INTRODUCTION
Toothpaste is a gel or paste formulation product and is used to clean and maintain oral hygiene with the aid of toothbrush. It is a common product used by the community for dental care. Although it is recommended by most dentist to brush the teeth twice daily and it is highly effective for plaque removal but it is not possible for bacterial infection. By removing the plaque it will helps in decrease the chances of periodontal inflammation which mainly caused by bacteria or oral flora. To overcome this problem, it is recommended for the patient to use toothpaste with better antibacterial activity. Most of the toothpaste available in the market nowadays contain two types of ingredient which are the active and non-active toothpaste ingredient or the excipient. One of the active toothpaste ingredients is abrasive which helps in removing the plaque. It constitutes at least 50% from the total toothpaste ingredients is abrasive which helps in removing the plaque. It constitutes at least 50% from the total

Formulation, Evaluation and Antibacterial Properties of Novel Polyherbal Toothpaste for Oral Care

Mahendran Sekar*, Noor Jasmin Shamsul Ariffin

Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh - 30450, Malaysia.

ABSTRACT
The demand for herbal based product such as toothpaste is high these days. Consumers believed by using herbal based toothpaste are safe, effective and less toxicity because less and only safe chemical used as compared to the synthetically produced toothpaste. Therefore, this study was aimed to formulate and evaluate new polyherbal toothpaste which containing herbal extracts that available in Malaysia to treat periodontal problem. The polyherbal toothpaste was formulated using three herbal extracts namely banana peel (Musa acuminata), guava leaves (Psidium guajava) and orange peel (Citrus arranticum) and tested against Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa with different concentrations varying from 100 mg/mL, 250 mg/mL and 500 mg/mL. The significant inhibition has seen against Staphylococcus aureus (12-18 mm) followed by Bacillus cereus (11-15 mm) and there is no inhibition for both Escherichia coli and Pseudomonas aeruginosa. The formulated toothpaste was also evaluated with the standard physiochemical parameters along with the antimicrobial activity. The formulated toothpaste showed potent inhibition against gram positive bacteria but not against gram negative bacteria. Thereby, it opens a window for future study to enhance the ability of the toothpaste and to prove the efficacy and safety of the formulated toothpaste.

**Keywords**: Polyherbal toothpaste, antibacterial activity, Musa acuminata, Psidium guajava, Citrus arranticum

INTRODUCTION
Toothpaste is a gel or paste formulation product and is used to clean and maintain oral hygiene with the aid of toothbrush. It is a common product used by the community for dental care. Although it is recommended by most dentist to brush the teeth twice daily and it is highly effective for plaque removal but it is not possible for bacterial infection. By removing the plaque it will helps in decrease the chances of periodontal inflammation which mainly caused by bacteria or oral flora. To overcome this problem, it is recommended for the patient to use toothpaste with better antibacterial activity. Most of the toothpaste available in the market nowadays contain two types of ingredient which are the active and non-active toothpaste ingredient or the excipient. One of the active toothpaste ingredients is abrasive which helps in removing the plaque. It constitutes at least 50% from the total preparation of the toothpaste. It really helps in minimized periodontal disease. The commonly used abrasives are sodium bicarbonate, calcium carbonate and aluminium hydroxide. Whitening agents helps to remove stain on the teeth but the effect is temporary. The commonly used whitening agents for toothpastes in the market are peroxide and bleach. Fluoride and its derivatives are used to strengthen the enamel and prevent cavities. The most common used fluorides are sodium fluoride, sodium monofluorophosphate, olaflur and stannous fluoride. Between them, stannous fluoride shows effective controls of gingivitis. The problem now is most people do not know the long term consequence of using the commercial toothpastes. This is because the marketed toothpastes contain substance which consider as unhealthy and could harm the body in the future. Recently there is some issues arise on the harmful effect of fluoride when being used for a longer period of time. The bleach and peroxide used as whitening agent in the toothpaste is consider as hazardous as they may cause irritation to the mouth and skin in small amount and chemical burn in large amount. The flavouring agents used are synthetically and chemically produced and commonly derived from petrochemicals. After studying the drawbacks of commercial toothpastes, people are now more inclined toward the use of herbal formulations. Herbal toothpaste does not contain the artificial colors, flavors or fluoride that many, when compare the contents of artificial products. The antimicrobial activity of Musa acuminata as known as banana, Malus domestica known as apple and Citrus arranticum also known as orange against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Porphyromonas gingivalis have been proven recently. Psidium guajava or known as guava and its leaf exhibits antimicrobial activity against dental pathogens. This study is selected to overcome the problems associated with the usage of unhealthy ingredient used in commercial toothpastes which widely used around the world. In addition, to reduce the cases of periodontal cases which mainly caused by dental pathogen. Hence, in
the present study we are interested to formulate and evaluate new polyherbal toothpaste and to study its antimicrobial potency.

**MATERIALS AND METHODS**

**Plant collection and indentification**

The fresh banana fruits (Musa acuminate) and orange fruits (Citrus arranticum) were purchased from local market, Ipoh and identified. The fresh guava fruits leaves (Psidium guajava) were collected in Ipoh and Identified.

**Extraction**

The peels of banana and orange fruits were removed off using a peeler and shade dried at room temperature for a week. The dried peels and guava fruit leaves were coarsely powdered and then defatted with petroleum ether. The defatted materials were separately subjected to extraction by cold maceration separately by agitation using 70% methanol for 7 days. The marc was squeezed out and filtered off. The combined filtrates were concentrated separately by allowing it to be evaporated from the petri dish. The concentrated extracts were stored in air-tight containers in a refrigerator at 4 °C until further use.

