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Development of Hand Sanitizer Formulated with Essential Oil from Piper betle Grown in Yogyakarta, Indonesia

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Abstract

The purpose of this study was to characterize the metabolites in *Piper betle* essential oil and to determine the antibacterial activity against *S. aureus* and *E. coli* in order to develop into a hand sanitizer. Betel leaves were collected in five districts in Jogjakarta (Sleman, Kota Madya, Kulon Progo, Gunung Kidul, and Bantul). The chemical compounds in essential oil was identified using GC-MS. The antibacterial activity was determined using the Minimum Inhibitory Concentration (MIC) and Minimum Bacterisidal Concentration (MBC). There were 34 components identified in *betel* oils, with the eugenol, acetyl eugenol, and chavicol acetate being the most abundant. Betel oil from Sleman district showed the highest inhibitory activity against *S. aureus* with MIC 0.313% v/v and MBC 0.625%v/v, and against *E. coli* with MIC 0.078%v/v and MBC 0.156%v/v. The essential oil from Sleman District was used in the production of a gel and cream hand sanitizer. The gel product was transparent, soft, and homogeneous, whilst the cream was beige in colour and homogenous. Both the gel and cream hand sanitizers meet the standard for topical preparations. The gel and cream were also well-accepted, as evidenced by the average value of respondents' responses.

Keywords: *Piper betle* essential oil, metabolic profiles, quality control, hand sanitizer

Introduction

Infectious diseases are featured among the top ten diseases affecting Indonesia, including upper respiratory tract infections, diarrhoea, tuberculosis, dengue fever, intestinal worms, skin diseases, malaria, HIV/AIDS, and diphtheria. Hand hygiene is regarded as a critical factor in the fight against infection, particularly when it comes to preventing the spread of bacteria that cause gastrointestinal and respiratory diseases. Despite repeated emphasis on the importance of hand hygiene, recent studies indicate that populations have low hand hygiene compliance. According to the Ministry of Health of the Republic of Indonesia, Indonesians continue to have poor hand hygiene practices, with an average of only 12% of the population practicing proper hand hygiene (Ministry of Health RI, 2020). The issue persists, and it has been established that the availability of water in households and public facilities correlates with the frequency of handwashing. When soap and water are unavailable, hand sanitizers and disinfectants are the best choices for preventing infection transmission by inactivating and limiting the growth of microorganisms (Azelee *et al*., 2020; Golin *et al.*, 2020).

Alcohol-based hand sanitizers (ABHS) have become a popular alternative to traditional handwashing with soap and water in both clinical and community settings. ABHS have been shown to be an effective alternative to handwashing in preventing the spread of bacterial and viral diseases, making them an integral part of the healthcare burden reduction routine (Golin *et al*., 2020). When the COVID-19 pandemic spread over the world, the use of ABHS grew tremendously, resulting in a market shortage of sanitizers. Consumer habits and patterns are rapidly changing in response to the COVID19 issue, and the exceptional demand for hand sanitizers is expected to persist for a lengthy period of time as the "new normal" (Alghamdi, 2021; Fallica *et al*., 2021). However, excessive use of hand hygiene products can result in skin reactions including a common irritant contact dermatitis and cause skin irritation, dryness, and pore enlargement, making delicate skin more prone to infection (Azelee *et al.*, 2020; Fallica *et al*., 2021). Today, herbal disinfectants are being promoted in the industrial sector as an alternative and environmentally acceptable method of disinfection. Numerous researchers from a variety of businesses are investigating novel antimicrobials for use in the production of phytochemicals (Alghamdi, 2021; Booq *et al*., 2021).

