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Antibacterial potential of liquid hand soap with *Piper aduncum* leaf extract

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ABSTRACT

Liquid soaps containing antimicrobial active ingredients take away more bacteria as compared to plain soaps. This study was conducted to formulate a liquid hand soap with *P. aduncum* leaf extract as an organic antibacterial component. The physico-chemical properties was evaluated to determine the quality, efficiency, and cleansing properties of the liquid soap. The antibacterial potential was measured using the disc diffusion method. The pH values of the different formulated liquid hand soaps are within the accepted pH range of 8.5-10.5. In foam retention capacity, the 25% concentration of *P. aduncum* has the longest time duration of 110 minutes whereas the 75% concentration of *P. aduncum* has the shortest time duration of 75 minutes. Results revealed that the zone of inhibition of commercial liquid hand soap significantly vary as compared to the formulated liquid hand soaps with *P. aduncum* crude extract. However, among the formulated liquid hand soaps with *P. aduncum* crude extract, it all exhibited antibacterial activity in terms of zone of inhibition up to 16 hours, but not as greater as the commercial liquid hand soap. It was therefore proven that *P. aduncum* crude extract is not effective as an antibacterial component in the formulation of liquid hand soap.

Keywords: Aseptic technique, Bacterial growth, Disc diffusion test, Saponification, Zone of inhibition

INTRODUCTION

Skin, specifically hands are being the most exposed part of the body which carries large numbers of bacterial flora picked up from various objects that come into contact, thus proper hygiene is the single most important, simplest, and least expensive means of preventing health care associated with infection and the spread of antimicrobial resistance. The emergence of bacterial resistance to the currently available antimicrobial drugs

necessities further research in the discovery of new safe and effective antimicrobial agents (Londhe *et al*., 2015; Sajed *et al.,* 2014). Medicinal plants have been used in developing countries as an alternative treatment to health problems (Duarte *et al*., 2005). According to Selvi *et al*. (2012), medicinal plants constitute an effective source of both traditional and modern medicine. In the Philippines, *Piper aduncum* is locally known as Buyo-buyo. It is a shrub native to tropical regions of America and was introduced in Asia during the 19th century (Pacheco *et al.,* 2016), a medicinal plant used traditionally in South America and the Caribbean Basin (Abreu *et al*., 2015). In addition, *P. aduncum* has a wide range of traditional uses and its essential oil is a well-known insecticide, molluscicide, and bactericide (Mee *et al*., 2009). It has a great potential for economic exploration because of the proven use of its essential oil in the agriculture and in human health.

Hastuti *et al.* (2017) proved that the antibacterial compounds of the leaves of *P. aduncum* can inhibit the growth of *B. subtilis, M. lutues, and E. coli.* Ethanolic extract from aerial part of *P. aduncum* were more active against Gram-positive bacteria than against Gram-negative bacteria (Kloucek *et al*., 2005; Brazao *et al.,* 2014). Although many studies have been done regarding the antibacterial properties of *P. aduncum* and specific phytochemicals (Lucena *et al*., 2017), only Mamood *et al*. (2017) formulated a product which is a cream containing 10% of the essential oil of *P. aduncum* for irritation and skin sensitization assays used on New Zealand white rabbits and guinea pigs as test animal. It was revealed that the formulated cream containing *P. aduncum* essential oil caused slight irritation on rabbit skin due to their sensitive skin. However, no positive response was detected in the skin sensitization assay. In addition, the effectiveness of *P. aduncum* essential oil was evaluated in the form of three formulated semisolid products such as ointment, cream, and gel. As the formulated essential oil cream and ointment displayed better repellent properties than the gel, both ointment and cream appear to be the most promising *P. aduncum* formulations to be developed and commercialized as alternatives to synthetic repellents (Mamood *et al.,* 2016).

The *P. aduncum* has been noted for its antibacterial properties which has a potential for commercial use. Since, it is widely distributed in the country, making a product out of it could be a good source of income serve as the formulation of antibacterial liquid soap with *P. aduncum* leaf extract and a great use as an antibacterial liquid hand soap for the local consumers to help prevent the spread of infections through hand washing with a liquid hand soap. Thus the objective of the study aims to formulate a liquid hand soap with *P. aduncum* leaf extract as an organic antibacterial component. Specifically, evaluate the quality of the soap base and determine which concentrations in the formulated liquid soap of *P. aduncum* will yield a significantly greater zone of inhibition.

