ORIGINAL PAPER

ANTI-MICROBIAL EFFECT OF *PIPER BETLE* CRUDE EXTRACT AND ESSENTIAL OIL INCORPORATED INTO SHORT-TERM SOFT LINING MATERIAL

Potsawat Poolkerd[1], Noppavan Nagaviroj[2], Ratchapin Laovanitch Srisatjaluk[3], Trinuch Eiampongpaiboon[2]

[1]Department of Prosthodontics, Residency Training Program, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[2]Department of Prosthodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[3]Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

ABSTRACT

Introduction: Using soft lining material to treat denture stomatitis for an extended period of time may result in oral bacterial and fungal accumulations. Several additives, including natural products, have been identified as anti-microbial agents.

Objectives: The aim of the present study was to evaluate the anti-microbial activity of *Piper betle* crude extract (PBC) and *Piper betle* essential oil (PBO) incorporated into soft liner with different concentrations against *Candida albicans* and *Streptococcus mutans*. Cytotoxicity of various additives applied into soft lining material was also investigated.

Material and methods: Anti-microbial activity of PBC and PBO were assessed using agar disc diffusion method. Agar well diffusion method was also applied to evaluate inhibitory effect of PBC and PBO incorporated into soft lining material. Cell viability of human gingival fibroblast (HGF) cell line after exposing to soft lining materials with PBC was investigated.

Results: With the same minimum inhibitory concentration (MIC) of 2.5% w/w, PBC showed anti-fungal and anti-bacterial activities against *C. albicans* and *S. mutans*. However, PBO had a 2.5% v/v MIC against *C. albicans*, but a 10% v/v MIC against *S. mutans*. Accordingly, GC soft liner with PBC had MICs of 5% w/w against *C. albicans* and 10% w/w against *S. mutans*, whereas GC soft liner with PBO had MICs of 20% v/v and 60% v/v against *C. albicans* and *S. mutans*, respectively. Statistical analysis revealed that there was no significant difference in cell viability among GC soft liner, with 5% and 10% w/w PBC and GC soft liner without additive ($p > 0.05$).

Conclusions: Both PBC and PBO showed anti-bacterial and anti-fungal activity; however, the 10% w/w PBC incorporated into GC soft liner demonstrated the optimal concentration to serve as an anti-microbial agent against both *C. albicans* and *S. mutans*, with no toxicity to HGF cells.

Key words: *Candida albicans*, *Streptococcus mutans*, *Piper betle*, denture liners, bactericidal and fungicidal properties.

J Stoma 2023; 76, 4: 226-234
DOI: https://doi.org/10.5114/jos.2023.133636

INTRODUCTION

Denture stomatitis is an inflammatory lesion of oral mucosa commonly found in denture wearers. The etiology of denture stomatitis is multifactorial [1]. Generally, *Candida* species, in particular *C. albicans*, are the etiology of denture stomatitis, although denture plaque bacteria may be involved. Bacteria can contribute to the colonization and growth of *Candida* strains in the oral cavity, according to a prior study [2]. Additionally, soft

JOURNAL OF STOMATOLOGY
CZASOPISMO STOMATOLOGICZNE

Address for correspondence: Trinuch Eiampongpaiboon, Department of Prosthodontics, Faculty of Dentistry, Mahidol University, 6 Yothi Street, Rajthewee, 10400, Bangkok, Thailand, e-mail: trinuch.eia@mahidol.ac.th

Received: 10.03.2023 • Accepted: 05.06.2023 • Published: 15.12.2023

tissue trauma should be properly considered when treating denture stomatitis. Since there are many contributing factors affecting denture stomatitis, there could be more than one solution required for the treatment. As a result, materials for denture lining are frequently used as an adjunctive treatment in combination with medications [1]. Previous studies demonstrated that various denture liners showed typically no effect on bacteria, but the biofilm on the denture surface could infect mucosal tissue [3, 4]. Therefore, the anti-microbial activity of soft lining materials has been still improving. Schneid reported that it is effective to incorporate chlorhexidine, clotrimazole, fluconazole, and nystatin into a soft liner as the sustained-release delivery system for the treatment of denture stomatitis. The results revealed that *C. albicans* growth was suppressed by all medications released from the tissue conditioner [5]. Since the overuse of antibiotics leads to a variety of side effects, including drug resistance, many researchers have been studying the novel anti-fungal agents. Consequently, numerous plants have undergone thorough research in an effort to discover more potent and harmless anti-microbial properties [6].

Piper betle (*Piperaceae*) is a traditional herb that contains various pharmacological properties, such as anti-microbial, anti-oxidant, anti-carcinogenic, and anti-inflammatory effects [7]. Kumpanich *et al.* reported that *Piper betle* crude extract (PBC) can be used as an additive in the denture soft liner with the optimum composition of 5% w/w against *Candida albicans* [8]. Clinical application of PBC is still somewhat restricted due to its dark green color and difficulty in preparation. *Piper betle* essential oil (PBO) is an additional extracted form that appears as a clear liquid; however, its anti-microbial effect on oral pathogens is currently unknown [8-12].

OBJECTIVES

The aim of the present study was to evaluate the anti-microbial activity of PBC and PBO incorporated into soft liner with different concentrations against *C. albicans* and *S. mutans*. Cytotoxicity of various additives applied into soft lining material was also investigated.

