ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 11, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

KASTURI TOBACCO LEAF FLAVONOIDS AS BIOACTIVE COMPOUNDS IN PERIODONTAL ANTIBACTERIAL MUCOADHESIVE GEL

QONITA NAFILAH FEBI[1], MALIHATUL ROSIDAH[1], DERI ABDUL AZIS[1], ENDAH PUSPITASARI[2], ARI SATIA NUGRAHA[3], BANUN KUSUMAWARDANI[1]*

[1]Department of Biomedical Sciences, Faculty of Dentistry, University of Jember, Jawa Timur 68121, Indonesia. [2]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Jember, Jawa Timur 68121, Indonesia. [3]Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, University of Jember, Jawa Timur 68121, Indonesia. Email: banun_k.fkg@unej.ac.id

Received: 17 July 2019, Revised and Accepted: 21 September 2019

ABSTRACT

Objective: The tobacco leaves are a rich source of many biologically active substances. Flavonoids are one of the bioactive substances contained in tobacco leaves with a wide range of pharmacological properties. This study has been aimed to formulate the antibacterial periodontal gel containing the flavonoids fraction of Kasturi tobacco (*Nicotiana tabacum*) leaves.

Methods: Fraction flavonoids obtained from methanolic extracts of tobacco leaves, which have been purified from nicotine. Different concentrations of flavonoids fraction (0, 25, 0,5, 1, 2, and 4 mg/ml) were prepared with Carbopol-974P. The strength of flavonoid gel adhesion in *ex vivo* mucosa was tested by the falling liquid film method. Evaluation of *in vitro* antibacterial agent was followed by the disk diffusion method.

Results: The flavonoid gels have better adhesion parameters (time, strength, and distance) than gel bases. The antimicrobial by the zone of inhibition studies proved that flavonoids periodontal gels with concentrations of 4 mg/ml are highly active against *Porphyromonas gingivalis* with the zone of inhibition >10 mm which was higher than quercetin gel formulations (control groups).

Conclusion: Gel formulation containing the flavonoids fraction of Kasturi tobacco (*N. tabacum*) leaves could be a good candidate for periodontal gel with good mucoadhesive gel and antibacterial agent.

Keywords: Antibacterial periodontal gel, Flavonoid, Periodontitis, Tobacco leaves.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4. 0/) DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i11.34967

INTRODUCTION

Gingivitis and periodontitis are the most common oral diseases which it initial stage is characterized by inflammation of the gingival tissue called gingivitis [1], further periodontitis is characterized by inflammation of the supporting tissues of the teeth, progressive damage to the periodontal ligament, and alveolar bone together with the formation of periodontal pockets and gingival recession. The immune response to the interaction of microorganisms and inflammatory cells in the tissues around the teeth contributes to the development of this disease. The enzymes and toxins released by periodontal pathogenic bacteria exaggerate the destruction of the periodontium [2]. Anaerobic Gram-negative bacteria involved in the development of periodontal disease are *Porphyromonas gingivalis, Bacteroides forsythus*, and *Actinobacillus actinomycetemcomitans* [3]. The bacteria colonization in the periodontal pocket constructs a firmly attached biofilm on the teeth and gingiva surface which the film helps bacteria fight antibiotics. Bacteria can be interconnected in biofilms, and this action helps gene transmission that results in resistance to antibiotics and the growth of resistant microorganism species [4].

In most cases, the important treatment modality is by controlling inflammation through dental plaque and calculus removal to reduce pathogenic microorganisms [5], but this procedure is not able to inhibit bacterial growth by systemic antibiotic therapy. As the etiologies of periodontal disease correlate to various types of bacterial infection, there is no ideal and effective antibiotic for all pathogens, and the improper different antibiotics uses can lead to medical side effects [6]. The short-term efficacy and inconsistency of antiseptic mouthwash and also the side effects of systemic antibiotics encourage the use of a therapeutic system locally. The use of minocycline gel and metronidazole gel in the subgingival area is the development of drug delivery systems [7]. This system has several advantages including reducing prescription drugs, increasing drug concentration in the target tissue, reducing drug side effects, and lowering the dosage frequency [8]. Natural products such as flavonoids are capable to prevent the development of infections. Flavonoids are safe and cost-effective with neglectable side effects. Local drug delivery of natural products to the periodontal pocket is an additional therapy other than dental root planing and scaling for the treatment of periodontal disease [9].

With the increasing prevalence of antibiotic-resistant bacterial infections, the use of natural ingredients, such as flavonoids, is increasingly attracting potential utility as antibiotic replacement therapy or adjuvant therapy. Flavonoids have been known to possess antibacterial potency against various pathogenic microorganisms [10]. Therefore, the development of flavonoids as herbal mucoadhesive active compounds is very necessary and important to improve the success of periodontal disease treatment. The bioactive fraction used in this development of mucoadhesive gel is flavonoids from Kasturi tobacco leaf wastes in which our preliminary research on the crude extract showed antimicrobial activity. Nicotine constituent removal was the most important part in producing a satisfactory antimicrobial assay [11]. The preliminary research data encourage us to further carry out extraction and fractionation of nicotine-free flavonoids from the waste of Kasturi tobacco leaves.

