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Preliminary assessment of *Polytrichum commune* extract as an antimicrobial soap ingredient

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ABSTRACT

Mosses have long been used in traditional Chinese medicine due to the presence of secondary metabolites which have shown high biological activities. In particular, these secondary metabolites have demonstrated effective antibacterial activity against pathogenic microorganisms. In this study, the influence of different extraction solvents on the antibacterial activities of the *Polytrichum commune* was carried out using the disc diffusion method. Results showed that both 12.5 mg/mL of methanol moss extract and 6.25 mg/mL of ethanol moss extract were the most effective concentrations against *Bacillus cereus* and *Pseudomonas aeruginosa*. Additionally, the *P. commune* extracts were included as an added ingredient in soap bases to produce antibacterial soap prototypes where the effectiveness of the soaps containing the extracts in removing microorganisms from actual test individuals was carried out. Results of the thumb impression test of test individuals showed that the growth of microbial reduced after washing hands with the usage of both liquid and solid soap with the addition of *P. commune* extracts. Moreover, the antibacterial soaps performed better in eliminating microorganisms in comparison to control soaps without *P. commune* extracts. Taken together, *P. commune* extract could be a good candidate as a value-added ingredient utilized to produce antibacterial soaps due to its antibacterial properties.

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1 Introduction

Soap is an essential daily product that is mainly used for cleaning purposes, particularly for hand washing. Handwashing with soap and water is necessary for one's hygiene to avoid getting sick and spreading infections to others for generations (Yawson and Hesse 2013). However, washing hands with plain soap alone is often not enough. It has been shown that hand washing with plain soap resulted in almost triple the percentage of bacterial infectious diseases transmitted to food as compared with antibacterial soap (Sajed et al. 2014). In addition, the antibacterial activities of the soap can be enhanced by adding some natural active ingredients from botanicals to reduce the negative effect on human skin (Riaz et al. 2009).

Bryophytes are essential in pharmaceutical uses as they are the source of many biologically active compounds (Mishra et al. 2014; Hanif et al. 2014). Many moss species contain unique secondary metabolites such as terpenoids, alkaloids, flavonoids, phenols, and other aromatic compounds with therapeutic potential (Greeshma and Murugan 2018). These secondary metabolites have been reported to possess effective antibacterial and antifungal activities against various pathogenic microorganisms (Chauhan et al. 2014). Among the Bryophytes, *Polytrichum commune* (also known as common haircap, great golden maidenhair, great goldilocks, common haircap moss, or common hair moss) extract carries antimicrobial potential capabilities to inhibit the proliferation of pathogenic cells (Klavina et al. 2015). In addition, *P. commune* extract was demonstrated to have antioxidation potential that was strongly correlated with total phenolic contents found in the extract (Hanif et al. 2014). The antioxidants present in *P. Commune* were able to neutralize the reactive oxygen species and prevent oxidative damage to human cells and tissues, which in turn were able to help in the treatment of skin diseases (Addor 2017). There are a few reports on the antibacterial, cytotoxicity, and antimicrobial activities of solvent extract of *P. commune* grown in different parts of the world including Turkey (Klavina et al. 2015; Nikolajeva et al. 2012; Sevim et al. 2017).

Pseudomonas aeruginosa is an opportunistic pathogen that can colonize a healthy person, causing severe infections, especially in those who have weak immune systems or hospitalized patients (Nguyen et al. 2018). Additionally, *Bacillus cereus* can quickly spread to food products that cause food-borne diseases, emetic or diarrheal syndrome (Savini 2016). These bacteria strains are commonly found in the environment such as water, soil, plants, and animals (including human beings) (Savini 2016; Nguyen et al. 2018), serving as a continuous threat to human health. As such, eliminating these harmful bacteria through the use of antibacterial soap can reduce the chances of infections. The most crucial element in preventing nosocomial infections by preventing touch and fecal-oral transfer of pathogens is hand hygiene, which is frequently equated with handwashing (Boyce and Pittet 2002). An essential public health measure is hand washing and it has long been acknowledged as a practical, useful, and economical method of preventing infectious infections (Burton et al. 2011; Tao et al. 2013). In this research, the antimicrobial activity of *P. commune* extract with methanol and ethanol against *P. aeruginosa* and *B. cereus* was evaluated to assess the potential of methanol and ethanol extract of *P. commune* as an ingredient of antibacterial soap.