MATERIAL AND METHODS

PIPER BETLE ESSENTIAL OIL AND CRUDE EXTRACT

PBC was provided from the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. PBO was purchased from Thai Betel Leaf Products Co., Ltd., Bangkok, Thailand. Chemical composition of PBC and PBO are shown in Table 1.

SOFT LINING MATERIAL

Soft lining material (GC Soft Liner™, GC Dental Products Corp., Tokyo, Japan) was used in this study. The standard powder/liquid ratio was 1.22 g/1 g (500 µl). Its chemical compositions are listed in Table 2 [13].

MICROBIAL CULTURE

C. albicans (ATCC® 10231™, Manassas, VA, USA) and *S. mutans* (ATCC® 25175™, Manassas, VA, USA) were used in this study. *C. albicans* and *S. mutans* were inoculated into Sabouraud dextrose broth (Becton, Dickinson and company, Sparks, MD, USA) and brain heart infusion broth (Becton, Dickinson and company, Sparks, MD, USA), respectively. After 24 h, the turbidity of suspension of *C. albicans* and *S. mutans* were diluted to 1.0 McFarland unit (1.5×10^8 CFU/ml) and 0.5 McFarland unit (0.75×10^8 CFU/ml), respectively. *C. albicans* and *S. mutans* were inoculated on an agar plate using a spread plate method.

ANTI-MICROBIAL ACTIVITY OF PBC AND PBO

Anti-microbial activity of PBC and PBO were assessed by an agar disc diffusion method. Final concentrations of PBC and PBO were prepared in dimethylsulfoxide (DMSO) (Sigma-Aldrich, Inc., St. Louis, MO, USA) using serial dilution technique to obtain 0.675,

TABLE 1. Composition of *Piper betle* crude extract (PBC) and *Piper betle* essential oil (PBO) used in this study

Compounds	Chemical formula	Total %
PBC		
4-chromanol	$C_9H_{10}O_2$	62.33%
Eugenol	$C_{10}H_{12}O_2$	17.10%
PBO		
Eugenol	$C_{10}H_{12}O_2$	27.84%
Acetyl-eugenol	$C_{12}H_{14}O_3$	20.98%
4-allyl-1,2-diacetoxybenzene	$C_{13}H_{14}O_4$	17.57%
Hydroxychavicol	$C_9H_{10}O_2$	1.08%
Caryophyllene	$C_{15}H_{24}$	4.29%

Note: The GC-MS of PBO was provided by manufacturer.

TABLE 2. Composition of GC soft liner

Product	Compositions
GC soft liner powder	Poly (ethyl methacrylate), PEMA (100%)
GC soft liner liquid	Butyl phthalyl butyl glycolate, BPBG (80.9%), Dibutyl phthalate, DBP (4.3%), Ethanol, EtOH (14.8%)

1.25, 2.5, 5, 10% w/w and v/v, respectively. DMSO was utilized as a negative control for both species. 30% v/v nystatin oral suspension, 100,000 IU/ml or 33.3 mg/ml (NYS) (Continental Pharm Co., Ltd., Bangkok, Thailand), and 5% v/v of 2% chlorhexidine gluconate (CHX) (Faculty of Dentistry, Mahidol University, Bangkok, Thailand) were used as positive controls for anti-fungal and anti-bacterial assays, respectively. 20 µl of tested solution was soaked into a 6 mm diameter paper filter disc before placing on the agar. Plates were subsequently incubated at 37°C for 48 h, and the mean value of inhibition zones was recorded. Each experimental group (n = 5) was replicated five times independently.

ANTI-MICROBIAL ACTIVITY OF SOFT LINING MATERIAL INCORPORATED WITH PBC AND PBO

Anti-microbial activities of GC soft liner incorporated with PBC and PBO were assessed with the agar well diffusion method. The wells with a dimension of 6 mm in diameter and 4 mm in depth were created in each agar plate using a punch hole technique. According to a manufacturer's recommendation, GC soft liner with 1.22 g of powder and 1 g (500 µl) of liquid was manually mixed with various amounts of PBC and PBO in a sterile beaker for 30 seconds at ambient temperature. Regarding anti-fungal assay, the final concentrations of soft liner with PBC were 2.5%, 5%, 10%, 20%, 30%, 40% w/w, and with PBO were 2.5%, 5%, 10%, 20%, 30%, 40% v/v. As for anti-bacterial assay, the final concentrations of PBC and PBO in GC soft liner were 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70% w/w, and 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70% v/v, respectively. Final concentration of 30% v/v nystatin oral suspension and 5% v/v of 2% chlorhexidine gluconate in GC soft liner were prepared as positive controls for anti-fungal and anti-bacterial assays, respectively. Negative control was soft lining materials without additives. The samples were added into each well and incubated at 37°C for 48 h, and the mean inhibition zone was calculated. Each experimental group (n = 5) was replicated five times independently.

CYTOTOXICITY TEST

According to ISO 10993-5, the eluting method was adapted to evaluate cytotoxicity of various additives incorporated into GC soft liner [14]. Cell viability of human gingival fibroblast (HGF) cell line (ATCC® CRL-2014, Manassas, VA, USA) was performed using the eluting extract. Specimens were prepared in dimension of 20 × 5 × 2 mm (3 cm^2/ml extracted ratio) with stainless steel mold, in accordance with ISO 10993-12 [15]. GC soft liner was mixed with 5% and 10% w/w PBC, 30% v/v nystatin oral suspension, and 5% v/v of 2% chlorhexidine gluconate. GC soft liner without additives was utilized as a control. Each side of the specimens was sterilized with UV light for 15 min. Each specimen was immersed in the 1 ml Dulbecco's modified Eagle's medium (DMEM) (HyClone™, Logan, UT, USA) with 10% v/v fetal bovine serum (FBS) and 1% v/v penicillin-streptomycin solution, and incubated in a shaking incubator at 37°C for 24 h. The incubated culture media was collected for cell cytotoxicity test. As a negative control, culture medium without samples was also incubated in the same manner.

