International Journal of Applied Pharmaceutics

ISSN - 0975 - 7058 Vol 11, Special Issue 5, 2019

Research Article

ANTIBACTERIAL ACTIVITY OF CELERY LEAVES (*APIUM GRAVEOLENS* L.) FORMULATED IN TOOTHPASTE AGAINST *STREPTOCOCCUS MUTANS*

ERZA GENATRIKA*, FITA SATRIANI, INDRI HAPSARI

Department of Pharmaceutics and Technology, Pharmacy Faculty, Universitas Muhammadiyah Purwokerto, Central Java, Indonesia.
Email: erzagenatrika@gmail.com

Received: 15 November 2018, Revised and Accepted: 06 December 2018

ABSTRACT

Objective: The objective of this research was to determine the antibacterial activity of the toothpaste from an extract of celery leaves on *Streptococcus mutans*.

Methods: The toothpaste was formulated with various concentrations of celery leaves, F1 with concentration of extract (6.25%), F2 (12.5%), and F3 (25%). Each formula was tested the physical characteristics and antibacterial activity toward *S. mutans*. The antibacterial activity was determined by the agar well diffusion method using brain heart infusion agar plates. Furthermore, the antibacterial activities were assessed by the presence or absence of inhibition zones after the plates were incubated at 37°C for 24 h.

Results: The results from this test illustrate that all toothpastes under study at various concentrations of celery leaves extract exhibited antibacterial activity. Maximum inhibition zone in antibacterial activity test was shown by F2 (12.5%). Therefore, we can use these toothpastes as natural antibacterial on prevention of dental caries caused *S. mutans*.

Conclusion: The toothpaste from an extract of celery leaves showed significant antibacterial activity against *S. mutans*.

Keywords: *Streptococcus mutans*, Celery leaves, Toothpaste, *Apium graveolens*, Inhibition zone.

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s5.T0028

INTRODUCTION

Dental and oral diseases that are commonly found in Indonesia are dental caries. Dental caries is human dental decay that starts from the process and develops toward the teeth, the process of demineralization of the teeth [1]. Prevention of dental caries can be done by *maintaining* oral hygiene [2]. The microorganism associated with dental caries is *Streptococcus mutans* [3,4].

Toothpaste is a dosage form that is used together with a toothbrush to clean the teeth. Toothpaste used when brushing teeth serves to reduce plaque formation, strengthening teeth against caries [5]. The addition of herbs to toothpaste is expected to inhibit the growth of plaque. This is related to the ability of several types of herbs that can inhibit microbial growth. In addition, because herbs come from plants, they are safe and natural [6].

Celery leaves contain flavonoids, tannins, and saponins which have antibacterial effects. Majidah *et al.* (2014) stated that celery leaves extract (*Apium graveolens* L.) at a concentration of 12.5% has an inhibitory effect on *S. mutans* [7]. Celery leaves that have been proven activity, for easy use, it is necessary to develop in dosage form of toothpaste. This toothpaste will be determined the antibacterial activity on *S. mutans* to ascertain that it can prevention of dental caries.

METHODS

Plant materials

Fresh leaves of *A. graveolens* L. were collected from one source in Purbalingga district, Central Java, Indonesia. The leaves of the plant were authenticated by Faculty of Biology, Jenderal Soedirman University, Indonesia.

Microorganisms

Pathogenic bacterial isolates of *S. mutans* were obtained from the Department of Microbiology, Universitas Muhammadiyah Purwokerto. *S. mutans* was cultured in brain heart infusion agar (BHI-A) (HiMedia) at 37°C for 24 h.

Extraction of plant materials

The cleaned materials were dried in shade, grinded to fine powder with the help of a mixer grinder and ethanolic extract was prepared using 96% ethanol by maceration method followed by steam evaporation in rotary flask evaporator [8]. Ethanol was obtained from Bratachem (Indonesia). The extract was transferred into clean and dried airtight vial and stored at 20–80°C until ready for use. A net yield of 278.3 g (27.83% w/w) was obtained by macerating 1000 g of dry powder of the leaves.