2 Materials and Methods

2.1 Plant Material

P. commune moss sample was brought from Terra Living Gallery & Farm House, Malaysia. Nutrient agar, nutrient broth, Petri dishes, 100% methanol, and 95% ethanol were bought from Sigma-Aldrich, USA.

2.2 Preparation of Plant Extract

Fresh leaves of *P.commune* were cleaned and air-dried under room temperature until a consistent weight was achieved and ground using a blender to obtain a fine homogenous powder. Thereafter, the extraction process was carried out by mixing 1 g of the dried *P. commune* powder with 100mL of 95% ethanol and 100% methanol, respectively. The mixed samples were then further incubated in a shaking incubator for 48 h at 150rpm at 37°C (Oyesiku and Caleb 2015). After the incubation period, the extracts were centrifuged (4,696 × g, 20 min, 4°C) and filtered using Whatman filter paper. The filtrates were concentrated over a hot plate stirrer at 60 °C until a concentration of 100 mg/mL was attained and stored at 4 °C for further use (Dulger et al. 2009; Sharma et al. 2013).

2.3 Preparation of Bacterial Standard Inoculum

B. cereus and *P. aeruginosa* were streaked on a fresh nutrient agar (NA) plate and incubated for 48 h at 37°C (Kazemian et al. 2015). At the end of the incubation period, single colonies of both the bacteria were picked from the respective plates and inoculated into the fresh nutrient broth (NB) then incubated for an additional 24 hrs, at 150 rpm and 37°C temperature in a shaking incubator. Subsequently, the broth cultures were centrifuged at 2,860 ×g, for 10min at 4 °C and the bacterial pellet was further rinsed thrice using 0.85% NaCl to remove the residual of NB and finally resuspended in 0.85% NaCl. The cell densities of the final suspensions were determined using a spectrophotometer at OD_{600nm} to obtain a reading of approximately OD of 0.5, which corresponds to $\times 10^8$ CFU/mL. The bacterial cell suspensions were used as standard inoculum for the anti-microbial activity assays (Modarresi et al. 2015).

2.4 Antibacterial Assay of P. commune Extracts

A 2-fold serial dilution method was carried out for both extracted solutions. The *P. Commune* extracts in both ethanol and methanol solutions were diluted in a range from 0 to 100mg/mL.

2.5 Disc Diffusion Assay

Six filter paper discs (about 6 mm in diameter) were soaked with *P. Commune* extracts of different diluted concentrations and the control solutions (95% ethanol and 100% methanol) for 15 min. The soaked discs with *P. Commune* extracts and a Ciprofloxacin disc (10 µg/mL) used as control were then placed on nutrient agar plates lawn with 30 µL of the standard inoculum bacteria (OD_{600nm} = 0.5) for *B. cereus* and *P. Aeruginosa*in triplicates for each of the bacteria. The plates were then incubated for 48 hrs at 37 °C (Balouiri et al. 2016). Followed by the measurement of the diameter of the clearing zones.

2.6 Preparation of Soap with the Plant Extracts

Liquid and solid soaps were prepared with added *P. commune* extracts. For liquid soap, the optimal concentration of *P. commune* extracts which depicts the highest antibacterial effect shown from the diffusion assays was chosen in making liquid soap by diluting the *P. commune* stock extract with a liquid soap base (Craftiviti, Selangor, Malaysia). A liquid soap base without any addition of *P. commune* extract was used as a control. For the solid soap, a solid soap base (Craftiviti, Selangor, Malaysia) was used by melting the solid soap base in a water bath at 70 °C before mixing with the *P. commune* extract stock to achieve the desired concentration. Thereafter, the mixture was poured into a silicon mold and placed at room temperature for 24 hrs to solidify. The solid soap base without any addition of *P. commune* extract was used as control.

2.7 Antimicrobial Assay of P. commune Extract Soaps

An antimicrobial assay was carried out with six test subjects. All the test subjects were requested to homogenize the microbes on both hands by rubbing them together before the test. For each test subject, nutrient agar plates were used to evaluate the soaps' effectiveness by which each plate was separated into four compartments with two compartments allocated for 'before washing' as control replicates while the remainder of the two compartments were then used for the washing with control soap (right hand) and washing with *P. commune* extract soaps (left hand). For each of the compartments, the subjects performed thumb impressions on the agar plate. Thereafter, the plates were incubated at 37 °C for 24 hrs (Wijetunge and Perera 2016). Subsequently, the amount of microbial growth obtained after incubation was recorded and differentiated using qualitative indicators of 0, +, ++, and +++ (Strigáč et al. 2018) as shown in Figure 1, to compare and evaluate the efficacy testing of handwashing and antimicrobial handwash.