Piper betle is a medicinal plant with antimicrobial and other pharmacological properties, including antioxidant, antimicrobial, antifungal, antiplatelet, anti-inflammatory, immunomodulatory, gastroprotective, and antidiabetic activity (Lubis & Wahyuni, 2020; Sanubol, *et al*., 2017; Srinivasan *et al*., 2016). *Piper betle* has been extensively researched as a nutraceutical/commercial product due to its diverse range of applications and widespread cultivation worldwide (Nayaka *et al*., 2021). Because betel essential oil has been shown to be effective against bacteria and fungi (Karak *et al*., 2018; Musdja *et al*., 2019; Nayaka *et al*., 2021; Singh *et al*., 2021) it is frequently used in hand sanitizers. It contains terpenoid compounds such as eugenol, chavicol, linalool, eucalyptol, and others terpenoid compounds (Karak *et al*., 2018; Madhumita *et al*., 2019; Murata *et al*., 2009; Singh *et al*., 2018). The concentration and composition of biologically active chemicals in betel essential oil vary depending on plant species, geographical location, growth, soil/mineral composition, season, climate, and extraction methods (Alighiri *et al*., 2018; Bhattacharya *et al*., 2016; Ghani *et al*., 2016; Islam *et al*., 2020; Purba & Paengkoum, 2019) which may cause variations in product efficacy and safety (Kunle *et al*., 2012; Lee *et al*., 2017; Wang *et al*., 2015). In this study, to ensure the effectiveness of the hand sanitizer, a quality control using metabolic profiling was created and applied to betel essential oils from five districts in Yogyakarta Special Region. Multivariate analysis using PLSDA with GC-MS fingerprint data were used to further evaluate the similarity and differences between the samples. The essential oil with the greatest antimicrobial activity was chosen to be developed further into gel and cream hand sanitizers. To project market acceptance of the products, the physicochemical properties and consumer hedonic test were also evaluated.

Materials and Methods

Chemicals

Na_2SO_4 anhydrite was purchased from Merck, Trypticase Soya Agar (TSA) was purchased from Oxoid. Carbopol 940, stearic acid, glycerine, cetyl alcohol, propylene glycol, and propyl paraben were purchased from Sigma-Aldrich. C*era alba* was purchased from Brataco.

Isolation of essential oils

Piper betle leaves were collected from five districts at Jogjakarta (Sleman, Kota Madya, Kulon Progo, Gunung Kidul, and Bantul), 3 each. The voucher specimen was deposited at the Laboratory of Pharmaceutical Biology, Department of Pharmacy, Universitas Islam, Indonesia. Leaves were washed thoroughly, sliced into 3-4 parts subjected to Indonesian Herbal Pharmacopoeia. Extraction of essential oil was performed using water-distillation apparatus for 3 hours. The essential oil layer was then dried over anhydrous sodium sulphate. Then, the essential oils were taken in a dark glass flask and stored at 4°C until analysis. Essential oil yield (%) was calculated using the formula:

$$\boldsymbol{Yield}\ (\%) = \frac{\boldsymbol{mass\ of\ essential\ oil\ (g)}}{\boldsymbol{mass\ of\ dry\ matter\ (g)}}\ \boldsymbol{x\ 100}$$

Analysis of the essential oil components

The essential oils were analysed by Shimadzu GCMS-QP2010 SE equipped with Rtx-5MS capillary column (0. 25 mm × 30 m, 0. 25-µm film thickness) (Restek, Bellefonte, USA) in Integrated Laboratory, Universitas Islam Indonesia. GC temperature program: 60.0°C; Injection temperature: 200.0°C; Injection mode: split; Flow control mode: Pressure 36.2 kPa. Total flow 101.3 mL/min. Column Flow: 0.75 mL/min. Linear velocity: 31.6 cm/sec. Purge flow: 3.0 mL/min. Carrier gas: helium. Detector: MS Ion source temperature: 200.0oC. Interface temperature: 250.0oC. MS mode: EI. Detector voltage: 0.1 kV. Mass range: 40-400 Da. Scan speed: 1250 Da/s.

Aliquots of essential oil samples from five districts in Jogjakarta were automatically injected into the GC-MS system. Data were collected in full scan mode (m/z 40-400). Identification of the volatile components was determined by matching their recorded mass spectra with those stored in the Wiley GC-MS library data system and based on the comparison of their mass spectra with those in the NIST05 database. The relative content of each constituent was determined by area normalization.

Metabolic profiling of essential oils

The resulting peak list was then exported to Microsoft Excel to generate matrix consist of name of the compound; RT, and area under curve (AUC). Peaks were retained in the sample if appeared in the minimum 2 out of 3 samples peaks. The matrix was exported to comma-separated value (CSV) format prior analysis using MetaboAnalyst (Xia & Wishart, 2016).