**Formulation of polyherbal toothpaste**

The extracts were used to formulate toothpaste. The composition of the polyherbal toothpaste were stated in Table 1. Hydroxypropylmethylcellulose (HPMC) was triturated together with methyl paraben and propyl paraben. Glycerin was added and mixed well by triturating them all together. Calcium carbonate was added and mixed well. Then, all the extracts were added and again triturate to mix the ingredients. Water was added until the desired consistency achieved.

**Evaluation of the formulated toothpaste**

**Organoleptic evaluation**

Organoleptic evaluation (colour, taste, odour) were done by sensory and visual inspection.

**Moisture content**

10 g of toothpaste was weighted and dried it in the oven at 105 °C then it was cooled. The loss of weight was recorded as percentage moisture content and calculated by the given formula.

\[
\% \text{ moisture} = \frac{\text{Original sample weight} - \text{dry sample weight}}{\text{Original sample weight}} \times 100
\]

**Foaming character**

For the evaluation of foaming character 5 g of sample was soaked with 10 mL of distilled water in 100 mL of beaker and closed with paraffin for 30 min to ensure the toothpaste disperse in the water. After 30 min, the mixture was stirred and transfer the slurry into a 250 mL measuring cylinder a make sure there is no foam formed. Rinsed the excess slurry in the beaker with 5-6 mL of distilled water and then again transferred into the measuring cylinder slowly. To the measuring cylinder, the volume was marked upto 50 mL volume. The measuring cylinder was heated in water bath and maintain the temperature at 30 °C. The slurry was stirred to make a uniform suspension. Then, the measuring cylinder was closed with paraffilm and the measuring cylinder was shaken with 12 complete cycles and allowed it to stand for 5 min. After 5 min, the volume of foam with water (V₁) and volume of water only (V₂) were measured.

Foaming power = V₁ - V₂

V₁ - volume in mL of foam with water
V₂ - volume in mL of water only

**Determination of pH**

5 g of sample was diluted with 45 mL of distilled water in a 150 mL beaker. This was stirred well to make a thorough suspension. The pH of the sample with pH meter within 5 min was measured.

**Storage stability**

For the evaluation of the storage stability, the toothpaste was filled in a toothpaste tube for storage and stored for 45 days at each of 5 °C, room temperature and 40 °C. The tube was then cut through and checked whether the liquid component was separated from the toothpaste.
Table 4: Zone of inhibition for antimicrobial activity testing.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Gram Positive</th>
<th>Gram Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>Zone of inhibition (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>250</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>500</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Positive control (Ciprofloxacin 5 µg)</td>
<td>31</td>
<td>26</td>
</tr>
</tbody>
</table>

Antimicrobial screening
Test microorganisms
Panels of common pathogenic microorganisms were used in the study, which includes two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) and two gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa).

Disc-diffusion method
The antibacterial activity of the toothpaste was evaluated using disc-diffusion method. A suspension of the tested microorganisms were uniformly swabbed on agar plates using sterile cotton swabs. Sterile blank discs were individually impregnated to the different concentrations of formulated paste (100, 250 and 500 mg/mL) were placed onto the inoculated agar plates. The plates were inverted and incubated at 37 °C for 24 h. The antibacterial activity was measured by measuring diameter of the resulting zone of inhibition against the tested organisms. The test for positive control and negative control was performed in duplicate.

RESULTS AND DISCUSSION
The percentage yield of methanolic extract of Musa acuminata, Psidium guajava and Citrus arranticae were shown in Table 2.

Evaluation of the formulated toothpaste
The formulated toothpaste was evaluated with the standard evaluation parameters for toothpaste. The results of the evaluation test were shown in Table 3 below:

Antimicrobial activity
The main objective of this study is to formulate and evaluate the antimicrobial activity of the prepared herbal toothpaste. Various extracts have been used in formulating the toothpaste such as orange peel extract, banana peel extract and guava leaves extract which are believed to have antibacterial properties. The toothpaste was evaluated for antimicrobial activity as given in the table above. The positive result was shown for Gram-positive bacteria, Bacillus cereus and Staphylococcus aureus at all level of dilution from 100 mg/mL up to 500 mg/mL and the zone of inhibition increased with increasing of concentration, whereas there is no zone of inhibition for Gram-negative bacteria, Pseudomonas aeruginosa and Escherichia coli. The most significant result can be seen against Staphylococcus aureus (12-18 mm) followed by Bacillus cereus (11-15 mm) and there is no inhibition against Escherichia coli and Pseudomonas aeruginosa. The zone of inhibition for antimicrobial activity testing have been shown in Table 4. A study has been carried out on antimicrobial property of orange peel extract. The result shown for methanolic extract was positive against S. aureus and E. coli. For banana peel extract, the antimicrobial activity has been established. The methanolic extract shows positive result against S. aureus, Bacillus sp, P. aeruginosa and E. coli and the zone of inhibition increased with increasing of the extracts concentration. The same goes to methanolic extract of guava leaves where the antimicrobial property has been known against S. aureus and E. coli. It is said to have antimicrobial activity due to flavonoid present in the leaves. From the studies as mentioned above, the results for Gram-positive bacteria has been well correlated with our present study results. The activity may be due to the presence of various phytoconstituents.

CONCLUSION
Herbal-based products have growing demand in the market. People believed using product made up of natural sources ingredient are safer with fewer side effects than the synthetic one. It is a good attempt to establish such herbal toothpaste containing orange peel extract, banana peel extract and guava leaves extract which helps in reducing bacterial growth in the mouth. Further study are warranted to improve the stability of the formulation, prove the efficacy and safety of the formulated toothpaste and to improve the antimicrobial activity of the toothpaste.

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CONFLICT OF INTEREST
All the authors declared that there is no conflict of interest.

REFERENCES


