

FORMULATION, EVALUATION AND ANTIMICROBIAL PROPERTIES OF POLYHERBAL TOOTHPASTE

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ABSTRACT

Objective: The present study was aimed to formulate and evaluate polyherbal toothpaste using commonly available medicinal plants in Malaysia in order to treat oral problems.

Methods: Polyherbal toothpaste containing methanolic extract of pomegranate, lemon and mango peels was formulated and tested for antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* with different concentrations of toothpaste were used (100, 250 and 500 mg/ml).

Results: Among all the tested bacteria used *Staphylococcus aureus* was found to be most sensitive to the formulated toothpaste as seen by zone of inhibition (10-15 mm) followed by *Escherichia coli* (9-12 mm), *Bacillus cereus* (7-12 mm) and *Pseudomonas aeruginosa* (9-11). The formulated polyherbal toothpaste was successfully evaluated using different standard parameters to ensure its quality and physicochemical properties.

Conclusion: The results showed that the formulated polyherbal toothpaste is promising antimicrobial effects against both gram positive and gram negative organisms. It may be safer compared to fully synthetic toothpaste. Further studies are warranted to prove safety and efficacy of the formulated polyherbal toothpaste.

Keywords: Herbal toothpaste, *Punica granatum*, *Citrus limon*, *Mangifera indica*, Antibacterial activity

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INTRODUCTION

Herbal medicines are referred to the use any part of the plants for healing and treating diseases purposes. Herbal medicines have been used widely throughout human history and according to World Health Organization (WHO) about 80% of the human population used herbal medicine for primary healthcare [1]. In addition, more than 35,000 plant species have been reported to be used in various human cultures around the world for medical purposes [2]. Some of them are potent antimicrobial, antidiabetic, antiviral, anticancer and antifungal.

Oral hygiene refers to the cleaning of mouth by brushing and flossing to prevent oral infections and gum diseases. In another word, oral hygiene is the practice of keeping the mouth and teeth clean to prevent dental problems, most commonly, dental cavities, gingivitis, periodontal (gum) diseases and bad breath [3]. One of the purposes to maintain oral hygiene is to prevent oral infections. The oral infections are caused by plaque forming bacteria and yeast which reside in the oral cavity such as *Actinomyces*, *Actinobacillus*, *Streptococcus* and *Candida* species [4]. Herbal medicines with antimicrobial properties can be added in the formulation of toothpaste because they help in prevent oral infections and diseases by fighting with those oral bacteria flora.

Punica granatum or its common name pomegranate is a well-known medicinal plants for its antimicrobial properties. Elahé *et al.*, 2014 [5] reported that *Punica granatum* water extract had significant antibacterial properties against 5 oral bacteria and prevented orthodontic wire bacterial biofilm formation. *Citrus limon* or lemon is a citrus fruit commonly available in the market. Prabuseenivasan *et al.* (2006) [6] reported that the lemon oil was showed potent antibacterial activity. *Mangifera indica* (mango) is a tropical fruit that can be found easily in tropical countries. The previous study reported that *Mangifera indica* extract was significant inhibition towards most of the Gram-positive and Gram-negative bacteria [7].

All the above reports showed clear indication that *Punica granatum*, *Citrus limon* and *Mangifera indica*. So we decided that the combination of these three plant extracts may have great potential

to formulate into polyherbal toothpaste since it contains a natural chemical which is safer to use compared to commercial toothpaste. Hence in the present study, we are interested in formulating and evaluating polyherbal toothpaste and its antibacterial properties.

MATERIALS AND METHODS

Plant collection

The fresh pomegranate fruits (*Punica granatum*), lemon fruits (*Citrus limon*), mango fruits (*Mangifera indica*), were purchased from local market, Ipoh and identified.

Table 1: Composition of polyherbal toothpaste

Components	Amount (g)
Pomegranate peel extract	5.0 g
Lemon peel extract	5.0 g
Mango peel extract	5.0 g
Carboxymethyl cellulose	3 g
Calcium carbonate	20 g
Glycerine	5 g
Methylparaben	0.5 g
Propylparaben	0.25 g
Sodium Lauryl Sulphate	1 g
Mint oil	0.5%
Demineralized water	25 ml

Extraction

The peels of the fruits were removed off using a peeler and kept for shade drying at room temperature for two weeks to avoid chemical degradation due to sunlight. The dried peels were coarsely powdered using a mechanical blender. 100 g of powdered peels of lemon, pomegranate and mango were separately subjected to extraction by maceration in 70% methanol at room temperature with occasional shaking for five days. The macerates were filtered,

and the filtrate was dried at low temperature (40-50 °C) under vacuum. The extracts were stored in air-tight containers in the refrigerator at 4 °C until further use.

Formulation of polyherbal toothpaste

The composition of herbal toothpaste was shown in table 1.

Carboxymethyl cellulose was triturated with methyl paraben and propyl paraben by using mortar and pestle. Sodium Lauryl Sulphate was dissolved in 25 ml of demineralized water and added sufficiently along with 5 g of glycerin. The mixture was triturated well. Finally, calcium carbonate and peel extracts of pomegranate, mango, and lemon have added the mixture and triturated to form a paste. Lastly, few drops of mint oil were added to mask the taste.