Formulation of toothpaste

The formula of toothpaste was formulated as given in Table 1. All the formulations were filled in regular tubes used for toothpastes. The storage in tubes was done to avoid the problem of crusting and drying of toothpaste during studies. Toothpaste formulation was done; then, it was evaluated such as organoleptic, homogeneity, pH, disperse, and adhesiveness test.

Preparation of the culture media

BHI-A was prepared by dissolving 3.7 g in 100 ml of distilled water, by sterilizing in the autoclave for 15 min at 15 lb pressure (121°C) and by pouring 5–8 ml of the prepared medium to each Petri dish and then storing in refrigerator.

Antibacterial activity determination

The antibacterial activity of the toothpaste of celery leaves was determined by the agar well diffusion method [9,10]. Bacterial suspension adjusted to 0.5 McFarland standard (1.5×10^8 colony-forming units/ml) was made, and then, the BHI-A plates were streaked with a bacterial suspension using sterile swab. After that, the wells were made with a diameter of 6 mm by punched aseptically with a sterile cork borer. Approximately 200 mg of the toothpaste at different concentrations of 6.25, 12.5, and 25% were loaded into the wells, and the positive control was used an x-brand toothpaste and an F0 (no extract of celery leaves) was used as a negative control.

Each test plate contained three samples of toothpaste at various concentrations of celery extract placed about equidistant to each other. After that, the plates were incubated at 37°C for 24 h. The diameter of inhibition zones was measured in millimeter and the study was carried out in triplicate.

RESULTS AND DISCUSSION

Physical characteristic

The toothpaste was tested for physical characteristics including organoleptic, homogeneity, pH, disperse, and adhesiveness. The results of physical characteristics can be shown in Table 2.

The results of organoleptic testing on all three formulas were the same; the results of the characteristics of semisolid dosage form, white color, and smell were typical of celery.

Homogeneity test was to determine the homogeneity of extracts in toothpaste. Homogeneity of dosage form will affect the antibacterial power of toothpaste. This is because with a homogeneous toothpaste, the distribution of the active ingredients in toothpaste will be evenly distributed so that the release of the active compound by the base through the test media will be good and the antibacterial effect will be maximized.

The difference in the concentration of celery extract does not affect the homogeneity of toothpaste because the process or treatment of each formula is the same. And then, disperse test was conducted to determine the ability of ointment's diffusion on the skin. The easier the ointment is flattened on the skin, the greater the absorption of its active substances [11]. The addition of the extract can decrease the disperse of toothpaste; however, the addition of the extract can increase the adhesiveness of toothpaste. On the results of pH testing, it was found that the higher the concentration did not affect the pH value of each formula. The pH value of the three formulas still meets the SNI requirements of 4.5–10.5 [12].

Antibacterial activity determination

In the study, positive control (x-brand toothpaste) was found to produce zones against *S. mutans* that were insensitive to the negative control (F0); hence, no zone of inhibition was noted for the negative control.

The zones of inhibitions are shown by toothpaste of celery leaves at different concentrations against *S. mutans* depicted in Table 3 and Fig. 1. The test results showed that the higher the concentration of celery extracts, so the smaller the inhibitory power. Hence, F2 has the greatest inhibitory power than F1 and F3 ($p<0.05$). A compound in a pharmaceutical dosage form to cause an effect must bind to the receptor, whereas the ability of the receptor to be able to bind to compounds is different. If a receptor is saturated, even though the dosage is increased, then the receptor is no longer able to bind to active compounds so that the effect decreases at continuously increasing concentrations [13].

The antibacterial activity of toothpaste of celery leaves can be attributed to the different phytochemicals present in the celery leaves. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemicals are non-nutritive plant chemicals that may have protective or disease preventive antibacterial activities. Due to their structural differences from those of the more studied antibacterial sources, their mode of action may too differ [14].

