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Formulated Hand Sanitizer Utilizing Passion Fruit (*Passiflora edulis*) Leaf Extract as an Active Ingredient

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Abstract

Passiflora edulis leaf extracts were added as an active ingredient for alcohol-based hand sanitizer (ABHS). Matured leaves were air-dried, powdered, and soaked in methanol (1:10, w/v) for 24 hours. The solvent-free crude extract was used to formulate hand sanitizer utilizing varying amounts of leaf extract (1,3 and 5% w/w). The antimicrobial activities of the leaf extracts and the hand sanitizer formulations were determined by calculating the zone of inhibition against some common pathogenic microorganisms. Physicochemical properties of the formulated hand sanitizers like appearance, odor, color, density, pH, and viscosity were also analyzed. Results show that the crude leaf extract contains alkaloids, flavonoids, and tannins as confirmed by the phytochemical screening and FTIR spectroscopy. Active antimicrobial activity against 3 of 5 microorganisms, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Trichophyton mentagrophytes*, was observed in crude leaf extract and formulated hand sanitizer. The formulated hand sanitizers are characterized by gel-to-liquid appearance, yellow-greenish tinge, characteristic citrus scent, and slightly acidic pH. The density and the viscosity of the three formulations were dependent on the concentration of passion fruit extract in the formulation.

Keywords: Antimicrobial Activity, Hand Sanitizer, *P. edulis* Leaf Extract, Phytochemicals

1. Introduction

The emergence of the coronavirus disease 2019 (COVID-19) has become a global health concern which severely affected the economy, livelihood, and overall well-being of people (Golin *et al*., 2020; Wu *et al*., 2020; Wang *et al*., 2021). Among Western Pacific Region countries, the Philippines has faced one of the most severe cases of COVID-19. However, through continued adherence to the minimum health standards, such as appropriate use of face masks, proper hand hygiene, and physical distancing, the probability of COVID transmission per contact was reduced (Caldwell *et al*., 2021).

The World Health Organization (WHO) has advocated proper hand hygiene to reduce the transmission of pathogenic microorganisms from person-to-person and recommended regular hand washing and the use of sanitizers (WHO, 2009). Proper hand hygiene, particularly hand washing, has been regarded as the cheapest, most effective, and single most important way to prevent and control hand-acquired infection caused by pathogenic microorganisms (Mathur, 2011; Jain *et al*., 2016; Alsaidan *et al*., 2020). However, frequent hand washing or continued use of alcohol may cause skin dryness due to the removal of fatty acids or natural oils in the hands, and may result in a microscopic surface crack that will serve as an entry point for microorganisms. Hand sanitizers formulated with emollients were introduced to be an alternative to hand washing while preventing skin dehydration from occurring (Jain *et al*., 2016; Alsaidan *et al*., 2020). Alcohol-based hand sanitizers (ABHS) are more affordable and more predominant in most healthcare settings than its non-alcohol counterpart due to its efficacy in decreasing the transmission of infection (Golin *et al*., 2020).

Many studies have affirmed the efficacy of many tropical plants in folk medicine due to its excellent antimicrobial activities. An exploration study conducted by Valle *et al*. (2015) validated the potential of twelve (12) Philippine medicinal plants against gram-positive and gram-negative multidrug resistant bacteria. Another study by San Luis *et al*. (2014) verified the presence of many bioactive compounds and confirmed the antibacterial potential of different indigenous plant chosen as revegetation species in landslide scars in Benguet, Philippines. Yellow passion fruit (*Passiflora edulis*) is a tropical fruit commonly consumed to make cakes, juice, jam, jelly, wine and many more (He *et al*., 2020). In addition, the leaf infusions are commonly used as sedative or tranquilizers in some European countries and in America (Yuan *et al*., 2017). Recent studies highlighted the antioxidant activity and antimicrobial activity of the compounds identified in the leaves of *P. edulis* (da Silva *et al*., 2013; Xu *et al*., 2013; Cazarin *et al*., 2015). Flavone glycoside conjugates were identified as the major metabolites present in several species of *Passiflora.*

