Ethanolic Extract of *Garcinia mangostana* L. pericarp as Preservative in Antacid Suspension

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ABSTRACT

**Objective.** The study was conducted to determine the preservative activity of ethanolic extract of mangosteen (*Garcinia mangostana* L.) pericarp and its compatibility in an antacid suspension.

**Methods.** The extract was subjected to phytochemical screening and was used as preservative in a formulated antacid suspension. Compatibility with the active pharmaceutical ingredient (API) and excipients were analyzed using fourier transform-infrared spectroscopy. Preservative activity of the formulation against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* was assessed using the United States Pharmacopoeia (USP) antimicrobial effectiveness test, with methylparaben as positive control and suspension without preservative as negative control.

**Results.** The extract exhibited pharmaceutical compatibility with API and excipients. The formulation revealed comparable reduction in microbial count of *E. coli*, *S. aureus*, and *P. aeruginosa* with positive control at Day 14 (p=0.916, 0.624, 0.335). At Day 28, comparable activity with positive control was only observed against *E. coli* and *S. aureus* (p=0.999, 0.854). However, it displayed significant increase in activity against *P. aeruginosa* (p=0.010) at Day 28. These activities may be attributed to glycosides and reducing substances present in the extract.

**Conclusion.** The ethanolic extract from *Garcinia mangostana* L. pericarp acted as a preservative in the formulation of an antacid suspension. It conformed to the USP criteria for antimicrobial effectiveness test on bacteria.

**Key Words:** antimicrobial test, *Garcinia mangostana*, preservative, suspension

INTRODUCTION

Foodborne diseases and microbial spoilage are persistent concerns in both developing and developed countries, mainly driven by advances in production techniques, climate change, and widespread trade of food and drug products.1 To mitigate these concerns, preservatives are added to prevent decomposition or any undesirable effects in the finished products.2,3 The United States Pharmacopoeia (USP) and National Formulary (NF) define preservatives as substances added to non-sterile dosage forms to protect them from microbial growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process.4 They act as antimicrobial chemicals in cosmetics, pharmaceuticals, foods, and industrial products to protect the consumer against infections and the product itself against microbial spoilage.5,6 Synthetic preservatives are commonly used for these purposes, however, they are highly discouraged due to their ill effects in humans.7,8
such as their carcinogenic risks. Thus, there is a need to search for alternative preservatives that are effective and safer, such as natural preservatives from plants, to control biodeterioration and biodegradation in food, cosmetics and pharmaceutical products.

Plants have been widely used for treating and preventing diseases. They contain a wide variety of secondary metabolites with high structural diversity correlated with their function. Different studies revealed that extracts from plants have the ability to inhibit the growth of microorganisms. Therefore, plant extracts can be utilized for the discovery of new bioactive compounds that could be used in the development of food, cosmetic and pharmaceutical preservatives, in place of synthetic ones.

The Philippines has numerous plants which can be used as potential sources of preservatives. One of the plants known to possess pharmacological activities is mangosteen (Garcinia mangostana L.). Various researches revealed the antimicrobial activity of G. mangostana fruit extracts in different microorganisms. However, the use of these extracts as preservatives has not been studied. Thus, this study aimed to investigate the ethanolic extract of G. mangostana pericarp (EEGMP) as a potential preservative in pharmaceutical liquid preparation, specifically in antacid suspension.

MATERIALS AND METHODS

Plant collection and preparation of extract

Mature mangosteen fruits, with purple-colored pericarp, were bought from Paco Public Market, Manila, Philippines. A specimen was submitted to the National Museum of the Philippines-Botany Division and was identified as Garcinia mangostana L. (voucher number: 170-2014). The extraction of the sample was based on the method of Arollado et al with minor modifications. The samples were washed thoroughly and the pericarps were obtained, cut into small pieces, air-dried and milled into powder. The powdered sample was macerated in 95% ethanol for 72 hours, with intermittent shaking. The extract was then filtered and concentrated using rotary evaporator (MRC ROVA-2L). The concentrated extract was evaporated to dryness to obtain the EEGMP. It was stored in an amber bottle container at 4°C.