MATERIALS AND METHODS

Collection of Leaf Samples

Fresh green leaves of *P. aduncum* were collected at La Paz, Ayala, Zamboanga City, Philippines. The plant species was verified by an agriculturist from Bureau of Plant Industry- Plant Quarantine Service.

Figure 1. The *Piper aduncum* (A) plant morphology and (B) air-dried leaves.

Preparation of Leaf Powder

Five kilograms of *P. aduncum* fresh leaves were washed with water thoroughly to remove the dirt and unwanted particles. Air-drying was done for 2 weeks to remove the moisture of fresh leaves (Figure 1). Two kilograms of air-dried leaves were pulverized using a blender.

Preparation of Leaf Extract

The pulverized leaves of *P. aduncum* were extracted with 95% ethanol as its solvent. The maceration method done by Hastuti *et al.* (2017) was adapted for leaf extraction. For about 100 grams of *P. aduncum* pulverized leaves were mixed with 1,000 ml of 95% ethanol (1g:10ml) in an Erlenmeyer flask covered with cork in the opening. The entire flask was covered with aluminum foil and was allowed to stand under room temperature for 72 hours. The ethanolic extract was filtered using a filter paper and decanted using a cheese cloth served as a filtrate which was subjected to rotary evaporator with reduced pressure and controlled temperature. The final extract was stored in the refrigerator until the extract was used in the preparation of liquid hand soap.

Preparation of Soap Base

For the formulation of liquid hand soap, the hot process method by Debnath *et al.* (2011) was used in the study. A 700 ml of coconut oil was heated in a beaker at 72°C for 15 minutes and 280 ml of glycerin was heated separately. Both coconut oil and glycerin were heated at 60°C and gently stirred for 20-30 minutes. The temperature was checked using a thermometer. A 175 grams of KOH was weighed and 584.5 ml of water was prepared. The KOH was mixed with distilled water and stirred using a stirring rod to dissolve the KOH. The mixture served as the lye-water solution. Once the lye-water was completely mixed until it became clear, the lye-water solution was then slowly added to the heated coconut oil. The coconut oil and lye-water solution were poured into the heated glycerin in a beaker. The solution was heated at a maintained temperature of 70°C for 3-4 hours. After which, the solution was continuously stirred using a mechanical mixer. This served as the soap paste. The soap paste was then mixed to distilled water depending on the amount of the formulated soap paste. The liquid soap was neutralized and preserved by adding Borax 10 g powder per 1,000 ml of the solution.

Preparation of Treatments

The crude extract of *P. aduncum* was added to the soap base in a clean bottle, depending on the concentration needed to formulate a liquid hand soap and it was stirred thoroughly. The treatments of the formulated liquid hand soap are as follows: Treatment 1 (30 ml of 25% crude extract + 95 ml soap base), Treatment 2 (30 ml of 50% crude extract + 95 ml soap base), and Treatment 3 (30 ml of 75% crude extract + 95 ml soap base). Wherein Treatment 4 (125 ml soap base) serve as negative control and Treatment 5 (125 ml commercial liquid hand soap) as positive control. The formulated liquid hand soaps were poured into a clean container and stored at room temperature.

Determination of the Physico-chemical Properties of Liquid Hand Soap

a. Test of pH

A volume of 1 ml on each of the Treatments was dissolved in a 100 ml distilled water. The pH of the soap solution was verified using a calibrated pH-107 meter. The pH values of the formulated liquid hand must be in an acceptable limit pH range of 8.5-10.5 (Debnath *et al*, 2011).

b. Foam Retention Capacity

A volume of 1 ml on each of the formulated liquid hand soaps was added to 49 ml of distilled in a 100 graduated cylinder. The graduated cylinder was covered with hand and was shaken vigorously for 10 times. The same process was repeated for the negative and positive control in a separate graduated cylinder. The time when the foam disappeared was recorded (Debnath *et al,* 2011).

Preparation of Nutrient Broth Culture

A 1 gram of beef extract and 0.6 gram of peptone were added in a beaker containing 200 ml of distilled water which was stirred thoroughly to dissolve the beef extract and peptone and heated in a hot plate. The broth was sterilized at 15 psi at 121°C for 15 minutes.