Anti-microbial testing of essential oils

The antimicrobial activity of essential oils was assessed using two bacteria, *S. aureus* and *E. coli* using disk diffusion according to Manual of Antimicrobial Susceptibility Testing (Jorgensen & Ferraro, 2009; Ortez, 2005). *S. aureus* ATCC 25923 and *E. coli* ATCC 35218 were purchased from Fisher-Scientific. Sterile filter paper discs (6 mm in diameter, Whatman, Maidstone) was impregnated with 20 µL of essential oil. All discs were placed on TSA agar plates inoculated with bacteria 10^8 CFU/mL, Mc Farland standard) which were then incubated at 37°C for 24 h. The efficacy of the samples was determined by measuring the diameter of the zone of inhibition of microbial growth. The minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were observed according to Clinical and Laboratory Standards Institute (CLSI, 2012), using the microdilution method in 96-well microtiter plates. The essential oils were subtilized tripled for regulate a concentration range of 1.125–144 µg/mL. Then, 50 µL of each concentration of essential oils was added to 100 µL suitable broth growth medium in wells of a 96-well microplate, after 10 µL of regulated suspension of the organism was put to each well. Microplates of control and test were incubated for 37°C for 24 h.

Hand sanitizer formulation

Betel oil was developed into gel and cream hand sanitizers. For gel formulation, Carbopol was dissolved in 50 mL of hot distilled water, and propyl paraben was added. The betel essential oil was dissolved in glycerine and added to the Carbopol mixture. Propylene glycol was gradually added while the mixture was stirred at a higher speed until a homogeneous gel was formed.

For hand cream formulation, the oil phase, which contains stearic acid, *cera alba*, and cetyl alcohol, was stirred-heated at 65°C. The aqueous phase, which contained triethanolamine, glycerine, propyl paraben, and distilled water, was thoroughly mixed. The aqueous phase was then mixed with the oil phase until it was homogeneous, and a good cream mass was formed. The formed cream was mixed with betel essential oil until it formed a homogeneous cream mass.

Physical properties and stability of gel and cream handsanitizers

The handsanitizer were analyzed for physical properties to ensure they meet the requirements for topical preparations, which include organoleptic, homogeneity, dispersion, adhesion, viscosity, and pH. Physical stability of gel and cream were evaluated, including organoleptic properties, pH, homogeneity, and phase separation in temperature 4°, 25°, and 40°C until 8 weeks of storage (Bharath, 2013).

Sensory and irritation analysis of gel and cream handsanitizers

This research has obtained a letter of ethical clearance from the Faculty of Medicine UII with the number: 17/Ka.Kom.Et/70/KE/VI/2020 on June 30, 2020. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Thirty untrained panelists were given a questionnaire and asked to provide feedback on the sensory evaluation and irritation potential of the gel and cream hand sanitizers. Potential panelists must be at least 18 years old, physically and mentally healthy, without lesions on their skin, and with no history of allergies or sensitivity to the product under consideration. All volunteers provided informed consent. The gels or creams were applied to the palms and thoroughly rubbed until dry (this should take around 20 seconds). The responses were scored on a five-point hedonic scale, with 1 being the worst and 5 being the best: 1. Dislike very much; 2. dislike; 3. neither like nor dislike; 4. like; 5. like very much.

Results and Discussion

The yield of Piper *betel* essential oil

Betel essential oils obtained in this study has a distinctive odour, clear yellow, and slightly spicy bitter taste. The District Kota Madya has the highest yield of betel oil with 0.24 ± 0.05 compared to other districts (Table 1). The yield of betel oils obtained from Yogyakarta Special Province were lower compared to other Province. Previous report showed the yield of betel oils from Bogor District (West Java Province), Boyolali District (Central Java Province), and Makassar City (South Sulawesi District) were higher with 0.937 ± 0.003; 0.983 ± 0.004; and 1.055 ± 0.006 respectively (Alighiri *et al*., 2018). Differences in essential oil yield may be caused by altitude and climate parameters as a result of different sunlight exposure or climate change, which may account for the difference in decreasing nutrient absorption (Alighiri *et al*., 2018; Khorshidi *et al*., 2009). Furthermore, the method used to obtain the essential oil may have an impact on the extraction yield (Islam *et al*., 2020; Madhumita *et al*., 2019; Purba & Paengkoum, 2019). Alighri *et al*. (2018) used steam distillation in his study, whereas we used water and steam distillation.