2.8 Statistical Analysis

All the data collected from the antibacterial assay of moss extract was analyzed using a T-test with a 95% confidence level.

3 Results and Discussion

3.1 Disc Diffusion Assay

From the assay conducted, the results showed that the methanol and ethanol extracts of *P. commune* demonstrated clear zones of inhibition for *B. cereus* and *P. aeruginosa* for all the measured concentrations. On the other hand, there was no clear zone of growth inhibition observed for *B. cereus* and *P. aeruginosa* when control soap was used. Additionally, with *B. cereus*, methanol extraction of the *P. commune* showed the largest clear zone of inhibition with a diameter of 11.3 mm at a concentration of 12.5 mg/mL as compared to ethanol extraction, where the clear zone of inhibition demonstrated a diameter of 10.7 mm at a concentration of 6.25 mg/mL. Both methanol and ethanol extraction solutions were able to produce larger clear zones when compared to the control antibiotic, Ciprofloxacin, at 10 µg/mL (Figure 2) which has a clear zone diameter of 7.0 mm.

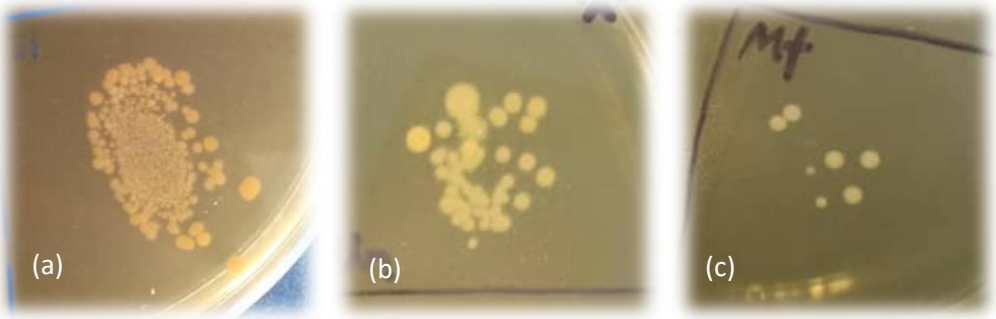

Figure 1 Microbial growth indicators representing their respective growth sizes as a form of qualitative measurement for the thumb impression test used as a form of an antimicrobial assay (a) High microbes growth was represented as + + +, (b) medium microbes growth was represented as + + while (c) low microbes growth was represented as +