DMEM supplemented with 10% v/v FBS and 1% v/v penicillin-streptomycin solution was used as a culture media for HGF cells. HGF cells were cultured in 96-well plates with seeding density of 10,000 cells/well/100 µl in 5% CO_2 incubator at 37°C for 24 h. Then, the media were discarded, and 200 µl aliquots of the prepared extracted solutions, including negative control media, were subsequently added into 96-well plates. The samples were incubated in 5% CO_2 incubator at 37°C for 24 h. MTT assay was conducted to assess cell viability using microplate spectrophotometer (µOuant™, BioTek instruments Inc., Winooski, VT, USA) at 570 nm. Cytotoxicity of GC soft liner combined with various additives was expressed as percentage of cell viability compared with GC soft liner without additive. Each experimental group (n = 3) was replicated three times independently.

STATISTICAL ANALYSIS

All statistical analyses were performed using PASW Statistics software (IBM Corp., released 2019, IBM SPSS Statistics for Windows, version 26.0, Armonk, NY, USA). One-way ANOVA and Tukey's multiple comparison tests were performed to analyze the inhibition zones and percentages of cell viability. Significance level was set at $\alpha = 0.05$.

RESULTS

ANTI-MICROBIAL ACTIVITY OF PBC AND PBO ON *C. ALBICANS* AND *S. MUTANS*

The means inhibition zone of PBC and PBO against *C. albicans* and *S. mutans* were investigated (Table 3). The inhibitory zones against *C. albicans* were detectable in 2.5% w/w PBC and 2.5% v/v PBO (Figures 1A, 1B), whereas the inhibitory zones of *S. mutans* were detectable in 2.5% w/w PBC and 10% v/v PBO (Figures 1C, 1D). As the concentration of PBC and PBO increased, the inhibition zones also increased. Furthermore, similar to the positive control (nystatin), 5% v/v PBO and 5% w/w PBC demonstrated remarkable anti-fungal activity against *C. albicans*.

TABLE 3. Mean inhibition zone ± SD ($n = 5$) of various concentrations of *Piper betle* crude extract (PBC) and *Piper betle* essential oil (PBO) against *Candida albicans* and *Streptococcus mutans*. Inhibition zone of less than 6 mm was defined as not detected (ND). Statistical difference in various concentration of additives was compared within the same column, and the same superscript capital letters indicate no significant difference in inhibition zone ($p > 0.05$)

Condition	*Candida albicans*, mean inhibition zone ± SD (mm)		*Streptococcus mutans*, mean inhibition zone ± SD (mm)	
	PBC	PBO	PBC	PBO
Control (DMSO)	ND	ND	ND	ND
Nystatin	12.41 ± 0.39^{A}	12.41 ± 0.36^{E}	–	–
5% v/v of 2% chlorhexidine	–	–	22.16 ± 1.15^{I}	23.55 ± 2.29^{M}
0.625%	ND	ND	ND	ND
1.25%	ND	ND	ND	ND
2.5%	9.02 ± 0.48^{B}	9.22 ± 0.34^{F}	7.99 ± 0.89^{J}	ND
5%	14.94 ± 0.49^{C}	13.99 ± 0.42^{G}	13.13 ± 0.84^{K}	ND
10%	21.77 ± 1.43^{D}	18.03 ± 0.55^{H}	16.14 ± 0.85^{L}	9.19 ± 0.77^{N}