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Cyanogenic glycosides (mandelonitrile-*O*-di-glucoside and mandelonitrile-*O*-rutinoside) were also detected while harman alkaloids were noted at lower quantities than flavonoids (Farag *et al*., 2016). In addition, the extracts from the fruit pericarp were shown to inhibit the growth of the majority of the bacterial strains examined with the lowest minimum inhibitory concentration (128 µg/mL) was recorded against *E. coli* (Dzotam *et al*., 2015). *P. edulis* seed extracts exhibited antimicrobial activity against *S. aureus, C. albicans*, and *E. coli*, which may be attributed to the alkaloids, flavonoids, tannins, steroids, and saponins present (Kanu *et al*., 2017). Indeed, the phytochemicals found in *P. edulis* had significant antibacterial properties which qualify it as a good active ingredient for the formulation of hand sanitizer.

The aim of this study was to develop and formulate hand sanitizer with *P. edulis* leaf extract. It also intended to determine the physicochemical and antimicrobial properties of the formulated hand sanitizer. It is envisioned that the outcome of this study will serve as a benchmark for future researchers and entrepreneurs to utilize extracts from underutilized plants and its parts in the production of antimicrobial products.

2. Methodology

2.1. Chemicals

Methanol (GR), HCl (AR), glacial acetic acid (AR), concentrated sulfuric acid, nitric acid, ferric chloride, Dragendorff's reagent were available in the CEAFA Laboratory and were used without further purification. The chemicals (*see Table 1*) used for hand sanitizer formulation were purchased from a local supplier.

2.2. Collection of Passion Fruit Leaves and Preparation of the Plant Extracts

Matured leaves of *P. edulis* with no visible damage or discoloration were collected in a farm at Lucban, Quezon, Philippines. The leaves were rinsed with water to take off the dirt and plant debris followed by final washing with distilled water.

Two hundred grams (200 g) of *P. edulis* leaves were selected and dried for seven (7) days until fully dried and brittle. The dried *P. edulis* leaves were reduced to a powder using a food processor and kept in an air-tight container. Powdered leaves were soaked in 2000 mL methanol (1:10, w/v) and stored in a cool, dry, dark place for 48 hours. Leaf residues were removed by filtration and the extracts were concentrated using rotary evaporator under reduced pressure at 60 °C. The extracts were collected and stored at 4 °C in an amber bottle.

2.3. Phytochemical Screening of P. edulis Leaf Extract

The phytochemical test used for *P. edulis* leaf extracts was based from the method adapted from Guevarra *et al.* (2005). The alkaloids in the leaf extracts were determined by the Munier and Macheboeuf modification test wherein the diluted leaf extract was added with 3 drops of Dragendorff's reagent. Flavonoids in the leaf extract were determined by the Bate-Smith and Metcalf test in which 3 to 5 drops of 12 M HCl was mixed with the extract followed by gentle heating for 15-60 minutes. Fehling's test was done to determine the presence of glycosides in the leaf extract. In this method, one drop of 5% $FeCl_3$, concentrated H_2SO_4 and glacial acetic acid were added onto 2ml of leaf extract. Froth formation was the basis for the presence of saponins. In this test, leaf extracts were mixed with 2 to 3 mL water and were shaken vigorously afterwards. To test for the presence of tannins, 1% $FeCl_3$ were added dropwise to 1 mL of the leaf extract.

2.4. FTIR Spectra of Crude Leaf Extract

The functional groups present in the crude *P. edulis* leaf extract were determined using an FTIR (Thermo Scientific Nicolet 6700 FT-IR) spectroscope. Analysis of the FTIR spectra was conducted at the De La Salle University, Manila.

2.5. Formulation of Hand Sanitizer with P. edulis Leaf Extract

Formulation of the hand sanitizer with *P. edulis* extract was adopted from the method described by Thombare *et al*. (2015). Three formulations of alcohol-based hand sanitizers (F1, F2, and F3) were prepared as presented in Table 1. In many alcohol-based hand sanitizer formulation, 60-95% (v/v) ethanol, isopropanol, or *n*-propanol was used as hand sanitizer's main ingredient (Hans *et al.,* 2021). Emollients such as glycerin were added to aid in skin moisturization while thickeners were used to enhance the viscosity. Fragrance and dyes are added to increase the product's marketability and safety (Filipe *et al.,* 2021).