Table 1. The yield of *Piper betle* essential oil from five districts in Yogyakarta

District	Leaves (g)	Essential oil (ml)	Weight of essential oil (g)	Yield	Yield average /district (w/w)
S1	2013	2,6	2.43	0,12%	
S2	1090	2.0	2.01	0,18%	0.15 ± 0.03
S3	1275	2,2	2.03	0,16%	
B1	1005	1.3	1.29	0,13%	
B2	1115	3.0	3.17	0,28%	0.21 ± 0.08
B3	1003	2.2	2.03	0,20%	
KM1	1005	2.0	1.87	0,19%	
KM2	1005	2.5	2.53	0,25%	0.24 ± 0.05
KM3	1008	2.9	2.85	0,28%	
KP1	1162	2.1	2.04	0,18%	
KP2	1000	2.1	2.08	0,21%	0.21 ± 0.04
KP3	1000	2.4	2.54	0,25%	
GK1	1002	1.8	1.57	0,16%	
GK2	1012	1.9	1.94	0,19%	0.17 ± 0.02
GK3	1007	1.6	1.50	0,15%	

S: Sleman; B: Bantul; KM: Kota Madya; KP: Kulon Progo; GK: Gunung Kidul

Essential oils composition

To evaluate the volatile compositions, aliquots of essential oil samples from five districts in Jogjakarta were automatically injected into the GC-MS system. Data were collected in full scan mode (m/z 40-400). Identification of the volatile components was determined by matching their recorded mass spectra with those stored in the Wiley GC-MS data system and based on comparison of their mass spectra with those in the NIST05 database. The relative content of each constituent was determined by area normalization. 34 chemical components were identified from the essential oil of the *Piper betle* leaves. Table 2 shows the 34 characterized components, their retention times, and their relative contents in each district. Chromatograms of essential oils from each district are shown in **Supplementary figure 1**.

Table 2. Relative area percentage of essential oils from *Piper betle* collected from five districts in Yogyakarta