Evaluation of the formulated toothpaste

Organoleptic evaluation

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

Moisture content

10 g of toothpaste weighted and dried it in the oven at 105 °C then it will be cooled until constant weight is achieved. The loss of weight will be 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

About 10 g of the sample was accurately weighed and placed in a 100 ml glass beaker. 10 ml of water was added and the beaker and covered with a watch glass and allowed to stand for 30 min. This operation was carried out to disperse the toothpaste in water.

The contents of the beaker were stirred with a glass rod and the slurry was transferred to a 250 ml graduated measuring cylinder, during this transfer ensured that no foam was produced, and no lump paste went into the measuring cylinder. The residue left in the beaker was transferred with a further portion of 56 ml of water to the cylinder. The content of cylinder was adjusted to 50 ml by adding sufficient water, and the content has to be maintained at 30 °C. Stirred the contents of the cylinder with a glass rod to ensure a uniform suspension. As soon as the temperature of the content reached 30 °C, the cylinder was stoppered, and 12 complete shakes were given to it.

The cylinder was allowed to stand for 5 min and the volume of foam with water (V1) and water only (V2) was noted for all samples.

Determination of foaming power:

Foaming power = V1-V2

V1-Volume in ml of foam with water.

V2-Volume in ml of water only.

Storage stability

The stability study was carried out for the prepared toothpaste at a temperature of 37 °C for 2 mo.

pH test

The pH was tested by dissolving 1 g product into 9 ml of water and shake vigorously then aqueous solution and pH is observed using pH meter.

Antimicrobial activity

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 was 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 [8]. The plates will be inverted and incubated at 37 °C for 24 h. The antibacterial activity was measured by measuring the diameter of the resulting zone of inhibition against the tested organisms. The test for positive control and the negative control was performed in duplicate.

RESULTS AND DISCUSSION

The nature and percentage yield of methanolic extract of *Punica granatum*, *Citrus limon* and *Mangifera indica* peels were shown in table 2.

The methanolic extract of *Punica granatum*, *Citrus limon* and *Mangifera indica* peels were used as active ingredient in the preparation of polyherbal toothpaste. The formulated toothpaste was evaluated for physicochemical parameters such as colour, odour and pH; the results were shown in table 3.

Table 2: Nature and yield of the methanolic extract of *Punica granatum*, *Citrus limon* and *Mangifera indica* peels

Name of the plant	Quantity of plant powder	Methanol	Yield
<i>Punica granatum</i>	100 g	500 ml	4.55%
<i>Citrus limon</i>	100 g	500 ml	3.85%
<i>Mangifera indica</i>	100 g	500 ml	5.05%

Table 3: Organoleptic evaluation of formulated polyherbal toothpaste

Organoleptic evaluation	Result
Colour	Yellowish brown
Taste	Bitter
Odour	Pleasant (mint)
Texture	Smooth
Moisture content	22.70%
Foaming character	10
Storage stability	After 2 mo, separation of a liquid component is observed slightly.
pH	4.5

The formulated toothpaste was tested for antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* with different concentrations of toothpaste were used (100, 250 and 500 mg/ml). The potency was qualitatively and quantitatively assessed by the presence or absence of a zone of inhibition and zone diameter values. Different concentrations show different reading in terms of zone of inhibition. The formulated toothpaste exhibited highly significant effect towards all the tested bacteria, whereas the negative control does not produce observable inhibitory effect for any of the tested bacteria (table 4).

Among all the tested bacteria used *Staphylococcus aureus* was found to be most sensitive to the formulated toothpaste as seen by a zone of inhibition (10-15 mm) followed by *Escherichia coli* (9-12 mm), *Bacillus cereus* (7-12 mm) and *Pseudomonas aeruginosa* (9-11 mm).

Table 4: Antibacterial activity of the formulated polyherbal toothpaste

Concentration (mg/ml)	Gram-positive		Gram-negative	
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
	Zone of inhibition (mm)			
100	13	10	9	10
250	10	7	10	9
500	15	12	12	11
Positive control (Ciprofloxacin 5 µg)	35	32	36	35

Earlier studies reported that the extracts of *Punica granatum*, *Citrus limon* and *Mangifera indica* were potential for inhibition of both gram positive and gram negative bacteria [5-7]. The antibacterial activity of the formulated polyherbal toothpaste also showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstituents present in the extract. Hence, the observed antibacterial activity of the toothpaste was due to the presence of active constituents of the extract and the activity also well maintained when it was converted to toothpaste. This was good sign to do further studies on that to make it as one of the commercial herbal toothpaste for the treatment of oral bacterial infections.

Conclusion and recommendation

The formulated polyherbal toothpaste was successfully evaluated using different standard parameters including antimicrobial properties. The extract showed promising antimicrobial effects against both grams positive and gram negative organisms. The formulated toothpaste may be safer compared to fully synthetic toothpaste. Further studies are warranted to prove safety and efficacy of the formulated toothpaste.

CONFLICT OF INTERESTS

Declare none

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