Hand sanitizer was formulated by mixing Carbopol 940 to deionized water until it became homogenous. Sodium citrate was then added to the mixture and left undisturbed for 24 hours. *P. edulis* leaf extracts, glycerin, polysorbate 20 were added to ethanol and were mixed with aqueous phase until homogenous. The hand sanitizers were kept in an air-tight HDPE container.

Table 1. Formulation of Hand Sanitizer

COMPONENTS	**Quantity given (% w/w)**			**Uses**
	F1	**F2**	**F3**	
Deionized water	32.5	30.5	28.5	Vehicle (Solvent)
Ethanol	62	62	62	Antimicrobial agent
***P. edulis* leaf extract**	**1**	**3**	**5**	Antimicrobial agent
Carbopol 940	0.5	0.5	0.5	Thickening agent
Sodium Citrate	0.7	0.7	0.7	Solubilizing agent
Glycerin	2.3	2.3	2.3	Emollient
Polysorbate 20	0.5	0.5	0.5	Emulsifier
Fragrance Oil	0.5	0.5	0.5	Fragrance
Total	100	100	100	

2.6. Antimicrobial Activity of P. edulis Leaf Extract and Formulated Hand Sanitizer

The disc-diffusion assay was conducted to determine the antimicrobial activity of *P. edulis* leaf extract, formulated hand sanitizers, and commercial hand sanitizer. Analysis was done at the Microbiological Research and Services Laboratory (MRSL) of the University of the Philippines-Natural Science Research Institute (UP-NSRI). The antimicrobial activities of the aforementioned samples were tested on five (5) microorganisms: *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Pseudomonas aeruginosa* (UPCC 1244), *Candida albicans* (UPCC 2168), and *Trichophyton mentagrophytes* (UPCC 4193).

The microbial suspension used in the assay were made from the test organisms subcultured for 24 hours. Culture plates, about 3 mm thick, were prepared by pouring nutrient agar, glucose yeast peptone agar, potato dextrose agar onto the plates, followed by inoculation of the microbial suspension by swabbing the agar surface evenly. Using a sterile cork borer, 3 wells (about 10 mm) were made at equal distance and about 200 µg of samples were placed on each well. Culture plates with nutrient agar and glucose yeast peptone were incubated at 35° C and observed after 24 hours while the potato dextrose agar plates were incubated for 5 to 7 days at room temperature.

The inhibitory activity of the leaf extracts and hand sanitizer samples were determined by obtaining the diameter of the clearing zone (in mm). Ciprofloxacin (1 µg), tetracycline (30 µg), doxycycline (5µg) and Canesten solution (100 µL with 1% clotrimazole) were used as positive control. The antibacterial index was computed using the formula:

$$Antimicrobial\ Index = \frac{(Diameter\ of\ clearing\ zone - Diameter\ of\ well)}{Diameter\ of\ well} \quad (1)$$

The following interpretative range of standard zone was adopted from Guevarra *et al*. (2005).

Table 2. Interpretative Range of Standard Zone

Zone of Inhibition, mm	Inhibitory Activity	Interpretation
> 19	+ + +	Very Active
14 - 19	+ +	Active
10 - 13	+	Partially Active
< 10	–	Inactive

2.7. Physicochemical Analysis of Hand Sanitizers

The physicochemical properties of the formulated hand sanitizers and commercial hand sanitizer were analyzed at the Chemical Engineering Laboratory of Batangas State University. Sensory analysis was used to evaluate the organoleptic characteristics of the formulated hand sanitizers. Visual test was made to check the appearance and color while an olfactory test was done to determine the odor of the samples. The pH of the samples were analyzed using a pH meter (Eutech, Thermo Scientific), wherein the average of three trials was used to compute the pH. The density was determined by pycnometer method as prescribed in ASTM D854. The average of three trials was used to compute the density. To determine the viscosity, Oswald viscometer was utilized in which the average of the three trials was used to calculate the viscosity.