Metabolites	RT (min)	Relative contents				
		S	KP	GK	KM	B
α-pinene	4.30	0.45 ± 0.34	-	0.45 ± 0.21	0.14 ± 0.13	-
camphene	4.51	-	-	0.28 ± 0.14	0.14 ± 0.12	-
β-phellandrene	4.79	1.6 ± 1.3	0.48 ± 0.45	2.00 ± 0.96	1.38 ± 1.29	0.21 ± 0.06
β-myrcene	4.95	0.27 ± 0.27	-	0.30 ±0.13	0.19 ± 0.17	-
α-terpinene	5.37	-	-	0.13 + 0.11	-	-
sabinene	5.58	0.58 ± 0.24	0.17 ± 0.02	0.53 ± 0.27	0.32 ± 0.02	-
eucalyptol	5.60	-	-	-	0.13 ± 0.03	-
γ-terpinene	5.96	0.29 ± 0.27	0.11 ±0.09	0.29 ± 0.07	0.18 ± 0.15	-
linalool	6.51	0.38 ± 0.24	0.24 ± 0.03	0.24 ± 0.21	0.25 ± 0.07	0.13 ± 0.02
4-terpineol	7.78	1.42 ± 0.36	1.07 ± 0.30	1.03 ± 0.20	0.99 ± 0.31	0.71 ± 0.27
methyl chavicol	8.06	0.68 ± 0.14	0.73 ± 0.16	0.81 ± 0.31	0.71 ± 0.29	0.39 ± 0.10
chavicol	8.88	4.43 ± 0.37	4.55 ± 0.98	5.58 ± 0.46	6.21 ± 0.49	3.26 ± 1.47
E-citral	9.08	-	0.37 ± 0.29	-	-	-
chavicol acetate	10.23	18.23 ± 1.92	25.21 ± 6.83	26.98 ± 4.83	26.93 ± 3.05	23.98 ± 2.63
3-allylguaiacol	10.41	0.10 ± 0.08	-	-	-	-
eugenol	10.64	23.61 ± 2.56	18.92 ±5.74	16.81 ± 3.79	21.28 ± 4.52	21.73 ± 3.34
α-copaene	10.73	0.50 ± 0.01	0.38 ±0.20	0.35 ±0 .05	0.31 ± 0.04	0.28 ± 0.04
β-elemene	10.91	0.98 ± 0.06	0.73±0.31	0.65 ± 0.03	0.63 ± 0.08	0.61 ± 0.07
methyleugenol	10.31	-	-	-	-	0.13 ±0.11
α-bergamotene	11.20	-	0.08 ± 0.06	-	-	0.06 ± 0.05
trans (beta)-caryophyllene	11.38	3.61 ± 0.24	2.52 ± 1.10	2.97 ± 0.27	2.61 ± 0.41	2.27 ± 0.74
zingiberene	11.48	-	-	0.14 ± 0.12	-	0.07 ±0.05
β-farnesene	11.65	0.17 ± 0.15	-	0.13 ± 0.11	-	0.09 ± 0.08
1,6,10-dodecatriene	11.71	-	-	-	0.10 ± 0.08	-
α-humulene	11.85	2.83 ± 0.06	2.08 ±0.87	2.38 ± 0.17	1.94 ± 0.55	1.92 ± 0.50
γ-muurolene	12.09	3.07 ± 0.54	1.94 ± 0.95	2.37 ± 0.27	1.17 ± 1.03	0.54 ± 0.47
α-morphene	12.10	-	-	-	-	1.27 ± 1.15
germacrene-D	12.20	4.39 ± 1.62	3.49 ± 1.13	4.64 ± 0.23	4.38 ± 0.57	3.67 ± 0.36
β-selinene	12.30	3.41 ± 0.18	2.60 ± 1.20	2.83 ± 0.25	2.26 ± 0.71	2.34 ± 0.61
α-selinene	12.40	4.43 ± 0.13	3.24 ± 1.50	3.77 ± 0.28	3.09 ± 0.63	3.06 ± 0.82
4-allyl-1,2-diacetoxybenzene	12.52	1.66 ± 0.83	3.01 ± 1.08	1.94 ± 0.26	1.73 ± 0.23	1.36 ± 1.18
acetyl eugenol	12.69	25.95 ± 0.89	28.13 ± 5.40	22.90 ± 2.37	23.24 ± 1.65	31.68 ± 5.39
germacrene B	13.23	-	0.11 ± 0.09	-	-	0.16 ± 0.05
caryophyllene oxide	13.59	-	-	-	-	0.07 ± 0.06

RT: retention time

Districts = S: Sleman; KP: Kulon Progo; GK: Gunung Kidul; KM: Kota Madya; B: Bantul

The five major components found in each district were acetyl eugenol (22.90% to 32.68%), chavicol acetate (18.23% to 26.98%), eugenol (16.81% to 23.61%), chavicol (3.26% – 6.21%), and germacrene D (3.49% −4.64%). According to Alighiri *et al.* (2018), the five major compounds in betel oil are eugenol (17.02% −19.21%); acetyl eugenol (9.62% −13.09%); chavicol (5.39% – 6.76%); β-caryophyllene (4.13 – 4.67); α-humulene (3.03%−3.52%). These variances could be due to the farming environment and the extraction method.

Multivariate analysis of the global metabolites in essential oils

We used partial least square discriminant analysis (PLSDA) to separate the groups in order to find the intrinsic variation in the data set. Using PLS-DA, group differentiation can be seen in Figure 1A. In PLS-DA, there are two importance measures: the variable importance in projection (VIP) and the weighted sum of absolute regression coefficients. We presented the top 15 features ranked by their contribution to group discrimination, as determined by the VIP score and analysis of variance (ANOVA, $p<0.05$) (Figure 1B). It is clear that chavicol, acetyl eugenol, γ-muurolene, trans beta caryophyllene, 4-terpineol, α-terpineol, eugenol, α-amorphen, α-selinene, β-selinene, α-humulene, β-humulene, β-elemene, α-copaene, sabinene, linalool, and β-phellandrene are varied highly. Other compounds are distributed homogeneously across areas.