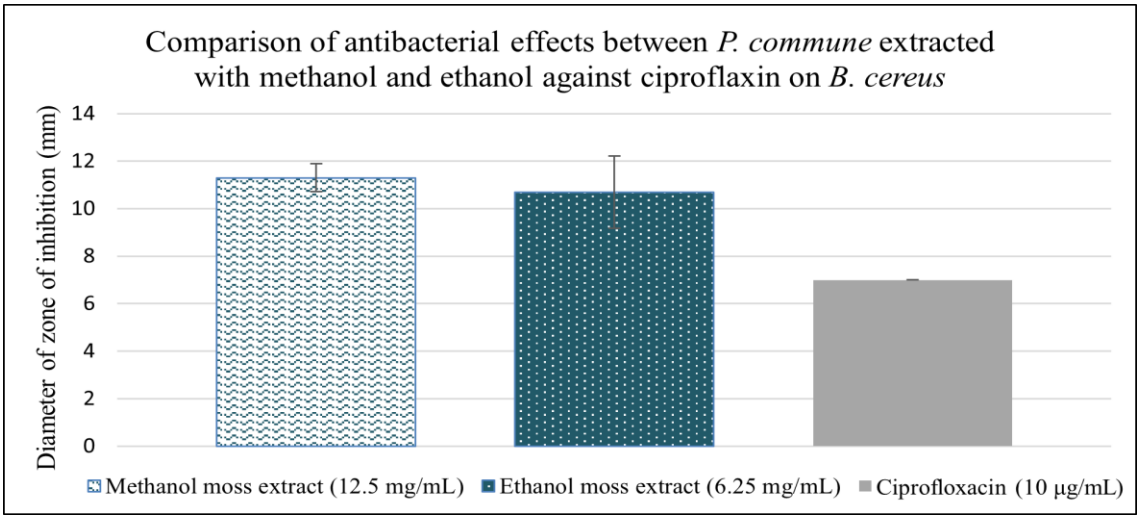

Figure 2 The comparison between the highest antibacterial effects of *P. commune* extracted with methanol at 12.5 mg/mL, *P. commune* extracted with ethanol at 6.25 mg/mL and ciprofloxacin at 10 µg/mL against *B. cereus* using disc diffusion assay.

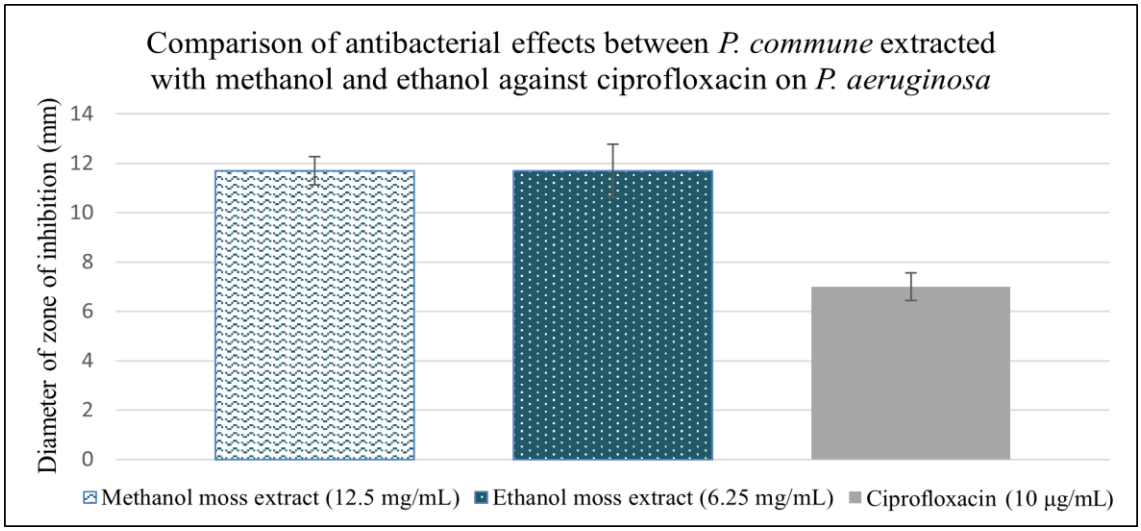

Figure 3 Comparison between the highest antibacterial effects obtained by *P. commune* extracted with methanol at 12.5 mg/mL, ethanol at 6.25 mg/mL and ciprofloxacin at 10 µg/mL on *P. aeruginosa* using disc diffusion assay

From the results obtained, this study demonstrated that the highest antibacterial effect against *B.cereus* and *P.aeruginosa* was seen with methanol extract of *P. commune* at a concentration of 12.5 mg/mL and for ethanol extract, this was reported highest at a concentration of 6.25 mg/mL. Although higher concentrations of methanol and ethanol extract of *P. commune* were also introduced into the agar medium, these concentrations failed to show a larger diameter of the growth inhibition zone, which suggests that an increase in concentration was unable to promote an additional antimicrobial effect. This may be because the amount of phenolic compounds that are present in the extraction is limited; further research is needed to confirm. The polarity of the compounds extracted under different solvents might affect their intrinsic bioactivity (Do et al. 2014). Thus, the polar solvent can be used to increase the solubility of the phenolic compound which increases the concentration of phenolic compounds that are present in *P. commune* extract to enhance the antimicrobial effect. In addition, the ability of extracted compounds to diffuse in different media that are used might also lead to differences in antibacterial efficiency (Do et al. 2014).