Preparation of Cultured Bacteria

A consent was prepared and signed by the respondent in obtaining the hand-swab samples. The hands of the respondent were washed with tap water then wiped with clean cloth right after. Inside the laminar flow, sterile swab stick was used to swab the surface of the hands, specifically on the palm and in between fingers and it was inoculated directly into a beaker which

contains the nutrient broth. The beaker was incubated for 24 hours. Cloudy appearance in the nutrient broth indicates bacterial growth which served as the test organisms from one source of respondent only.

Preparation of Mueller- Hinton Agar

In the Erlenmeyer flask, 38 grams of Muller-Hinton agar powder was added in 1000 ml of distilled water. The mixture was boiled while stirring thoroughly to dissolve fully the agar in a hot plate. For 15 minutes, the dissolved mixture was placed in an autoclave at 121ºC to avoid contamination. The Muller-Hinton agar was poured into 50 petri plates while still molten by aseptic technique. There are 10 replicates for each Treatment and each contains 20 ml of the medium.

Preparation of Filter Paper Disc

Whatman filter paper were cut into discs (6mm in diameter) using a puncher. The discs were wrapped in an aluminum foil and sterilized in an autoclave at 15 psi at 121°C for 15 minutes.

Evaluation of the Antibacterial Property of the Different Treatments

Spread plate method was used in inoculating the cultured bacteria. Using a micropipette, 100 microliter of cultured bacteria from a beaker was transferred in each of the 50 different petri plates containing the MH Agar. The L-tube was aseptically sterilized before and after use. The inoculum was spread evenly by using the L-tube, without letting the L-tube touch the sides of the petri plate. The antibacterial potential of the formulated liquid hand soap with different concentrations of *P. aduncum* crude extract was measured using the disc diffusion method. The sterile filter paper discs were dipped in the formulated liquid hand soap with different concentrations of *P. aduncum* crude extract and as well as in the positive and negative control. The sterile filter paper discs were aseptically placed at the center of each prepared MH agar plates containing the cultured bacteria using a sterile forceps. The medium which contained cloudy colonies indicate the presence of the bacteria. The petri plates were incubated at room temperature in an inverted position and observed after 4 hours, 8 hours, 12 hours, and 16 hours. Post Hoc analysis was done using Tukey's B Homogeneous Subsets to determine the mean zone of inhibition of the formulated liquid hand soap observed. Results with $p<0.05$ were considered statistically significant. The standard zone diameter was based within the recommended ranges of EUCAST (European Committee on Antimicrobial Susceptibility Testing).

Statistical Analysis

Complete Randomized Design (CRD) was used in the study having 10 replicates. Randomization was simply done by lottery method wherein the five treatments contains 10 petri plates that represents as replicate on each treatment with a total number of 50 petri plates. The results of the study were analyzed using One-Way ANOVA to determine if there is a significant difference on the mean zone of inhibition of the formulated liquid hand soap after 4 hours, 8 hours, 12 hours, and 16 hours. Post Hoc analysis was done using Tukey's B Homogeneous Subsets to determine the mean zone of inhibition of the formulated liquid hand soap observed.

RESULTS AND DISCUSSION

The physico-chemical properties of soap actually determine its quality, efficiency, and cleansing properties. According to Narkhede (2010), the range of pH for liquid hand soap is within 8.5-10.5. In the evaluation of physico-chemical parameters as shown in Table 1, all the formulated liquid hand soaps including the commercial liquid hand soap fall within the accepted limit pH range. These pH values were might due to the process of saponification resulting to partial alkali hydrolysis, which could be possible by the addition of excess fat or oil or any super fatting agent used to decrease the harshness of the soap (Warra *et al.,* 2011). The human skin has an acidic pH of 5.4 to 5.9, which is an important factor in the protection against microorganism wherein alkaline substances such as soaps neutralize the body's protective mantle that acts as barrier against bacteria (Onyango *et al.,* 2014). Furthermore, highly alkaline pH could damage the acid mantle and as well as the disruption of the lipid lamellae of the epidermis, that could possibly result to skin dryness due to higher trans-epidermal water loss allowing the access of potential irritants and allergens. (Mendes *et al.,* 2015). Higher pH values make the soap basic and lather easily (Habib *et al.,* 2016). However, the pH does not influence the bacteriological activity of the soap (Onyango *et al.,* 2014).