Table 1. Formulation of oral antacid suspension

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition (%w/v)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum hydroxide</td>
<td>6</td>
<td>Active pharmaceutical ingredient (API)</td>
</tr>
<tr>
<td>Sorbitol solution</td>
<td>40</td>
<td>Stabilizing agent, humectant</td>
</tr>
<tr>
<td>Syrup</td>
<td>10</td>
<td>Filler/Flavor enhancer</td>
</tr>
<tr>
<td>Glycerine</td>
<td>20</td>
<td>Co-solvent, viscosity enhancer</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>0.5</td>
<td>Suspending/gelling agent</td>
</tr>
<tr>
<td>Methylparaben or EEGMP or none</td>
<td>0.2</td>
<td>Antimicrobial preservative</td>
</tr>
<tr>
<td>Strawberry flavor</td>
<td>4</td>
<td>Flavoring agent</td>
</tr>
<tr>
<td>Purified water</td>
<td>qs</td>
<td>Diluent</td>
</tr>
</tbody>
</table>

qs - quantum sufficiat; EEGMP - ethanolic extract of Garcinia mangostana pericarp

Phytochemical screening

The EEGMP was tested for the presence of tannins, glycosides, reducing substances, plant acids, saponins, and flavonoids as described in the methods of Camposano et al. The results were recorded as (+) for the presence and (-) for the absence of the specific secondary metabolite. The pH of the extract was also determined using a pH meter (Trans BP3001).

Preparation of antacid suspension

Oral antacid suspension, with aluminum hydroxide as active pharmaceutical ingredient (API) and EEGMP as preservative, was prepared using the formulation described in Table 1. Positive control suspension was also prepared with methylparaben as preservative, while negative control suspension was prepared without any preservative. Good compounding practices (i.e., use of sterile glassware and clean work area) were observed to limit the suspension's microbial load.

Compatibility studies

The interaction of the API and each excipient with EEGMP was determined using fourier transform infrared (FTIR) spectroscopy. The FTIR spectra of each excipient, API, EEGMP, and their mixtures were determined using Thermo Nicolet 6700 FTIR spectrometer in the range of 4000 – 500 cm⁻¹. Each excipient or API and EEGMP was mixed in a 1:1 weight ratio and kept in refrigeration for seven days. The powdered samples were then analyzed by potassium bromide (KBr) pellet method while liquid samples were evaluated as films placed on KBr plates.

Antimicrobial effectiveness test

The antimicrobial effectiveness test for bacteria described in the USP was used to determine the preservative activity of the EEGMP on isolated cultures of Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853). The test organisms were bought from the Department of Medical Microbiology of the College of Public Health, University of the Philippines Manila. The microorganisms were diluted in buffered sodium chloride-peptone solution pH 7.0 and turbidities were compared to 0.5 McFarland standard equivalent to a
count of $1 \times 10^8$ colony-forming units (cfu)/mL. A 0.1-mL aliquot of each standardized inoculum was transferred to the formulated suspensions and mixed thoroughly. The resulting solutions were incubated at 22.5 ± 2.5°C. A 0.1-mL aliquot of each solution was sampled at Days 0, 14 and 28, spread over the surface of the soybean-casein digest agar medium, and incubated at 32.5 ± 2.5°C. The number of cfu present in each solution was determined and the change in $\log_{10}$ values of the concentration in cfu/mL was computed at the applicable sampling intervals. These changes were expressed in terms of log reductions. Suspensions without preservative and with methylparaben as preservative were also prepared, and served as negative and positive controls, respectively. An effective preservative should have no bacterial increase from the initial calculated count at Days 14 and 28, based on USP criteria for product category 4 (antacids made with an aqueous base).

**Statistical analyses**

The data gathered were recorded as mean (standard error of mean, SE), with all measurements done in triplicate. The computed log reduction values were used to determine the statistical differences among the formulated suspension, positive control, and negative control using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test of SPSS 23.0 software. Probability values, $p<0.05$, were considered statistically significant.

**RESULTS AND DISCUSSION**

Preservatives are either natural or man-made compounds added to foods, medicines and other products, to prevent degradation from microorganisms and oxidative products. These compounds protect the consumers against food-borne illnesses and sensitivity reactions that may result from such degradation. In this study, an antacid suspension was formulated using EEGMP as preservative. The compatibility of EEGMP with the API and excipients were explored, as well as its preservative activity against *E. coli*, *S. aureus*, and *P. aeruginosa*.

Compatibility study was done to determine the interaction of EEGMP with the API or excipients used in the formulation. The FTIR spectra of the components of the formulated antacid suspension and their mixtures were analyzed for their characteristic absorption bands. EEGMP...
showed characteristic bands at 3418 cm\(^{-1}\) (O-H stretch), 2921 cm\(^{-1}\) (C-H stretch), 1643 – 1458 cm\(^{-1}\) (aromatic C=C stretch), 1281 cm\(^{-1}\) (C-O stretch) and 1078 - 585 cm\(^{-1}\) (aromatic C-H bend) (Figure 1A). This was found to be in agreement with the reported spectrum of anthocyanins, occurring mainly as glycosides of anthocyanidins, found in the *G. mangostana* pericarp.\(^{17}\) The spectrum EEGMP-aluminum hydroxide mixture (Figure 1B) revealed the presence of signature peaks of EEGMP at 3651 – 3458, 2964 – 2923, 1641 – 1458, 1278 and 1029 – 561 cm\(^{-1}\).