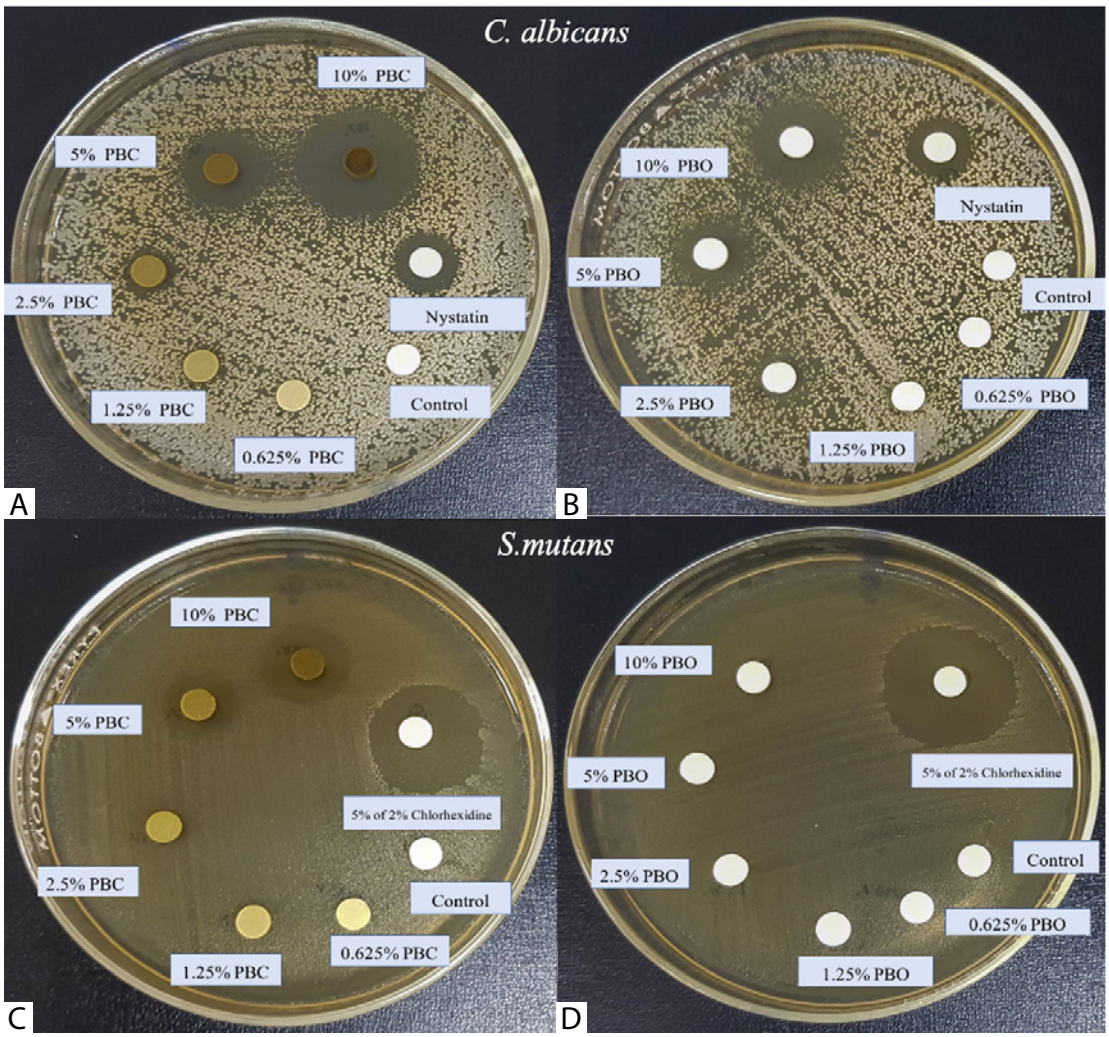

FIGURE 1. Agar disc diffusion showing inhibition zone of various concentration of *Piper betle* crude extract (PBC) and *Piper betle* essential oil (PBO) against *Candida albicans* (**A, B**) and *Streptococcus mutans* (**C, D**)

ANTI-MICROBIAL ACTIVITY OF GC SOFT LINER INCORPORATED WITH PBC AND PBO

The anti-fungal and anti-bacterial activities of GC soft liner combined with various concentration of PBC and PBO are illustrated in Figures 2 and 3, respectively. The mean inhibition zone of PBC and PBO against *C. albicans* and *S. mutans* is shown in Table 4. GC soft liner with additive demonstrated inhibitory effect on *C. albicans* with 5% w/w PBC and 20% v/v PBO, while the inhibitory zone of *S. mutans* was detected in GC soft liner with 10% w/w PBC and 60% v/v PBO. The inhibition zones increased as the concentration of PBC and PBO also increased.

EFFECTS OF PBC INCORPORATED INTO GC SOFT LINER ON CYTOTOXICITY OF HGF CELL LINE

The results of anti-bacterial and anti-fungal tests in this present study showed that 5% and 10% w/w PBC presented anti-bacterial and anti-fungal effects. Therefore, MTT assay was conducted to investigate the cell viability of HGF cell line after exposing to soft lining materials with 5% and 10% w/w PBC, 5% v/v of 2% chlorhexidine gluconate, and 30% v/v of nystatin oral suspension comparing with soft lining materials without additives. Statistical analysis with one-way ANOVA test showed no significant difference among soft lining materials with additives, compared with no additive (Figure 4).