Foam retention capacity is one of the important attributes in the cleansing efficacy of a liquid soap. As shown also in Table 1, Treatment 1 (25 %

concentration of *P. aduncum*) liquid hand soap has the longest time duration of 110 minutes, whereas Treatment 3 (75% concentration of *P. aduncum*) liquid hand soap has the shortest time duration of 75 minutes. These observations are most likely could be attributed to the presence of the coconut oil which is commonly used in the formulation of liquid soap that contains fatty acid specifically lauric acid which may contribute to the lathering and cleansing properties of liquid soap (Omwoyo *et al.,* 2014). According to Essien *et al.* (2013), soap lather effectively holds particles in colloidal suspension, which can be easily rinsed with clean water. Therefore, the lathering ability of soap could further increased bacterial reduction (Fuls *et al.,* 2008).

Table 1. The physico-chemical properties of the formulated liquid hand soaps in terms of pH and foam retention capacity.

TREATMENT (T)	Physico-chemical properties	
	pH	Foam Retention Capacity
T1 (30ml of 25% crude extract + 95ml soap base)	9.7	110 minutes
T2 (30ml of 50% crude extract + 95ml soap base)	9.1	109 minutes
T3 (30ml of 75% crude extract + 95ml soap base)	9.4	75 minutes
T4 (125ml soap base)	9.0	95 minutes
T5 (125ml commercial liquid hand soap)	8.4	63 minutes

Table 2. Mean zone of inhibition (mm) of the formulated liquid hand soaps as tested against the cultured bacteria observed after 4 hours, 8 hours, 12 hours, and 16 hours.

TREATMENT (T)	Zone of Inhibition (Mean ± SD)			
	After 4 hours	**After 8 hours**	**After 12 hours**	**After 16 hours**
T1 (30ml of 25% crude extract + 95ml soap base)	8.40 ± 1.58	8.30 ± 1.25	8.50 ± 2.88	7.40 ± 2.17
T2 (30ml of 50% crude extract + 95ml soap base)	8.50 ± 1.18	8.70 ± 1.16	9.20 ± 2.62	8.20 ± 2.25
T3 (30ml of 75% crude extract + 95ml soap base)	9.30 ± 1.83	10.90 ± 1.79	8.80 ± 1.75	8.40 ± 0.70
T4 (125ml soap base)	9.10 ± 1.97	9.10 ± 1.66	10.00 ± 2.21	6.90 ± 2.03
T5 (125ml commercial liquid hand soap)	17.90 ± 5.88	17.10 ± 4.72	18.10 ± 4.93	16.50 ± 4.14

Table 3. One-Way Analysis of Variance (ANOVA) on the mean zone of inhibition (mm) of the formulated liquid hand soaps as tested against the cultured bacteria after 4 hours, 8 hours, 12 hours, and 16 hours.

		Sum of Squares	df	Mean Square	F	Sig.
After 4 hours	Between Groups	664.720	4	166.180	18.204	**.000****
	Within Groups	410.800	45	9.129		
	Total	1075.520	49			
After 8 hours	Between Groups	584.000	4	146.000	23.422	**.000****
	Within Groups	280.500	45	6.233		
	Total	864.500	49			
After 12 hours	Between Groups	657.080	4	164.270	17.328	**.000****
	Within Groups	426.600	45	9.480		
	Total	1083.680	49			
After 16 hours	Between Groups	630.680	4	157.670	25.001	**.000****
	Within Groups	283.800	45	6.307		
	Total	914.480	49			

**Highly significant if $p<0.05$

Table 2 shows the antibacterial potential of the formulated liquid hand soap in terms of the mean zone of inhibition (mm) as tested against the cultured bacteria after 4 hours, 8 hours, 12 hours, and 16 hours of observation. The result showed that among the formulated liquid hand soaps with *P. aduncum* crude extract, it all yielded zones of inhibition. However, the commercial liquid hand soap (T5) has the greater mean zone of inhibition after 4 hours, 8 hours, and 12 hours as compared to the formulated liquid hand soaps. Results of One-Way Analysis of Variance (ANOVA) presented in Table 3 reveal that there is highly significant difference (p=0.000** at α=0.05) between the mean zone of inhibition of the formulated liquid hand soaps as tested against the cultured bacteria after 4 hours, 8 hours, 12 hours, and 16 hours of observation. Post Hoc Analysis using Tukey's B Homogeneous Subsets presented in Table 4 revealed that the zone of inhibition of commercial liquid hand soap (T5) significantly vary among all formulated liquid hand soaps.