Kasturi tobacco leaf waste is a tobacco leaf located in the lower stem, brownish-yellow in color, damaged or perforated leaf shape, and has a low selling value; hence, it is often discarded and destroyed by burning. Disposal of this waste will cause serious problems because tobacco leaf waste contains highly toxic nicotine. Thus, efforts need to be made to use controlled tobacco leaf waste to avoid harmful effects on the environment and make Kasturi tobacco leaf waste as a source of flavonoids that can be used as a basis for biopharmaceutical development to treat periodontal disease.

METHODS

Fractionation of flavonoids from the waste of Kasturi tobacco leaf

Kasturi tobacco leaf waste was collected from tobacco plantations in Pakusari, Jember, Indonesia. Tobacco leaves are dried at room temperature for 2 days before further drying in an oven for 24 h at 40°C. To dried tobacco leaf powder (50 g), methanol (400 mL) was added and the mixture was then stirred with an orbital shaker at 150 rpm for 24 h. The solution was obtained through filtration which the supernatant was volume reduced (200 mL) through vacuum evaporation. To the methanol extract (200 mL), hexane (20 mL) was added before stirring with a magnetic stir for 10 min to form two layers. The top layer was removed, while the bottom layer is washed again with hexane (20 mL). This process is repeated 15 times. All the upper layers were evaporated with a rotary evaporator until a thick extract (20 mL). A portion of the thick extract (10 mL), hydrogen chloride (10%, 20 mL), distilled water (10 mL), and ethyl acetate (20 mL) was added and the mixture was stirred under a magnetic stir for 10 min to form two layers. The top ethyl acetate layer was separated and collected. This fractionation process was repeated 3 times. The top layer is a solution rich in flavonoids, while the lower layer is a solution rich in nicotine. The top layer that is rich in flavonoids is taken and tested for the nicotine content with the Dragendorff test to make sure there are no nicotine traces in the layer. The result of fractionation of flavonoids (the top layer formed) is taken and evaporated. The alkaloid removal followed a standard procedure by Docheva *et al.* with a modification [12,13].

Flavonoid mucoadhesive gel formulation

Flavonoids with a series concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL were dispersed in dimethyl sulfoxide 1% along with a gel base consisting of Carbopol-974P, triethanolamine, and aquades (2:5:100). This mixture is slowly stirred to homogenize and forms a thick gel mass. The gel is poured into a petri dish and left overnight for stabilization. Metronidazole (2%) and quercetin (50 µM) gels were used as the control.

Ex vivo flavonoid gel adhesion

The strength of the flavonoid gel adhesion was performed through the falling liquid film method. In this study, the rat gastric mucous layer was used as a model to determine the strength of gel adhesion. The gastric mucosa of rats was cleaned with 0.9% sodium chloride and was cut (1.5 cm × 3 cm). The rat's gastric mucosa is placed between two glass plates and the top of which was a hole with a 2 cm diameter. Flavonoid gel (200 mg) was attached to the steel plate as a buffer. The steel plate was then attached to the texture analyzer tool pipe. The texture analyzer was managed using a computer with stable microsystem activation. The steel plate will slowly drop into the glass

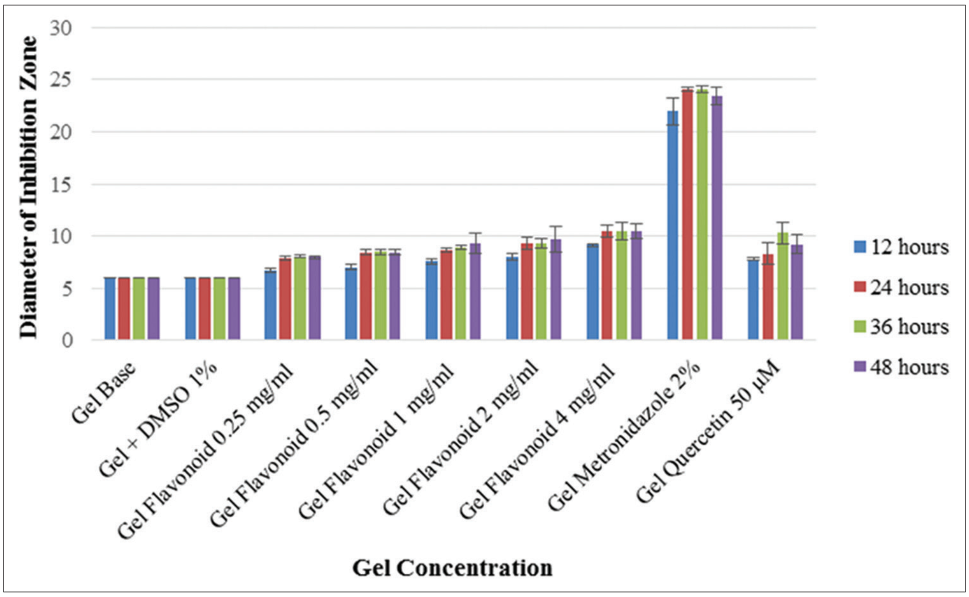

Fig. 1: *Porphyromonas gingivalis* inhibition zone in incubation 12, 24, 36, and 48 h in all control and treatment groups. Significance values in all groups at each time of exposure showed a value of $p<0.05$