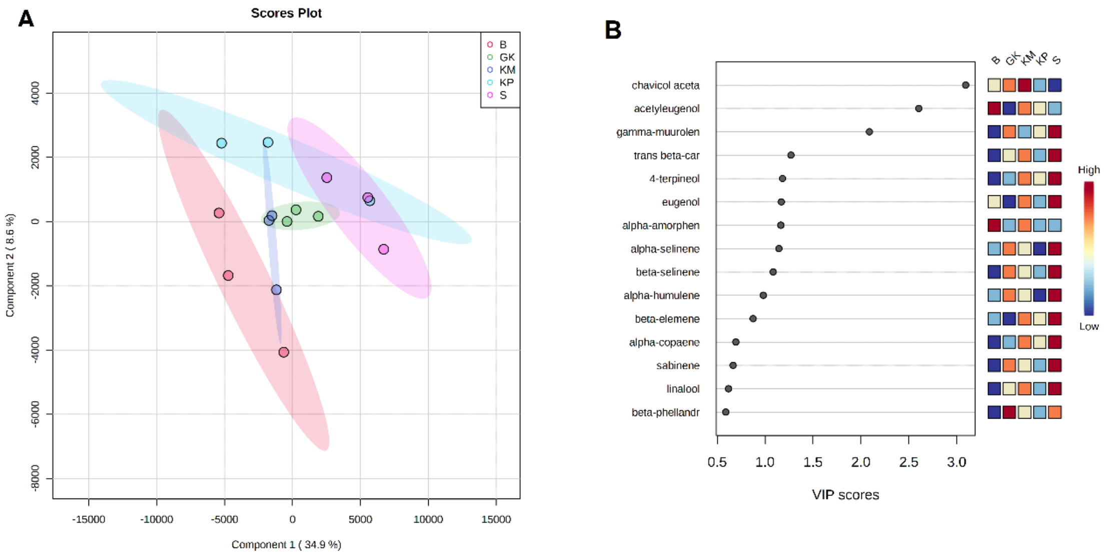

Figure 1. PLS-DA analysis and 15 compounds analysed by variable importance in projection (VIP) score (A). PLS-DA analysis of essential in five districts (regions display of 95% confidence) (B). The top 15 compounds ranked based on the VIP score. The coloured boxes on the right indicate the relative concentrations of the corresponding metabolite in each group. S: Sleman; B: Bantul; KM: Kota Madya; KP: Kulon Progo; GK: Gunung Kidul

According to VIP analysis, Bantul is rich with acetyl eugenol and α-amorphene. The least compound from Bantul area are γ-muurolene, *trans* β-caryophyllene, 4-terpineol, β-selinene, α-copaene, sabinene, linalool, and β-phellandrene. Gunung Kidul is rich with β-phellandrene and β-elemene. Kulon Progo is rich with E-citral, bergamotene, and germacrene B. The least compound from Kulon Progo area are α-selinene and α-humulene. Sleman is rich with γ-muurolene, *trans* β-caryophilene, 4-terpineol, α-selinene, β-selinene, γ-humulene, β-elemene, α-copaene, sabinene, and linalool. The least compound from Sleman district are 3-allylguaiacol and β-farnesene. Kota Madya is rich with chavicol acetate, eugenol, and germacrene D. The least compounds from the Kota Madya area are α-pinene and camphene.

A wide variety of volatile active compounds from betel leaves oil have been reported in the literature, which vary depending on the type of landrace, cultivation location, environment, and soil. Each country and possibly each region may have a distinct betel leaf essential oil, which may be classified as chemotypes (17,19). Volatile compounds are responsible for the aroma, taste and bioactivity, and are also employed for chemical fingerprinting. The PLSDA from GCMS data showed the characteristic of essential oil from each district. Essential oil from Sleman and Kulon Progo showed similarity of the metabolite profiles.

Antibacterial activities against *S. aureus* and *E. coli*

Antibacterial activity testing was performed on essential oils to determine the antibacterial activity against *S. aureus* and *E. coli*. Additionally, this test will compare the antibacterial activity of betel essential oil extracted from five districts in the Yogyakarta Province in order to determine which essential oil has the strongest antimicrobial activity that can be formulated into a hand sanitizer. The antibacterial activity of betel oils from five districts against *S. aureus* and *E. coli* is shown in Table 3.