The FTIR spectrum of syrup displayed distinct absorption bands at 3396 and 3324 cm\(^{-1}\) (O-H stretch), 2966 – 2882 cm\(^{-1}\) (C-H stretch), 1633 cm\(^{-1}\) (H\(_2\)O vibrational bend), 1431 – 1333 cm\(^{-1}\) (O-H bend), 1210 cm\(^{-1}\) (C-H bend), 1155 cm\(^{-1}\) (C-C bend in plane), 1109 –1016 cm\(^{-1}\) (C-O-C bend) and 915 – 721 cm\(^{-1}\) (absorption for α-D-glucopyranoside), which was congruent with the reported fingerprint regions of glucose.\(^{18,19}\) The characteristic peaks of EEGMP at around 1642 – 1457 and 1282 cm\(^{-1}\) (Figure 2A) and glucose at around 3398, 2966 – 2932, 1377, 1156, 1015 and 914 – 715 cm\(^{-1}\) (Figure 2B) were observed for the mixture of EEGMP and glucose (Figure 2C).

The FTIR spectrum of the glycerine sample revealed similar peaks at 3378 cm\(^{-1}\) (O-H stretch), 2939 and 2864 cm\(^{-1}\) (C-H stretch), 1456 – 1412 cm\(^{-1}\) (O-H bend) and 1110 – 1042 cm\(^{-1}\) (C-O stretch) described in the study of Indran et al.\(^{20}\) The respective signature peaks of EEGMP at around 1643 – 1457, 1283 and 992 – 581 cm\(^{-1}\) (Figure 3A), and peaks at around 3388, 2933 – 2886, 1106 –1044 cm\(^{-1}\) of glycerine (Figure 3B) were present in the EEGMP-glycerine mixture (Figure 3C).

Strong absorption bands at 3394 cm\(^{-1}\) (O-H stretch), 2982 – 2875 cm\(^{-1}\) (C-H stretch), 1471 – 1253 cm\(^{-1}\) (C-H bend) and 1093 – 1002 cm\(^{-1}\) (C-O stretch) appeared in the FTIR spectra of the sorbitol sample, which were in agreement with the reported data.\(^{21}\) The main peaks of EEGMP at around 1642, 1615, 1455 and 1284 cm\(^{-1}\) (Figure 4A) and sorbitol at around 3393, 2932, 1081 and 1047 cm\(^{-1}\) (Figure 4B) were found in the IR spectra of the EEGMP-sorbitol mixture (Figure 4C).

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**Figure 3.** IR spectra of (A) ethanolic extract of *G. mangostana* pericarp, (B) glycerine and (C) mixture of ethanolic extract of *G. mangostana* pericarp and glycerine.

**Figure 4.** IR spectra of (A) ethanolic extract of *G. mangostana* pericarp, (B) sorbitol and (C) mixture of ethanolic extract of *G. mangostana* pericarp and sorbitol.
In the case of xanthan gum, the FTIR spectrum exhibited sharp peaks at 3424 cm\(^{-1}\) (O-H stretch), 2923 cm\(^{-1}\) (C-H stretch), 1726 cm\(^{-1}\) (C=O stretch of esters, acids, aldehydes and ketones), 1620 cm\(^{-1}\) (C=O stretch of enols), 1455 – 1380 cm\(^{-1}\) (C-H bend) and 1091 cm\(^{-1}\) (C-O stretch), analogous to the spectrum of xanthan gum in previous literature.\(^{22}\)

Evaluation of the spectrum of EEGMP (Figure 5A) and xanthan gum (Figure 5B) revealed their characteristic peaks at 3418, 2920, 1643 – 1586, 1458, 1281 and 1078 – 1047 cm\(^{-1}\) in the EEGMP-xanthan gum mixture (Figure 5C).