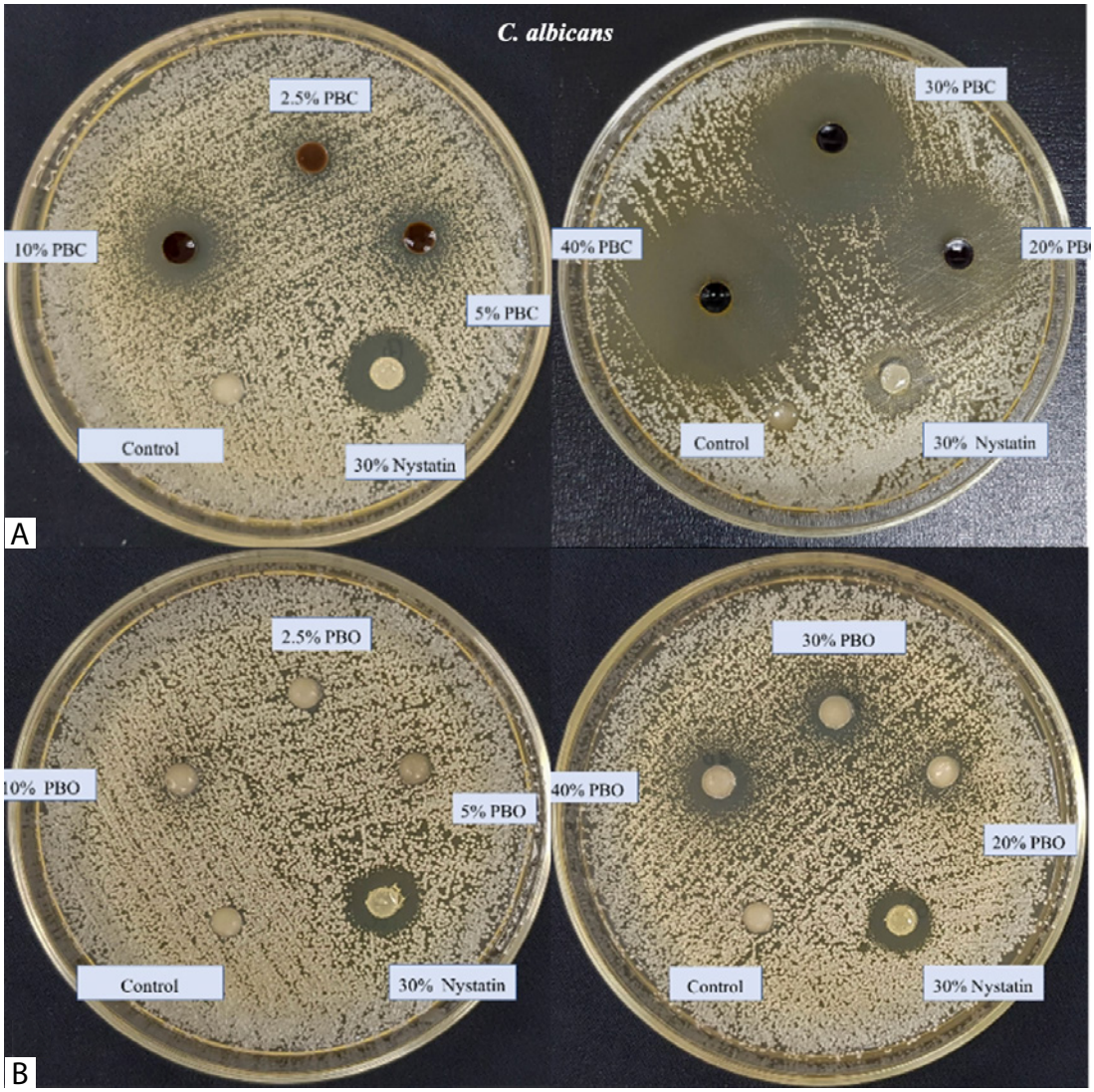

FIGURE 2. Inhibition zones of various concentration of GC soft liner incorporated with (**A**) *Piper betle* crude extract (PBC) and (**B**) *Piper betle* essential oil (PBO) against *Candida albicans*

