds.), *Information Literacy: Research and Collaboration across Disciplines* (pp. 287–304). https://doi.org/10.37514/PER-B.2016.0834.1.3

[60] Yusuf, B., Abraha, B., Salih, K., Abdurahman, A., Alemu, S., Wondimu, A., & Bayu, Y. (2022). In vitro antibacterial evaluation of four selected medicinal plants against *Staphylococcus aureus* isolated from bovine mastitis in Mieso District, West Hararghe Zone, Oromia Regional State, Ethiopia. *The Open Microbiology Journal, 16*, 128–134. https://doi.org/10.2174/1874285816161421128 4

[61] Zhang, L., Lin, I., & Ye, W. (2022). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine, 13*(20). https://doi.org/10.1186/s13020-018-0177-x

[62] Zhen, X., Lundborg, C., Zha, M., Sun, X., Li, Y., Hu, X., & Dong, H. (2020). Clinical and economic impact of methicillin-resistant *Staphylococcus aureus*: A multicenter study in China. *Scientific Reports, 10*, 11838. https://doi.org/10.1038/s41598-020-60825-6