Flavonoids, alkaloids, and saponins are found to be associated with antibacterial effects in various studies using plant extracts [15]. These phytochemicals have been found to be present in the celery leaves in other scientific studies [16]. Flavonoids have been found to exhibit antibacterial activity through various mechanisms such as inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, and energy

Table 1: Formula of toothpaste selected for study

Material	Formulation code (w/w)			
	F1 (6.25%)	F2 (12.5%)	F3 (25%)	F0 (-)
Extract	6.25	12.5	25	-
Calcium carbonate	40	40	40	40
Glycerol	18	18	18	18
Sorbitol	10	10	10	10
Sodium carboxymethyl cellulose	1	1	1	1
Saccharin	0.2	0.2	0.2	0.2
Sodium benzoate	0.1	0.1	0.1	0.1
Sodium lauryl sulfate	1	1	1	1
Oleum menthae piperitae	0.3	0.3	0.3	0.3
Purified water	ad 100	ad 100	ad 100	ad 100

F1: Toothpaste with 6.25% extract concentration, F2: Toothpaste with 12.5% extract concentration, F3: Toothpaste with 25% extract concentration, F0: Toothpaste with 0% extract concentration as negative control

Table 2: Physical characteristic test result

Test	Formulation code			
	F0	F1	F2	F3
Organoleptic				
Form	Semi-solid	Semi-solid	Semi-solid	Solid
Smell	None	Specific	Specific	Specific
Color	White	Light green	Green	Dark green
Homogeneity	Homogen	Homogen	Homogen	Homogen
Disperse (m)	0.053±0.001	0.047±0.001	0.043±0.0015	0.039±0.0005
Adhesiveness (s)	25.67±15.9	35.67±2.0	46.67±1.5	51.33±15.9
pH				

F1: Toothpaste with 6.25% extract concentration, F2: Toothpaste with 12.5% extract concentration, F3: Toothpaste with 25% extract concentration, F0: Toothpaste with 0% extract concentration as negative control

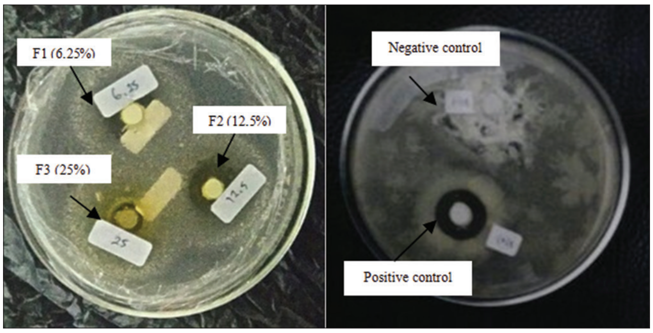

Fig. 1: Inhibition zone of the toothpaste formulation (FI, F2, and F3) and control group

Table 3: Antibacterial activity result

Replication	Formulation code (mm)				
	F0	F1	F2	F3	F4
I	0	18	19	12	24
II	0	17	18	12	25
III	0	18	18	13	24
Average±SD	0	17.6±0.57	18.3±0.57	12.3±0.57	24.3±0.57

F1: Toothpaste with 6.25% extract concentration, F2: Toothpaste with 12.5% extract concentration, F3: Toothpaste with 25% extract concentration, F4: X-brand toothpaste as positive control, F0: Toothpaste with 0% extract concentration as negative control. SD: Standard deviation

metabolism [17]. The mode of action of antibacterial effects of saponins seems to involve membranolytic properties [18]. Saponins might increase the permeability of bacterial cell membrane without destroying them [19]. The mechanism of the action of highly aromatic quaternary alkaloids is attributed to their ability to intercalate with DNA [20]. These may explain the probable mechanism of antibacterial activity of the plant.