Statistically, the zone of inhibition of commercial liquid hand soap significantly vary as compared to the formulated liquid with *P. aduncum* crude extract (Figure 2). However, among the formulated liquid hand soaps with *P. aduncum* crude extract, it all exhibited antibacterial activity in terms of zone of inhibition, but not as greater as the commercial liquid hand soap. This could be possible because of the potassium hydroxide used for saponification of fatty acids (oils) which may interfere in the antibacterial activity of plant extract (Londhe *et al.,* 2015). In this study, the addition of potassium hydroxide for the formulation of liquid hand soap which involves the process of saponification of fatty acids (oils) affects the antibacterial activity of *P. aduncum* crude extract which results to lesser zone of inhibition as tested against the cultured bacteria.

Table 4. Post-Hoc Analysis using Tukey's B Homogenous Subsets on the mean zone of inhibition (mm) of the formulated liquid hand soaps as tested against the cultured bacteria after 4 hours, 8 hours, 12 hours, and 16 hours.

TREATMENT (T)	After 4 hours		After 8 hours		After 12 hours		After 16 hours	
	1	2	1	2	1	2	1	2
T1 (30ml of 25% crude extract + 95ml soap base)	8.40		8.30		8.50		7.40	
T2 (30ml of 50% crude extract + 95ml soap base)	8.50		8.70		9.20		8.20	
T3 (30ml of 75% crude extract + 95ml soap base)	9.30		10.90		8.80		8.40	
T4 (125ml soap base)	9.10		9.10		10.00		6.90	
T5 (125ml commercial liquid hand soap)		17.90		17.10		18.10		16.50

Subset for α = 0.05

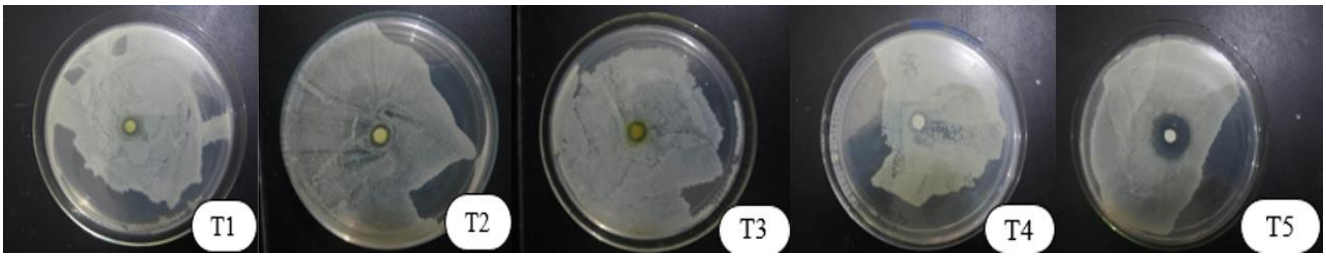

Figure 2. Zone of inhibition of the Treatments after 12 hours tested against the cultured bacteria.