Table 3. Zone of inhibition and MIC-MBC of the *Piper betle* essential oils

District sample	Zone of inhibition (mm) (mean ± SD)		MIC (v/v)	MBC (v/v)	MIC (v/v)	MBC (v/v)
	S. aureus	*E. coli*	S. aureus		*E. coli*	
Sleman	24.02 ± 2.0	14.67 ± 0.58	0.313%	0.625%	0.078%	0.156%
Bantul	21.67 ± 1.53	14.67 ± 0.58	0.313%	0.625%	0.313%	0.625%
Kota Madya	23.33 ± 1.15	15 ± 0.8	1.25%	2.5%	1.25%	2.5%
Kulon Progo	21.50 ± 3.04	16.33 ± 1.53	1.25%	2.5%	1.25%	2.5%
Gunung Kidul	22.67 ± 1.53	15 ± 1	1.25%	2.5%	1.25%	2.5%

Betel oil from Sleman district has the lowest MIC and MBC value against *S. aureus* and *E. coli* compared to other districts so it was chosen for the hand sanitizer formulations. The Sleman betel oil is rich with eugenol (23.61%), acetyl eugenol (25.95%), chavicol acetate (18.23%), and chavicol (4.43%).

Numerous reports show that eugenol has beneficial effects on common food source Gram-negative bacteria as *Escherichia coli*, Salmonella, Pseudomonas aeruginosa, and Gram-positive bacteria as *Staphylococcus*, *Streptococcus*, and *Listeria* (Hu *et al*., 2018). Eugenol and acetyl eugenol have been reported to cause membrane damage (Abdulhamid *et al*., 2021; Musdja *et al*., 2019) lysis of cells (Chouhan *et al*., 2017) and interfere in quorum sensing mediated virulence factors production and biofilm formation in bacteria (Srinivasan *et al*., 2016). Study in *E. coli*, eugenol has been reported to inhibit bacterial migration, inhibit bacterial adhesion, inhibit expression of virulence factors, and inhibit fimbriae formation (Hu *et al*., 2018; Kim *et al*., 2016). Hydroxy chavicol, a chavicol derivates has been reported to have bactericidal effects against *S. mutans, E. faecium, E. faecalis, S. sanguis, A. viscosus, H. actinomycetemcomitans, P. intermedia, F. nucleatum* and *P. gingivalis* (Nayaka *et al*., 2021). Hydroxy chavicol also inhibits biofilm formation in *S. mutans* (Sharma *et al*., 2009) blocking MurA that causes bacterial cell wall disruption. Betel oil from Sleman district was then formulated into gel and cream hand sanitizers.

Physical Evaluation of Hand Sanitizer

To meet the standards for topicals dosage form, the physical features of the gel and cream of betel oil hand sanitizer were examined. Table 4 shows the findings of organoleptic, homogeneity, pH, viscosity, dispersibility, and adhesion tests.

Table 4. Physical properties of *Piper betle* gel

Characteristics	***Piper betle* gel**	***Piper betle* hand cream**
Organoleptic	Slightly white transparent gel, thick, non sticky, smell of betel and citrus	Beige colour cream, soft, slightly sticky, smell of bubble gum

Homogeneity	Homogenous	Homogenous
Viscocity (cP)	5755 ± 78.07	6504 ± 94.48
pH	5.73 ± 0.11	5.76 ± 0.24
Dispersibility (weight 250 g)	6.47 ± 0.25	6.32 ± 0.15
Adhesion (s)	1.73 ± 0.10	5.42 ± 0,17

Value were expressed as mean ±SD

The resulting gel was transparent, thick, non sticky, and smell of betel and citrus (as the *corigen odoris*) (Figure 2). The cream was homogenous, smelled of buble gum (as the *corigen odoris*), and slightly sticky.

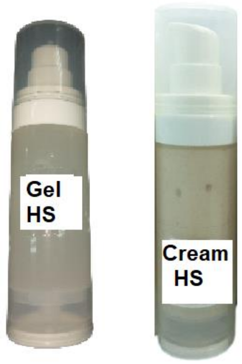

Figure 2. The gel and cream hand sanitizer of betel essential oil

The viscosity of the gel and cream was satisfactory because it fell within the 2000-50000 cP range stipulated by Indonesian National Standard (SNI) 16-4399-1996. A semisolid preparation requires a viscosity test to determine its flow qualities when applied to the skin. The high viscosity of the emulsion system in emulgel preparations will provide stability since it will decrease dispersion phase droplet movement, preventing droplet size changes to greater sizes and the potential of coalescence. The spreadability test was designed to determine the product's ability to spread well at free pressure so that it can be easily applied to the skin's surface. The active substance can be delivered more effectively if the gel/cream were well dispersed. The two products met with the criteria of dispersion range 5-7 cm (Bharath, 2013).