2.8. Data Analysis

Independent T-test and One-way analysis of variance (ANOVA) were used to analyze the results and were carried out using SPSS version 20.0

3. Results and Discussion

3.1. Antibacterial Activity of P. edulis Leaf Extract

Active antibacterial activity was demonstrated by *P. edulis* leaf extract against 3 of 5 pathogenic microorganisms, namely *S. aureus, P. aeruginosa,* and *T. mentagrophytes* as depicted by the clearing zone values of 15 mm, 17 mm, and 15 mm, respectively. On the contrary, no growth inhibition against *E. coli* and *C. albicans* was observed (Table 3).

Table 3. Antimicrobial Activity of the *P. edulis* Leaf Extract*

Test Organism	Sample	Clearing zone (mm)	Antimicrobial Index	Inhibitory Activity
E.coli	Leaf Extract	NI	0	-
	Control[a]	28	3.7	+++
S. aureus	Leaf Extract	15.0 ± 0.0	0.5	++
	Control[b]	45	6.5	+++
P. aeruginosa	Leaf Extract	17.0 ± 1.0	0.7	++
	Control[c]	18	2.0	++
C. albicans	Leaf Extract	NI	0	-
	Control[d]	40	3.0	+++
T. mentagrophytes	Leaf Extract	14.67 ± 0.58	0.5	++
	Control[d]	70	6.0	+++

Note: Leaf extract, 3%; NI means no inhibition of growth of the test organism. [a] Disc contains 1 µg Ciprofloxacin, 6 mm diameter. [b] Disc contains 30 µg Tetracycline, 6 mm diameter. [c] Disc contains 5 µg Doxycycline, 6 mm diameter. [d] Canesten solution, 100 µL (contains 1% clotrimazole)

Similar investigations on the antimicrobial activity of the methanolic extract of *P. edulis* leaf revealed its efficacy against *S. aureus, P. vulgaris, E. coli, S. faecalis, B. subtilis,* and *S. typhi* (Kanan *et al*., 2011). In a similar study, *P. edulis* leaf extract significantly inhibited the growth of *L. monocytogenes, S. gallolyticus, S. aureus, B. subtilis,* and *B. cereus*. Intermediate activity against *B. subtilis, S. aureus,* and *L. monocytogenes* were observed in methanol extracts, while a small zone of inhibition was noted in the petroleum ether and acetone extracts. In addition, partially active antimicrobial activity was observed against *P. aeruginosa* and *E. coli*. Moreover, *S. enteritidis* and *P. vulgaris* was observed to be sensitive towards methanolic extracts (Ramaiya *et al.,* 2014). In the investigation conducted by the group of Ripa (2009), chloroform extracts of *P. edulis* leaf exhibited moderate antibacterial activity, while no activity was determined from petroleum ether extract. The highest growth inhibition was observed against *P. aeruginosa*, *S. boydii*, and *S. dysenteriae*. The abovementioned results of the cited studies validate the antimicrobial action of *P. edulis* extract against pathogenic bacteria.

3.2. Phytochemical Screening of P. edulis Leaf Extract

Table 4. Phytochemical Screening of the *P. edulis* Leaf Extract*

Plant Constituent	Chemical Test	Result	Descriptive Result
Alkaloids	Munier and Macheboeuf Modification Test	+	Orange precipitate was formed.
Flavonoids	Bate-Smith and Metcalf Test	+	Generation of magenta color.
Tannins	Ferric Chloride Test	+	Generation of brownish-green color.
Glycosides	Fehling's Test	-	Brick-red colored precipitate was not observed.
Saponins	Froth Test	-	No formation of froth

Note: *Methanolic extract; The notations (+) and (-) indicates presence or absence, respectively.