Table 1: Diameter of *Porphyromonas gingivalis* growth inhibition zone

Groups	Diameter of inhibition zone (mm)				*P*
	12 h	24 h	36 h	48 h	
Gel base	6±0.00	6±0.00	6±0.00	6±0.00	-
Gel+DMSO 1%	6±0.00	6±0.00	6±0.00	6±0.00	-
Gel flavonoid 0.25 mg/ml	6.71±0.17	7.87±0.16	8.07±0.09	7.98±0.15	0.000
Gel flavonoid 0.5 mg/ml	7.05±0.32	8.44±0.266	8.50±0.29	8.47±0.24	0.000
Gel flavonoid 1 mg/ml	7.58±0.29	8.70±0.20	8.93±0.15	9.30±0.98	0.009
Gel flavonoid 2 mg/ml	8.05±0.33	9.35±0.61	9.33±0.49	9.75±1.24	0.078
Gel flavonoid 4 mg/ml	9.13±0.16	10.46±0.55	10.51±0.84	10.49±0.71	0.045
Gel metronidazole 2%	21.96±1.31	24.12±0.17	24.04±0.33	23.47±0.85	0.022
Gel quercetin 50 µM	7.79±0.14	8.31±1.03	10.29±1.03	9.24±0.90	0.019

DMSO: Dimethyl sulfoxide

antibiotics in periodontal pockets. J Babol Univ Med Sci 2011;13:82-9.

5. Mlachkova AM, Popova CL. Efficiency of nonsurgical periodontal therapy in moderate chronic periodontitis. Folia Med (Plovdiv) 2014;56:109-15.
6. Rams TE, Degener JE, van Winkelhoff AJ. Antibiotic resistance in human chronic periodontitis microbiota. J Periodontol 2014;85:160-9.
7. Zingale J, Harpenau L, Bruce G, Chambers D, Lundergan W. The effectiveness of scaling and root planing with adjunctive time-release minocycline using an open and closed approach for the treatment of periodontitis. Gen Dent 2012;60:300-5.
8. Medlicott NJ, Rathbone MJ, Holborow DW, Tucker IG. Delivery systems for the administration of drugs to the periodontal pocket. Adv Drug Deliv Rev 1994;13:181-203.
9. Bruschi ML, Jones DS, Panzeri H, Gremião MP, de Freitas O, Lara EH. Semisolid systems containing propolis for the treatment of periodontal disease: *In vitro* release kinetics, syringeability, rheological, textural, and mucoadhesive properties. J Pharm Sci 2007;96:2074-89.
10. Bakht J, Azra, Shafi M. Antimicrobial activity of *Nicotiana tabacum* using different solvents extracts. Pak J Bot 2012;44:459-63.
11. Putri RH, Barid I, Kusumawardani B. Daya hambat ekstrak daun tembakau terhadap pertumbuhan mikroba rongga mulut. Stomatognatic 2014;11:27-31.
12. Docheva M, Dagnon S, Statkova-Abeghe S. Flavonoid content and radical scavenging potential of extracts prepared from tobacco cultivars and waste. Nat Prod Res 2014;28:1328-34.
13. Nugraha AS, Pratoko DK, Damayanti YD, Lestari ND, Laksono TA, Addy HS, *et al.* antibacterial and anticancer activities of nine lichens of Indonesian Java Island. J Biol Act Prod Nat 2019;9:39-46.
14. Larsen T. *In vitro* release of doxycycline from bioabsorbable materials and acrylic strips. J Periodontol 1990;61:30-4.
15. Petti S, Scully C. Polyphenols, oral health and disease: A review. J Dent 2009;37:413-23.
16. Hägerström H, Edsman K, Strømme M. Low-frequency dielectric spectroscopy as a tool for studying the compatibility between pharmaceutical gels and mucous tissue. J Pharm Sci 2003;92:1869-81.
17. Woodley J. Bioadhesion: New possibilities for drug administration? Clin Pharmacokinet 2001;40:77-84.
18. Huang Y, Leobandung W, Foss A, Peppas NA. Molecular aspects of muco-and bioadhesion: Tethered structures and site-specific surfaces. J Control Release 2000;65:63-71.