Contrarily, Figure 3 demonstrated that both methanol and ethanol extract of *P. commune* at a concentration of 12.5 mg/mL and 6.25 mg/mL, respectively, showed similar clear zones of inhibition against *P. aeruginosa* which were with a diameter of 11.7 mm. Notably, both clear zones were found to be larger than the diameter of the clear zone of inhibition developed by ciprofloxacin (10 µg/mL) which was with a diameter of 7.0 mm. In this study, the

antibacterial effect of *P. commune* methanol extract at the optimal concentration of 12.5 mg/mL was better than the optimal concentration of *P. commune* ethanolic extract at the optimal concentration of 6.25 mg/mL. Greater diameters were seen from the distinct zones of inhibition formed against *B. cereus* and *P. aeruginosa* with methanol extraction. This could be attributed to the higher extraction capability of methanol in generating a higher extraction yield of the *P. commune* than ethanol due to the higher polarity of the solvent (Sultana et al. 2009; Bouarab-Chibane et al. 2019). In this context, a higher extraction yield would lead to an additional increase in the concentration of secondary metabolites such as phenolic and flavonoid compounds, which in turn could promote the antibacterial effect of the *P. commune* extract. The presence of the phenolic compounds will change the permeability of cell membranes and modify the rigidity of cell walls through different interactions, which can disrupt bacterial cell membranes and cause the loss of cell integrity (Kim et al. 2015). Moreover, the extracted flavonoid compounds would also demonstrate the ability to disrupt the bacteria cell membranes and inhibit both energy metabolism and DNA synthesis of the bacteria which consecutively inhibit their growth (Kim et al. 2015).

3.2 Antimicrobial Assay of Soaps containing P. commune Extract

3.2.1 Liquid Soap

Results obtained from the thumb impression tests showed that the use of liquid soap containing *P.commune* methanol extract produced a significantly higher reduction in microbial growth as compared to the liquid control soap (Table 1). Interestingly, only subject 3, showed a reduction of microbial growth from high to medium (unwashed to washed) using liquid control soap while other subjects remained unchanged, which can be deduced that the liquid control soap can eliminate microbial load but with low efficiency as compared to the liquid soap containing methanol extract of *P. commune*, subjects 1, 2, 3, 4, and 6 demonstrated a reduction of microbial growth while only subject 5 remained unchanged, which indicated that the liquid soap containing methanolic extract of *P. commune* has higher efficiency in eliminating microbial load.

Meanwhile, liquid soap containing the ethanol extract of *P.commune* demonstrated that subjects 1, 2, 3, 5, and 6 had a reduction in the amount of microbial growth after washing their hands (Table 2). The reduction in microbial growth indicated that the liquid soap containing *P. comuune* ethanolic extract possesses antimicrobial properties. Other than this, it can be deduced that the effectiveness of liquid soap containing ethanol extract of *P. commune* was similar to liquid soap containing *P. commune* extract with methanol. This is a result of a decrease (either reduced from high to medium or from medium to low) in microbial growth following hand washing with both liquid soaps.

3.2.2 Solid Soap

In the second experiment of hand washing by using the solid control soap, only subjects 1 and 3 showed a reduction in the amount of microbial from high to medium growth while other subjects remained relatively unchanged. Thus, it can be deduced that the solid control soap can eliminate microbial load but with low efficiency which is similar to the liquid control soap. On the other hand, subjects 1, 2, 3, 4, and 5 showed a notable reduction in the amount of microbial growth after all of them washed their

Table 1 Qualitative indicator data showing the amount of microbial growth on unwashed, liquid control soap-washed, and liquid soap-washed hands with *P. commune* extracted with methanol

Treatments	Subjects					
	1	2	3	4	5	6
Unwashed hands	+ + +	+ +	+ + +	+ +	+ +	+ + +
Washed hand with liquid control soap	+ + +	+ +	+ +	+ +	+ +	+ + +
Washed hand with liquid soap containing *P. commune* extracted with methanol	+ +	+	+ +	+	+ +	+ +

Table 2 Qualitative indicator data showing the amount of microbial growth on unwashed, liquid control soap-washed, and liquid soap-washed with *P. commune* extracted with ethanol-washed hands

Treatments	Subjects					
	1	2	3	4	5	6
Unwashed hands	+ + +	+ +	+ + +	+ + +	+ +	+ + +
Washed hand with liquid control soap	+ +	+ +	+ + +	+ + +	+ +	+ +
Washed hand with liquid soap containing *P. commune* extracted with ethanol	+ +	+	+ +	+ + +	+	+ +