Analysis of the *P. edulis* leaf extract through phytochemical screening showed that alkaloids, flavonoids, and tannins were present (Table 4). The phytochemicals in the methanolic leaf extract were confirmed by the Bate-Smith and Metcalf test, Munier and Macheboeuf modification test, and Ferric chloride test, respectively, wherein its concentration depends largely on the polarity of the solvent utilized for extraction. The phytochemical screening findings in the present study are comparable to the outcome of the study by the team of Doss (2008) and Johnson (2008).

The presence of flavonoid compounds such as vitexin, isovitexin, isoorientin, vicenin-2, and 6,8-di-*C*-glycosyl chrysin in *P. edulis* leaf extracts was identified and quantified by the team of da Silva (2013), Ayres (2015) and Cazarin (2015). Flavonoid compounds were identified to be present in high quantities in the leaves. In the study of Ferreres and coworkers (2007), sixteen (16) apigenin and luteolin derivatives including *O*-glycosyl-*C*-glycosyl, *O*-glycosyl, and mono-*C*-glycosyl and were identified and characterized to be present in *P. edulis* leaves. In addition, 4 flavones mainly 2,6-dideoxyhexose-*C*-glycosyl were also isolated and identified in the stem and leaves of *P. edulis* Sims (Xu *et al*., 2013). Solvent partitioning and chromatographic separation of the methanolic leaf extract led the team of Yuan (2017) to the identification of 12 new compounds such as benzenoids (3), flavonoids (4), quinol (1), amides (2), steroid (1) and lignan (1) in *P. edulis*.

Flavonoids, alkaloids, and tannins are reported to possess antioxidant activity and antibacterial activity by forming a complex with soluble and extracellular proteins, most especially bacterial cell walls, thereby negating their activity (Othman *et al*., 2019; Pizzi, 2021). Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds were found mostly throughout the *Passiflora* genus. Other phytoconstituents such as anthocyanins, γ-lactone, carotenoids, and volatile oil constituents were also reported to be isolated in *P. edulis* (Dhawan *et al*., 2004). The antimicrobial activity exhibited by the passion fruit leaf extracts against *S. aureus*, *P. aeruginosa*, and *T. mentagrophytes* may be due to the presence of these phytochemicals.

3.3. FTIR Spectrum of the Crude Leaf Extract

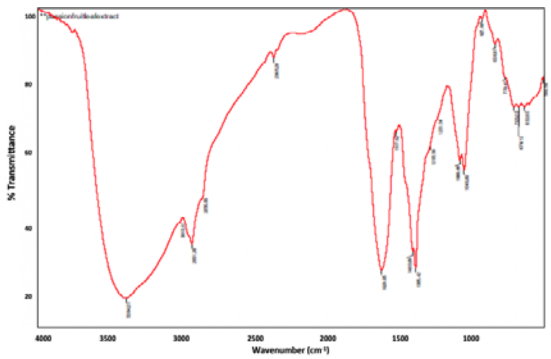

Figure 1. FTIR Spectrum of the P. edulis leaf extract

FTIR analysis of the methanolic leaf extract of *P. edulis* (Figure 1) revealed different peaks corresponding to different functional groups (Table 5). The result of FTIR analysis further confirmed that flavonoids, alkaloids, and tannins (Ricci *et al*., 2015; Ayalew, 2020) were present in the crude leaf extract and were accountable for the antimicrobial activity of the *P. edulis* leaf extract.

Table 5. FTIR Peak Values in *P. edulis* Leaf Extract

Wave Number, cm^{-1}	Compound Assignment
3342	O–H stretching vibration (presence of OH group in flavonoids)
2931	-CH, CH_2 asymmetric stretching
2365	CH_3 asymmetric and symmetric stretching
1620 & 1517	C=C aromatic ring stretching vibration (Presence of aromatic ring)
1403	Ring stretching vibration
1306	CH_3 and CH_2 scissoring (CH_3 and CH_2 groups in flavonoids and aromatics)
1060 & 1049	C–O stretching of alcohol and hydroxy compounds (phenols in tannins and flavonoids)
836	C–H deformation aromatic ring (attributed to tannins)
770	C–C stretching vibration
705	Ring deformation (attributed to tannins)
674 & 632	Aromatic C–H out of plane bending