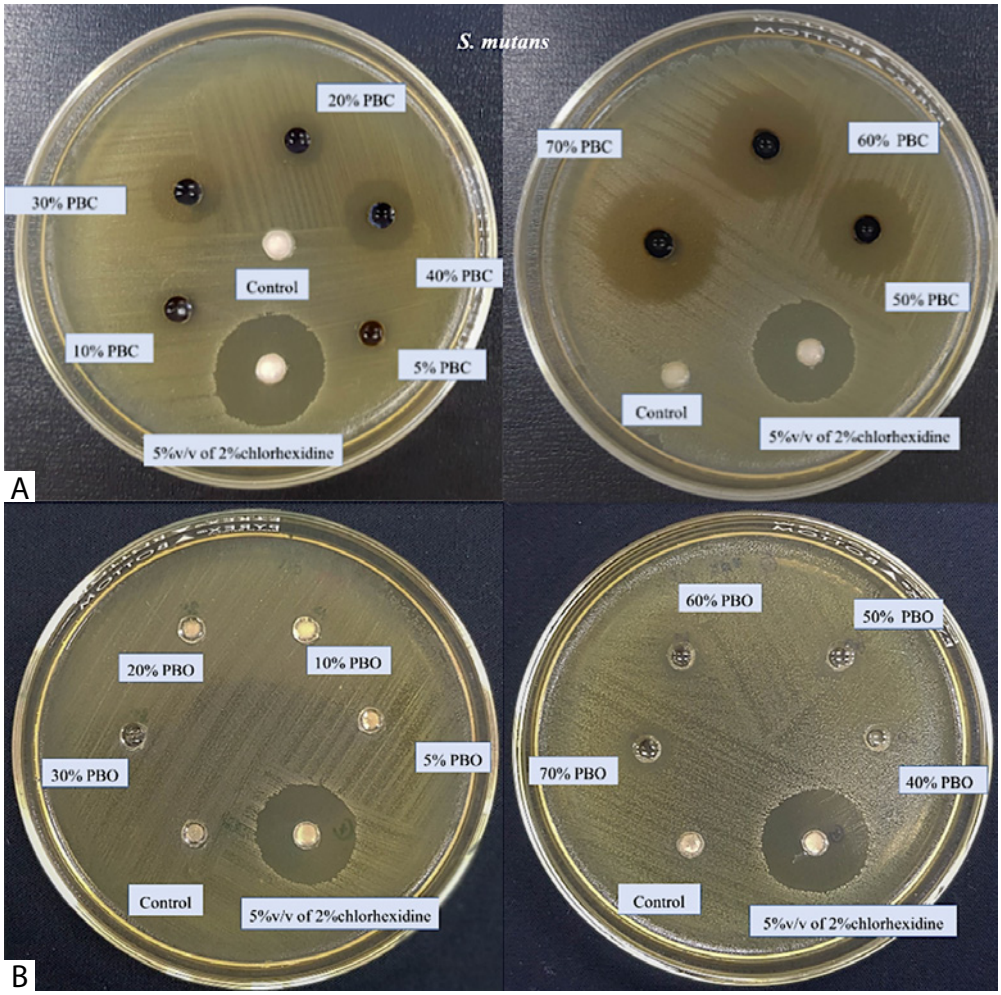

FIGURE 3. Inhibition zones of various concentration of GC soft liner combined with (**A**) *Piper betle* crude extract (PBC) and (**B**) *Piper betle* essential oil (PBO) against *Streptococcus mutans*

TABLE 4. Mean inhibition zone ± SD ($n = 5$) of various concentrations of GC soft liner incorporated with *Piper betle* crude extract (PBC) and *Piper betle* essential oil (PBO) against *Candida albicans* and *Streptococcus mutans*. Inhibition zone of less than 6 mm was defined as not detected (ND). Statistical difference in various concentration of additives was compared within the same column, and the same superscript capital letters indicate no significant difference in inhibition zone ($p > 0.05$)

Condition	*Candida albicans*, mean inhibition zone ± SD (mm)		*Streptococcus mutans*, mean inhibition zone ± SD (mm)	
	PBC	PBO	PBC	PBO
Negative control (no additive)	ND	ND	ND	ND
30% nystatin	12.78 ± 0.70^{A}	11.84 ± 0.88^{G}	–	–
5% of 2% chlorhexidine	–	–	21.19 ± 0.56^{K}	21.96 ± 0.76^{S}
2.5%	ND	ND	–	–
5%	7.75 ± 0.34^{B}	ND	ND	ND
10%	10.88 ± 1.20^{C}	ND	8.38 ± 0.47^{L}	ND
20%	18.63 ± 0.68^{D}	7.58 ± 0.36^{H}	11.67 ± 0.77^{M}	ND
30%	23.33 ± 1.07^{E}	9.60 ± 0.38^{I}	14.47 ± 0.60^{N}	ND
40%	26.63 ± 1.03^{F}	13.69 ± 0.86^{J}	17.02 ± 1.01^{O}	ND
50%	–	–	19.41 ± 0.35^{P}	ND
60%	–	–	25.01 ± 1.15^{Q}	8.31 ± 0.16^{T}
70%	–	–	26.76 ± 0.96^{R}	9.30 ± 0.12^{U}

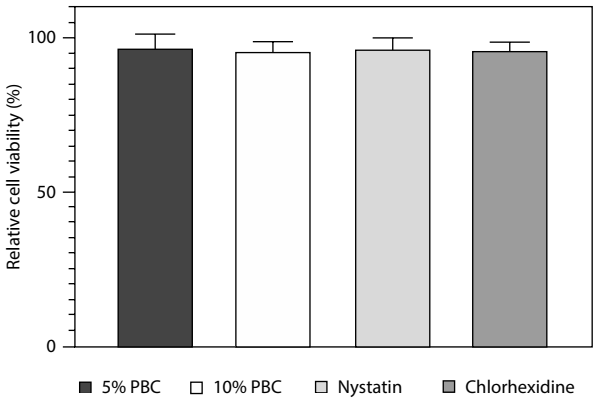

FIGURE 4. Percentages of cell viability of human gingival fibroblast (HGF) exposed to GC soft liner incorporated with *Piper betle* crude extract (PBC), nystatin, and chlorhexidine compared with GC soft liner with no additive. No statistical difference was found among the groups ($p > 0.05$)

DISCUSSION

Because of the overuse of conventional antibiotics, natural-derived medicines, such as many herbal plants with lower toxicity have been investigated. *P. betle* leaves containing 0.7-2.6% essential oils, mainly phenolic and terpenoid compounds, were examined as an antimicrobial agent [16, 17]. *P. betle* leaves can be processed through extraction to obtain the crude or distillation to obtain the oil. *P. betle* contains a variety of biologically active compounds, the concentration of which depends on the plant's diversity, climate, harvesting time, and geographic region [18].

TABLE 5. Molecular weight of *Piper betle* crude extract (PBC), *Piper betle* essential oil (PBO), and GC soft liner used in this study

Compounds	M.W. (g/mol)
PBC	
4-chromanol	150.17
Eugenol	164.20
PBO	
Eugenol	164.20
Acetyl-eugenol	206.24
4-allyl-1,2-diacetoxybenzene	234.25
Hydroxychavicol	150.17
Caryophyllene	204.35
GC soft liner liquid	
Butyl phthalyl butyl glycolate	336.38
Dibutyl phthalate	278.34
Ethanol	46.07