The gel and cream demonstrated a good adhesion time, allowing them to maximize the effect when comes into contact with the skin. The adhesivenes of the gel and the cream were 1.73 and 5.42 s respectively. The adhesivenes of cream to the skin was bigger compared to gel product due to the cera alba and cetyl alcohol. The pH of the gel and the cream were 5.73 and 5.76 met the requiremens of the topical product (Benson & Watkinson, 2012). Too alkaline or acidic a pH can cause skin problems such as dry skin and a burning sensation. In conclusion, the physical properties of the hand sanitizer gel and cream products meet the applicable requirements.

A pharmaceutical product may undergo change in appearance, consistency, uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability (Bajaj *et al*., 2012). The hand sanitizer products were subjected to high and low temperatures (4°, 25°, and 40° C) and the physical appearance of the products were observed during 8 weeks of storage (4). The color, PH, and homogeneity of the gel and cream were remain unchanged during the course. There was no phase separation of the products until 8 weeks showed the stability of the products (table 5).

Table 5. Stability test of the gel and cream

Parameter	Temp (°C)	Week															
		1		2		3		4		5		6		7		8	
		G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
Colour	4	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B

	25	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
	40	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
PH	4	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8
	25	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8
	40	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8	5.7	5.8
Homogeneity	4	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	25	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	40	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Phase separation	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

G: gel; C : cream; T: transparent; B: beige; H: homogenous

The Acceptability test of the gel and cream hand sanitizer

The acceptability test was performed to get the desirable attributes expected by the consumer through a sensory evaluation (hedonic test) perceived through the senses of sight, smell, and touch. Sensory aspects of topical preparations are critical in the consumer selection, acceptability, and loyalty. Consumer choice for a product is initially determined by their perception of its packaging, followed by their perception of its scent, appearance, and texture (tactile senses) (Mosquera *et al*., 2018; Sharif *et al*., 2017). The color, smell, texture, and sensation of the betel essential oil gel and cream preparation were evaluated overall, with five points scale: with 1 being the worst and 5 being the best: 1. dislike very much; 2. dislike; 3. neither like nor dislike; 4. like; 5. like very much. The skin condition of the respondent after using the betel essential oil gel product was also evaluated to observe the possibility of irritation. Tabel 6 showed what respondents thought of the resulting gel and cream.

Tabel 6. *Hedonic rating of essential oil gel and cream products*

	Color	**Odor**	**Texture**	**Moisturizing effect**	**Easy to use**	**Speed of dry**	**Non iritatif**	**Average score**
Gel	4.5	4.5	4.5	4.9	4.9	4.5	5	4.68
Cream	4.2	5	4	5	5	4.3	5	4.64

Description: The higher the points, the better the respondent's response. Point scale = 1. dislike very much; 2. dislike; 3. neither like nor dislike; 4. like; 5. like very much.

The responses of panelists to the betel essential oil hand sanitizers revealed a range of responses. Based on the total points from each assessment which is 4.68 for gel products and 4.64 for cream products, the respondent's opinion of these betel hand sanitizers products is favorable, or the respondent likes both products. According to the sensory evaluation (Table 6), gel was the preferred formulation over cream. Both gel and cream products did not cause skin irritation, so the product was acceptable and suitable for use. However, the panelists made recommendations to improve the odor of the gel and cream.

Conclusion

Metabolite profiling of betel essential oil and antimicrobial activity are important to determine the compositions of volatile compounds and to ensure the effectiveness of the hand sanitizers. The growing area and the methods of extraction of betel essential oils affects the active volatile compounds. The essential oil from Sleman District has the highest activity against *S. aureus* and *E. coli* and incorporated into gel and cream hand sanitizers. The physical properties, stability, and hedonic tests suggest that the gel and cream hand sanitizers were promising formulations as an alternative hand sanitizer.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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