3.4. Properties of Formulated Hand Sanitizers with P. edulis Leaf Extract (1%, 3% and 5%) and Commercial Hand Sanitizer

Table 6. Properties of Formulated and Commercial Hand Sanitizer

Properties	Formulated Hand Sanitizer			Brand A*
	F1	F2	F3	
Appearance	Gel	Gel-Liquid	Non-gel	Gel
Color	Translucent, light green with yellowish tinge	Translucent, dull green with yellowish tinge	Translucent, dark green with yellowish tinge	Translucent, green with yellowish tinge
Odor	Characteristic citrus scent	Characteristic citrus scent	Characteristic citrus scent	Sweet and Fruit-like Scent
pH	4.92 ± 0.02^{A}	4.59 ± 0.01^{B}	4.42 ± 0.01^{B}	6.18 ± 0.05^{C}
Density	0.8829 ± 0.0001^{A}	0.8830 ± 0.0003^{A}	0.8873 ± 0.0001^{B}	0.8813 ± 0.0015^{A}
Viscosity (Pa-s)	2.27 ± 0.02^{A}	0.07 ± 0.0^{B}	0.0005 ± 0.0^{C}	4.21 ± 0.15^{D}

*Brand A is the commercial hand sanitizer.

Mean values with different superscript within the same row indicate significant difference at 5% level of significance.

The properties of formulated hand sanitizers and commercial hand sanitizers are presented in Table 6. The appearance and color of formulated hand sanitizers varies as the amount of *P. edulis* extract increases (Figure 2). F1 is characterized as a gel with translucent light green, a yellowish tinge, and a characteristic citrus scent. On the other hand, F3 is a non-gel with translucent dull green with a yellowish tinge and characteristic citrus scent.

Compared to commercial hand sanitizer, significant differences in the pH and viscosity ($p < 0.05$) were noted for all the formulated hand sanitizer, while no significant difference in the density ($p > 0.05$) was noted. The formulated hand sanitizer is more acidic and less viscous than commercial hand sanitizer.

Figure 2. Formulated Hand Sanitizer with varying level of *P. edulis* Leaf Extract

The amount of *P. edulis* extract in the formulation greatly impacts the pH and viscosity of the formulated hand sanitizer. It was observed that when the amount of *P. edulis extract* in the formulation was increased, the density increased while the pH and viscosity decreased. The pH of the formulated hand sanitizer in this study is slightly lower than the pH of the hand sanitizer with *Calendula officinalis* and aloe vera (4.16 to 6.65) formulated by the group of Fallica (2021). Both hand sanitizers are slightly acidic and were within the skin's pH range (4.5 to 6.5), indicating that the skin can tolerate it. In addition, due to addition of aloe vera, xanthan gum, and hydroxyethyl cellulose as viscosity thickener, the viscosity of the hand sanitizer developed by the group of Fallica (2021) was higher than the viscosity in the present study (except for F1 which has gel-like appearance and has a viscosity of 2.27 Pa-s). It is also comparable to the viscosity of the formulated hand sanitizer with zinc-amino clay and *Opuntia humifusa* extract (1.4 to 1.5 Pa-s) formulated by Hoang *et al.* (2021). Hand sanitizer with reasonable viscosity is important since it contributes to skin feel, and spreadability of the product (Hoang *et al.*, 2021). In their study, Binder *et al*. (2019) concluded that penetrability of the active ingredient onto the skin was independent of the dynamic viscosity of the product. Hence, formulation with moderate viscosity is recommended to allow ease of application.