The compositions of 4-chromanol, eugenol, and hydroxychavicol play important roles in antiseptic and anti-microbial properties [7, 19]. Previous studies showed PBC containing 4-chromanol and eugenol, which acted as anti-candidal agents, and MIC against *C. albicans* ranged from 0.125 to 25 mg/ml [20-22]. Other studies found that MIC against *S. mutans* ranged from 1.56 to 10 mg/ml [18]. Correspondingly, MIC of PBC against *C. albicans* and *S. mutans* in this study was 2.5% w/w. Moreover, another study found that 4-chromanol within PBC contained anti-bacterial and anti-biofilm properties. Therefore, PBC had dual action in preventing and eradicating biofilm formation [23]. According to Nalina *et al.*, PBC could damage the bacterial cell membrane and reduce acid production [24]. In addition, Kawsud *et al.* demonstrated that 4-chromanol was a crucial component of PBC that acted as an anti-candidal agent by preventing biofilm formation and eliminating existing biofilm [12]. Regarding PBO, previous studies have found that MIC of PBO against *C. albicans* was ranging from 0.078% to 1.6% v/v [9-11]. PBO's main ingredient, hydroxychavicol, is responsible for both antifungal and anti-bacterial actions by altering cell membrane structure, which affects microbial membrane permeability [9, 25]. Furthermore, the eugenol in PBO and PBC has anti-bacterial effect. Compounds that react or interact with nucleophilic groups or solutes in the cell membrane and cause it to malfunction, have unspecific anti-candidal mechanisms of action [25]. On the contrary, other earlier studies found that eugenol did not exhibit anti-candidal and anti-bacterial activities [12, 23]. In this study, PBC contained 4-chromanol and eugenol, both of which have anti-bacterial and anti-candidal effects, whereas PBO only contains eugenol and some hydroxychavicol. This could explain why PBO presented lower anti-bacterial and anti-candidal properties than PBC.

Polyethyl or polymethyl methacrylate resin is a major component of soft lining materials, and it is combined with a plasticizer, such as dibutyl phthalate (MW = 278.34 g/mol) or ethanol (MW = 46.07 g/mol). Because of the low molecular weight (MW) of the plasticizer, the anti-bacterial agent was gradually released from soft lining material molecules, making the soft lining material itself a drug carrier. The low molecular weight composition of PBC used in this study (Table 5) could possibly leach out and maintain its effective dose.

Regarding the mechanical properties of GC soft liner, Kumpanich *et al.* demonstrated that 5% w/w PBC incorporated into GC soft liner did not significantly affect the material's gelation time and surface hardness. This 5% w/w PBC was the optimum concentration that should promote inhibitory effect against *C. albicans* without a dramatic change in material properties. This study also found that incorporating 10% w/w PBC into GC soft liner resulted in significantly longer gelation time and an alteration in the material's surface hardness. The gelation

time is related to the material setting and working times. As the gelation time increases, the working time also increases. Moreover, the longer gelation time also affect the hardness of GC soft liner. The study showed that the hardness of 10% w/w PBC incorporated into GC soft liner at 2-hr and 7-day incubation time was 13.44 ± 1.53 and 21.98 ± 1.40, respectively [8]. However, according to ISO:10139-1:2018 standard, the hardness of soft material should be within a 30-50 units at 2-hr incubation time, and should not exceed 60 units at 7-day incubation time, while the hardness of extra soft material should be lower than 30 units at 2-hr incubation time and 60 units at 7-days incubation time [26]. Therefore, the hardness of GC soft liner incorporating 10% w/w PBC was in accordance with ISO:10139-1:2018 standard.

Adverse reactions, including allergies and local chemical irritation, may occur from materials used in the oral cavity due to direct and indirect contacts with the oral mucosa [27]. Roy *et al.* [28] used murine (Ehrlich Ascites Carcinoma and Melanoma B-16 cells) and human cancer cell lines to investigate the cytotoxic effect of PBC extract. They found that the concentration of PBC had an effect on cell viability. Although PBC extract was cytotoxic to both normal and tumor cell lines, tumor cells were more lethal than normal cells. On the other hand, Valle *et al.* [29] concluded that PBC was not harmful to normal human dermal fibroblasts. Previous studies demonstrated that GC soft liner was less cytotoxic than other commercial products, such as Coe Comfort and Kerr FITT [30]. Consistently, the current study found that PBC incorporated into GC soft liner had no cytotoxicity on HGF cell line.

Therefore, denture wearers benefit from the incorporation of anti-fungal and anti-bacterial substances into soft lining material to avoid infection. As a result, it may reduce inflammation as well as injury of tissue. However, PBC has not been investigated in clinical trials due to its dark green color and difficulty in preparation. Purification of a PBC active ingredient should be examined in future research that could lead to its therapeutic application.

CONCLUSIONS

According to this i*n vitro* study, PBC and PBO promote anti-microbial activity against *C. albicans* and *S. mutans*. However, when compared to PBC, GC soft liner combined with PBO demonstrated lower anti-bacterial and anti-candidal efficacy. The application of GC soft liner incorporated with PBC at an optimal concentration of 10% w/w showed anti-microbial activity against *C. albicans* and *S. mutans*, with no difference in cytotoxicity. PBC is a natural agent that could be used to treat denture stomatitis and decrease secondary caries with no harmful effect.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Jindaporn Puripattanavong from Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand for providing *Piper betle* extract, and Dr. Widchaya Kanchanavasita for his advice on statistical analysis.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