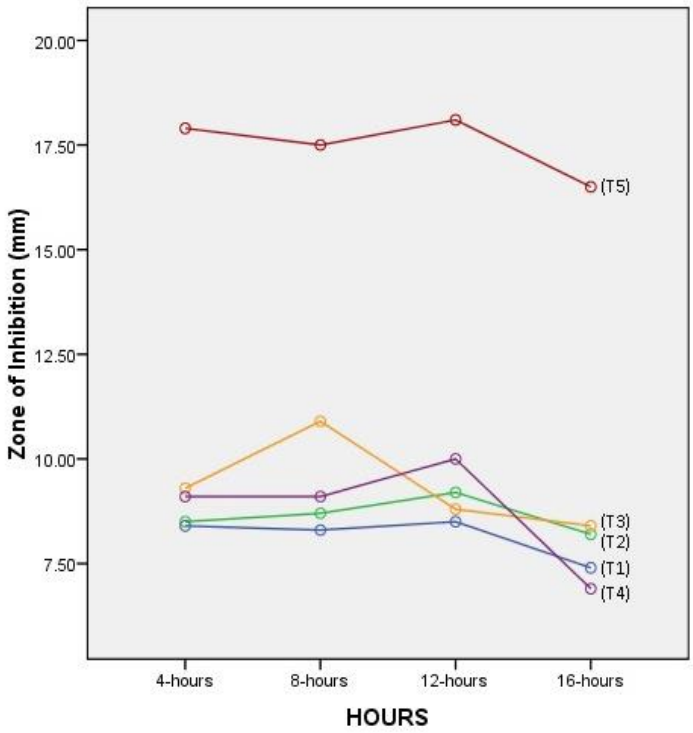

Figure 3. Line graph showing the mean zone of inhibition (mm) of the formulated liquid hand soap observed after 4 hours, 8 hours, 12 hours, and 16 hours.

The bacterial growth of inhibition of the antibacterial property of formulated liquid hand soap with *P. aduncum* crude extract may be attributed to the phytochemical constituent of *P. aduncum* which has bioactive compounds, amides alkaloids, phenylpropanoids, neo-lignans, terpenes, steroids, flavonoids, and phenolic compounds (Gopalakrishman, 2015). The flavonoids such as flavones, isoflavones, flavonones, chalcones, dihydrochalcone found on the leaves of *P. aduncum* are responsible on the direct disruption of the bacterial cell wall, inhibition of nucleic acid synthesis and energy metabolism, and alteration of the membrane permeability which mainly lead to bacterial cell death (Xie *et al.,* 2015). In addition, phenolic compounds present in *P. aduncum* could inhibit bacteria through novel mechanism, which involves membrane disruption of both Gram-positive and Gram-negative bacteria. These compounds actually altered the membrane stability by interfering with intracellular processes or by direct interaction with membrane components (Rempe *et al.,* 2017). Furthermore, *P. aduncum* leaf extract contains tannin and saponin compounds wherein tannin inhibit the peptidoglycan compounds that play a role in the bacterial cell wall and also in the enzyme activity in bacteria in which saponin bind with sterol in the cell membrane causing the bacterial cell to be damaged (Hastuti *et al.,* 2017).

Figure 3 shows T5 (commercial liquid hand soap) has greater mean zone of inhibition after 4 hours, 8 hours, and 12 hours compared to the formulated liquid hand soaps of T3 (75 % concentration *P. aduncum*), T4 (soap base), T2 (50% concentration *P. aduncum*) liquid hand soap, and T1 (25% concentration *P. aduncum*), respectively. However, all the treatments show a decreasing zone of inhibition after 16 hours of observation.

In the study of Hastuti *et al*. (2017), 70% concentration was the highest concentration of *P. aduncum* crude extract used, wherein the results showed that it has no significant effect against *C. albicans* colony. However, in this study it was increased to 75% concentration of *P. aduncum* crude extract and evaluated against bacteria, in which the results still showed no significant effect against bacterial growth. Nevertheless, all the formulated liquid hand soaps including the commercial liquid hand soap showed a decreased in zone of inhibition after 16 hours of observation, which implies an increasing growth of the cultured bacteria.

CONCLUSION

All of the formulated liquid hand soaps of *P. aduncum* fall within the normal pH range of 8.5-10.5 that indicates that it is probably safe to use. In terms of foam retention capacity, 25% concentration of *P. aduncum* liquid hand soap has the longest time duration of 110 minutes, which may suggest the potential of the soap lather to effectively hold particles in colloidal suspension that can be easily rinsed off with clean water. All the formulated liquid hand soap with *P. aduncum* crude extract has the property to inhibit bacterial growth up to 16 hours. However, its antibacterial property is not effective as the commercial liquid hand soap in terms of zone of inhibition. In this study, it was proven that *P. aduncum* crude extract is not effective as an antibacterial component in the formulation of liquid hand soap. So, it is recommended to further utilize *P. aduncum* for another product development which could be a good source for research purposes.

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