Table 3 Qualitative indicator data showing the amount of microbial growth on unwashed, solid control soap-washed, and solid soap-washed with *P. commune* extracted with methanol-washed hands

Treatments	Subjects					
	1	2	3	4	5	6
Unwashed hands	+ + +	+ + +	+ + +	+ +	+ + +	+ +
Washed hand with solid control soap	+ +	+ + +	+ +	+ +	+ + +	+ +
Washed hand with solid soap containing *P. commune* extracted with methanol	+	+ +	+	+	+ +	+ +

Table 4 Qualitative indicator data showing the amount of microbial growth on unwashed, solid control soap-washed, and solid soap-washed with *P. commune* extracted with ethanol-washed hands

Treatments	Subjects					
	1	2	3	4	5	6
Unwashed hands	+ + +	+ +	+ + +	+ +	+ +	+ +
Washed hand with solid control soap	+ + +	+ +	+ +	+ +	+ +	+ +
Washed hand with solid soap containing *P. commune* extracted with methanol	+ +	+	+	+	+	+ +

hands using the solid soap containing *P. commune* methanolic extracted. Moreover, for subjects 1 and 3, the reduction in microbial growth was reduced from high to low (Table 3). These results indicated that the soap containing *P. commune* methanolic extract contained antimicrobial properties and showed higher efficiency in eliminating microbial load by comparing to solid control soap, liquid control soap, as well as liquid soap containing *P. commune* extracted with methanol and ethanol.

From the thumb impression test, subjects 1, 2, 3, 4, and 5 showed a reduction in microbial growth with the usage of solid soap containing *P. commune* ethanolic extracted (Table 4), which indicated that the solid soap contained antimicrobial properties due to the addition of the *P. commune* extract. By comparing between subjects, only subject 3 showed a reduction of microbial growth from high to low. Therefore, it can be deduced that the efficiency of solid soap containing *P. commune* ethanolic extract has lower microbial elimination efficiency as compared to the methanolic extract of *P. commune* containing solid soap.

Based on the results from the thumb impression tests, the efficiencies of both solid and liquid control soaps in eliminating microbes were not as effective as the solid and liquid soaps containing *P. commune* extracts. As the control soaps used in this study did not have any known antimicrobial agent, our results have indicated that the improved reduction in microbial growth was due to the addition of *P. commune* extract as an ingredient that carries active antimicrobial activity (Lima et al. 2013). The outcomes of this study are in agreement with previous studies where it was found that the addition of herbal extract into soap has a higher antimicrobial effect than the soap without any additional herbal extract (Kareru et al. 2010; Blenkharn and Smales 2017). Meanwhile, by comparing the thumbprint test results between liquid and solid *P. commune* extract soaps, the solid soap was seen to have eliminated more microbial than the liquid soap. Similarly, other studies also revealed that using solid soap for hand wash was more effective than liquid soap and this was due to the mechanical movements involved in removing microbes that were transient on our hands (Sheikh 2018).

Interestingly, when compared, the antimicrobial effect between *P. commune* extracted using methanol and ethanol in solid soaps demonstrated that methanol extracted *P. commune* solid soap was able to eliminate more microbes through hand washing. This might be due to the higher amount of the secondary metabolites present as antimicrobial agents attributed to the higher extraction efficiency of methanol (Sultana et al. 2009). On this basis, it can be hypothesized that the solid soap containing methanolic extract of *P. commune* could eliminate more microbes on our hands than the solid soap containing ethanolic extract of *P. commune*.

Conclusion

In this study, it was demonstrated that *P. commune* extract was effective in inhibiting the growth of *B. cereus* and *P. aeruginosa* in which the optimal concentrations for *P.commune* extracted with methanol and ethanol extract was 12.5 mg/mL and 6.25 mg/mL, respectively. In addition, from the thumb impression tests, the results obtained showed that hand washing performed with both the solid and liquid soaps containing the *P. commune* extract can effectively reduce the presence of microbes in the hands. More specifically, it was demonstrated that the antimicrobial activity of soap containing *P. commune* extract was better in the solid form when compared with the liquid form. Taken altogether, the

addition of *P. commune* extract as an antimicrobial ingredient demonstrated the potential to increase the efficiency of microbial removal in hand washing.

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Conflict of Interest

There are no conflicts of interest.

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