References

1. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011; 20: 251-260.
2. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida-associated denture stomatitis. Aetiology and management: a review. Part 1. Factors influencing distribution of Candida species in the oral cavity. Aust Dent J 1998; 43: 45-50.
3. Odds FC. Candida and candidosis: a review and bibliography. 2nd ed. London: Baillière Tindall; 1988, p. 466.
4. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med 1999; 10: 99-116.
5. Schneid TR. An in vitro analysis of a sustained release system for the treatment of denture stomatitis. Spec Care Dentist 1992; 12: 245-250.
6. Nakamura CV, Ishida K, Faccin LC, et al. In vitro activity of essential oil from Ocimum gratissimum L. against four Candida species. Res Microbiol 2004; 155: 579-586.
7. Dwivedi V, Tripathi S. Review study on potential activity of Piper betle. J Pharmacogn Phytochem 2014; 3: 93-98.
8. Kumpanich J, Eiampongpaiboon T, Kanchanavasita W, Chitmongkolsuk S, Puripattanavong J. Effect of Piper betle extract on anti-candidal activity, gelation time, and surface hardness of a short-term soft lining material. Dent Mater J 2020; 39: 1016-1021.
9. Makkar N, Prasanna S, Singla H. Comparative evaluation of antifungal activity of Piper Betle leaf oil, Origanum vulgare essential oil and fluconazole suspension on Candida albicans; an in vitro study. J Indian Assoc Public Health Dent 2017; 15: 89-93.
10. Kaypetch R, Thaweboon S. Antifungal property of Piper betle leaf oil against oral candida species. MATEC Web Conf 2018; 242: 01021. DOI: https://doi.org/10.1051/matecconf/201824201021.
11. Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F. Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crops Prod 2015; 71: 197-204.
12. Kawsud P, Puripattanavong J, Teanpaisan R. Screening for anti-candidal and antibiofilm activity of some herbs in Thailand. Trop J Pharm Res 2014; 13: 1495-1501.
13. Hong G, Maeda T, Murata H, Sasaki K. The dynamic viscoelasticity and plasticizer leachability of tissue conditioners. Gerodontology 2012; 29: 284-291.
14. ISO 10993-5. Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity. International organization for standardization: Geneva; 2009.
15. ISO 10993-12. Biological evaluation of medical devices – Part 12: Sample preparation and reference materials. International organization for standardization: Geneva; 2021.

16. Datta A, Ghoshdastidar S, Singh M. Antimicrobial property of Piper betle leaf against clinical isolates of bacteria. Int J Pharm Sci Res 2011; 2: 104-109.
17. Alighiri D, Eden W, Cahyono E. Study on the improvement of essential oil quality and its repellent activity of Betle leaves oil (Piper betle L.) from Indonesia. Orient J Chem 2018; 34: 2913-2926.
18. Deshpande SN, Kadam DG. GCMS analysis and antibacterial activity of Piper betle (Linn) leaves against Streptococcus Mutans. Asian J Pharm Clin Res 2013; 6: 99-101.
19. Napisah H, Azmahani A, Zubaidi AL, Intan A, Nazifah A. A preliminary study on the antimicrobial properties of several plants collected from Terengganu, Malaysia. J Agrobiotech 2011; 2: 99-106.
20. Sivareddy B, Reginald BA, Sireesha D, Samatha M, Reddy KH, Subrahamanyam G. Antifungal activity of solvent extracts of Piper betle and Ocimum sanctum Linn on Candida albicans: an in vitro comparative study. J Oral Maxillofac Pathol 2019; 23: 333-337.
21. Gehan J, Charmalie LA, Buddhie SN. Anti-candidal activity of Piper betle (L.), Vitex negundo (L.) and Jasminum grandiflorum (L.). Afr J Microbiol Res 2014; 8: 2307-2314.
22. Himratul-Aznita WH, Mohd-Al-Faisal N, Fathilah AR. Determination of the percentage inhibition of diameter growth (PIDG) of Piper betle crude aqueous extract against oral Candida species. J Med Plant Res 2011; 5: 878-884.
23. Teanpaisan R, Kawsud P, Pahumunto N, Puripattanavong J. Screening for antibacterial and antibiofilm activity in Thai medicinal plant extracts against oral microorganisms. J Tradit Complement Med 2017; 7: 172-177.
24. Nalina T, Rahim Z. The crude aqueous extract of Piper betle L. and its antibacterial effect towards Streptococcus mutans. Am J Biotechnol Biochem 2007; 3: 10-15.
25. Ali I, Khan FG, Suri KA, et al. In vitro antifungal activity of hydroxychavicol isolated from Piper betle L. Ann Clin Microbiol Antimicrob 2010; 9: 7. DOI: 10.1186/1476-0711-9-7.
26. ISO 10139-1. Dentistry – Soft lining materials for removable dentures – Part 1: Materials for short-term use. International Organization for Standardization: Geneva; 2018.
27. Landayan JIA, Manaloto ACF, Lee JY, Shin SW. Effect of aging on tear strength and cytotoxicity of soft denture lining materials; in vitro. J Adv Prosthodont 2014; 6: 115-120.
28. Roy U, Vijayalaxmi K. Evaluation of cytotoxic activity of Piper betle L. using murine and human cell lines in vitro. J Sci Eng Res 2013; 4: 221-233.
29. Valle D Jr, Cabrera E, Puzon JJ, Rivera W. Antimicrobial activities of methanol, ethanol and supercritical CO2 extracts of Philippine Piper betle L. on clinical isolates of Gram-positive and Gram-negative bacteria with transferable multiple drug resistance. PLoS One 2016; 11: e0146349. DOI: 10.1371/journal.pone.0146349.
30. Okita N, Hensten-Pettersen A. In vitro cytotoxicity of tissue conditioners. J Prosthet Dent 1991; 66: 656-659.