For commercial strawberry flavor, the IR spectrum showed characteristic absorption bands at 3380 cm\(^{-1}\) (O-H stretch due to hydrogen bonding), 2973 – 2880 cm\(^{-1}\) (C-H stretch), 1647 cm\(^{-1}\) (bending vibration of H\(_2\)O), 1137 cm\(^{-1}\) (C-C bend in plane), which accounted for the propylene glycol and H\(_2\)O present in the strawberry flavor.\(^{23}\) The important peaks of EEGMP at around 3415, 1643 – 1458, 1283 and 1078 – 587 cm\(^{-1}\) (Figure 6A) and strawberry flavor at around 2969 – 2872 and 1133 cm\(^{-1}\) (Figure 6B) were well-preserved in the spectra of EEGMP-strawberry flavor (Figure 6C).

Overall assessment of the FTIR analyses indicated that no pharmaceutical incompatibilities occurred in the mixtures of EEGMP with the API or excipients used in the formulation.

To evaluate the EEGMP’s preservative activity in the formulated antacid suspension, antimicrobial effectiveness test was conducted. The formulated antacid suspension using EEGMP as preservative showed reduction in the microbial count of \(E.\) coli, \(S.\) aureus, and \(P.\) aeruginosa (Table 2) from the initial count to 28 days.

Comparable log reduction values were observed in the EEGMP suspension and positive control against \(E.\) coli at Day 14 (\(p=0.916\) and Day 28 (\(p=0.999\)). Comparison of negative control with EEGMP suspension and positive control revealed statistically different log reduction values at Day 14 (\(p=0.043\) and 0.018, respectively) and Day 28 \(p=0.040\) and 0.046, respectively). Log reduction values of EEGMP suspension against \(S.\) aureus were also comparable to that of positive control at Day 14 (\(p=0.628\)) and 28 (\(p=0.854\)). At Day 28, the negative control displayed no
Garcinia mangostana as preservative

Table 2. Antimicrobial effectiveness test of the formulated antacid suspension with ethanolic extract of Garcinia mangostana pericarp (EEGMP) or methylparaben as preservative against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Sample Formulation</th>
<th>Microbial count x 10^6, cfu/mL</th>
<th>Log reduction, log cfu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEGMP</td>
<td>9.05 (2.90)</td>
<td>4.34 (0.58)</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>13.40 (1.30)</td>
<td>5.91 (0.96)</td>
</tr>
<tr>
<td>Without preservative</td>
<td>4.56 (0.51)</td>
<td>7.91 (2.97)</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEGMP</td>
<td>6.29 (0.84)</td>
<td>3.81 (0.40)</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>7.17 (2.12)</td>
<td>5.28 (0.70)</td>
</tr>
<tr>
<td>Without preservative</td>
<td>5.03 (1.12)</td>
<td>8.99 (2.30)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEGMP</td>
<td>4.83 (0.28)</td>
<td>0.83 (0.18)</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>6.77 (0.83)</td>
<td>5.55 (1.31)</td>
</tr>
<tr>
<td>Without preservative</td>
<td>5.03 (1.12)</td>
<td>5.19 (1.63)</td>
</tr>
</tbody>
</table>

Methylparaben was used as standard preservative (positive control) while suspension without preservative served as negative control. The values were reported as mean (SE) of triplicate measurements. Significant difference of the formulation (p<0.05) with the positive control was marked with a while negative control was marked with b.

Table 3. Phytochemical screening of ethanolic extract of Garcinia mangostana pericarp (EEGMP)

<table>
<thead>
<tr>
<th>Test</th>
<th>EEGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4</td>
</tr>
<tr>
<td>Tannins</td>
<td>(-)</td>
</tr>
<tr>
<td>Glycosides</td>
<td>(+)</td>
</tr>
<tr>
<td>Reducing substances</td>
<td>(+)</td>
</tr>
<tr>
<td>Plant acids</td>
<td>(-)</td>
</tr>
<tr>
<td>Saponins</td>
<td>(-)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Experiment was done in triplicate. Symbols (+) and (-) signify the presence and absence of the secondary metabolite, respectively.

CONCLUSIONS

The EEGMP did not exhibit any pharmaceutical incompatibility with the API and excipients used in the formulation, as shown by the results of FTIR analyses. Moreover, the formulated antacid suspension with EEGMP as preservative inhibited the growth of E. coli, S. aureus, and P. aeruginosa up to 28 days, which conformed to the criteria of the USP antimicrobial effectiveness test for antacids made with an aqueous base. The glycosides and reducing substances present in the extract may be the compounds responsible for this bioactivity. Hence, the results of the study can be used as a starting point for the development of other pharmaceutical preparations utilizing EEGMP as preservative.

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Statement of Authorship

All authors approved the final version submitted.

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REFERENCES