CONCLUSION

The study reveals the antibacterial activity of the toothpaste of celery leaves against *S. mutans*. F2 has the greatest inhibitory power. The present study can pave a way for further research to develop newer antibacterial dosage form in this era of antibacterial resistance.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Silje S, dan Shilpi MO. A Text Books of Dentifrices and Mouthwashes Ingredients and their Use. Oslo: Oslo University of Andidatus; 2003.
2. Silva DD, Gonçalo Cda S, Sousa Mda L, Wada RS. Aggregation of plaque disclosing agent in a dentifrice. J Appl Oral Sci 2004;12:154-8.
3. Forssten SD, Björklund M, Ouwehand AC. *Streptococcus mutans*, caries and simulation models. Nutrients 2010;2:290-8.
4. Nugraha AW. The Plaques Everywhere. Microbial Article. Yogyakarta: Faculty of Pharmacy, USD; 2008. p. 1-3.
5. Perry DA, Beemsterboer P, Taggart EJ, Saunders WB. Periodontology for the Dental Hygienist. St. Louis: Saunders Elsevier; 2007. p. 249-50.
6. Okpalugo J, dan Ibrahim K. Toothpaste formulation efficacy in reducing oral flora. Trop J Pharm Res 2009;27:72.
7. Majidah D, Fatmawati DWA, Gunadi A. Antibacterial Power of Celery Leaf Extract (*Apium graveolens* L) against the Growth of *Streptococcus mutans* as an Alternative Mouthwash. New Jersey: Printice-HalI; 2014. p. 777-80.
8. Nairn JG. Solutions, emulsions, suspensions and extracts. In: Gennaro A, Marderosian AD, Hanson GR, Medwick T, Popovich NG, Schnarre RL, *et al*. editors. Remington: The Science and Practice of Pharmacy. 20th ed. Philadelphia, P.A: Lippincott Williams and Wilkins; 2000.p. 721-52.
9. Irobi ON, Moo-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (*Euphorbiaceae*). J Ethnopharmacol 1994;43:185-90.
10. Almawlah YH, Hadeel A, Al-Jelawi SO. Antibacterial activity of three plant extracts against multidrug resistance pseudomonas aeruginosa. Asian J Pharm Clin Res 2017;10:193-7.
11. Kamaliyah I. Comparison of the Effectiveness of Toothpaste Extract Formulations of Red Betel Leaves (Piper crocatum Ruiz and Pav) Between PGA, PEG and HPLC Bases Against *Streptococcus mutans* and *Lactobacilllus* [Thesis]. Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto; 2015.
12. Nursal FK, Indriani O, Dewantini LA. Use of Na CMC as a Gelling agent in Toothpaste Formulations of 70% Ethanol Extract of Guava Leaves (*Psidium guajava* L). Jakarta: Department of Pharmacy, UHAMKA; 2010.
13. Sujono TA, Wahyuni US. Effect of *Aloe vera* leave decocta (*Aloe vera* L) against the blood glucose levels of the rabbits who are burdened glucose. J Sci Technol Res 2005;6;26-34.
14. Panda S, Bandyopadhyay PK. Chemical information from GC-MS studies of methanolic leaf extract of *Andrographis paniculata* and *Datura metel* and their antibacterial activity against isolated *pseudomonas aeruginosa* (pb112) strain. Int J Pharm Bio Sci 2013;4:909-15.
15. Nwaogu LA, Alisi CS, Ibegbulem CO, Igwe CU. Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. Afr J Biotechnol 2007;6:890-93.
16. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J editor. Perspectives on New Crops and New uses. Alexandria: ASHS Press; 1999. p. 457-62.
17. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrobial Agents 2005;26:343-56.
18. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. Growing in Iraq. J Zhejiang Univ Sci B 2008;9:154-9.
19. Arabski M, Wegierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of saponins against clinical *E. coli* strains and *Eukaryotic* cell line. J Biomed Biotechnol 2011;2012:6.
20. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.