3.5. Antimicrobial Activity of the Formulated Hand Sanitizers with P. edulis leaf extract and Commercial Hand Sanitizer

Table 7. Antimicrobial Properties of Formulated Hand Sanitizer

Test Organism	Sample	Clearing zone, mm	Antimicrobial Index (AI)	Inhibitory Activity
E. coli	F1	NI	0	-
	F2	NI	0	-
	F3	NI	0	-
	Brand A*	12.3 ± 0.58	0.2	+
	Ciprofloxacin	38	2.8	+++
S. aureus	F1	21.6 ± 0.53^{A}	1.2	+++
	F2	26.0 ± 0.87^{B}	1.6	+++
	F3	25.9 ± 0.60^{B}	1.6	+++
	Brand A	18.7 ± 1.15^{D}	0.2	+++
	Ciprofloxacin	32	2.2	+++
P. aeruginosa	F1	14.6 ± 0.53^{A}	0.5	++
	F2	16.1 ± 0.60^{B}	0.6	++
	F3	17.4 ± 0.88^{C}	0.8	++
	Brand A	12.3 ± 0.58^{D}	0.9	+
	Ciprofloxacin	33	2.3	+++
C. albicans	F1	NI	0	-
	F2	NI	0	-
	F3	NI	0	-
	Brand A	16.3 ± 2.31	0.6	++
	Canesten[a]	35	2.5	+++
T. mentagrophytes	F1	15.8 ± 0.83^{A}	0.6	++
	F2	20.8 ± 1.72^{B}	1.1	+++
	F3	15.8 ± 0.44^{A}	0.6	++
	Brand A	31.3 ± 0.58^{D}	2.1	+++
	Canesten[a]	80	7.0	+++

NI means no inhibition of growth of the test organism. *Brand A is the commercial hand sanitizer.

[a] Contains 1% clotrimazole

Mean values with different superscript within the same column (per microorganism) indicates significant difference at 5% level of significance.

Results obtained for the antimicrobial activity of the formulated hand sanitizer with *P. edulis* leaf extract against pathogenic food-borne bacteria were presented in Table 7. From the result of the disc diffusion assay (Figure 3), the formulated hand sanitizer exhibited very active antibacterial activity against *S. aureus,* while active activity was noted against *P. aeruginosa* and *T. mentagrophytes* (except for F2, which has very active activity). On the other hand, the formulated hand sanitizer has inactive activity towards *E. coli* and *C. albicans*. Significant differences ($p < 0.05$) in the antimicrobial activity of formulated and commercial hand sanitizer were observed in the *S. aureus, P. aeruginosa* and *T. mentagrophytes*. In addition, no significant differences ($p > 0.05$) in the inhibition of *S. aureus* between F2 and F3 and in *T. mentagrophytes* between F1 and F3 were observed.

The antimicrobial activity of the formulated hand sanitizer can be attributed to the combined activity of phytochemicals present in the extracts. The high antibacterial activity of the leaf extracts against the 3 out of 5 pathogenic microorganisms tested in the present study may be due to the alkaloids, tannins, and flavonoids present in the extract. The antimicrobial activity of tannins and flavonoids can be ascribed to its ability to provide stable free radicals and inactivate protein due to complex formation with nucleophilic amino acids in the microbial cell wall (Suurbaar *et al*., 2017, Tura et al., 2017). The non-inhibitory activity of formulated hand sanitizers against *Candida albicans* may be due to the absence of glycosides which is the active phytochemical compound responsible for inhibiting the growth of the said microorganism (Park *et al*., 2010; Klunda *et al.*, 2016). The negative antimicrobial result of passion fruit leaf extract against the aforementioned microorganism further supports this. In the case of commercial hand sanitizers, excellent antimicrobial activity was exhibited by hand sanitizers with ethanol (80 %), isopropanol (75%) or benzalkonium chloride as the main active ingredient. However, significant variation in the efficacy against other pathogens was noted in different commercial hand sanitizers wherein *E. coli* is more susceptible to most of the samples evaluated than *S. aureus* (Chojnacki. *et al.,* 2021). In their study, Manaye *et al.* (2021) evaluated the effectiveness of ABHS marketed in Southwest Ethiopia. Commercially available ABHS with ethanol concentration ranging from 70% to 80% were utilized. Results revealed that the minimum inhibitory concentration (MIC) against the test organisms, *E. coli* and *S. aureus*, was at 45%, 55%, 65% and in undiluted form for Taflen. Five of the seven ABHS brands were successful in inhibiting the growth of test microorganisms.

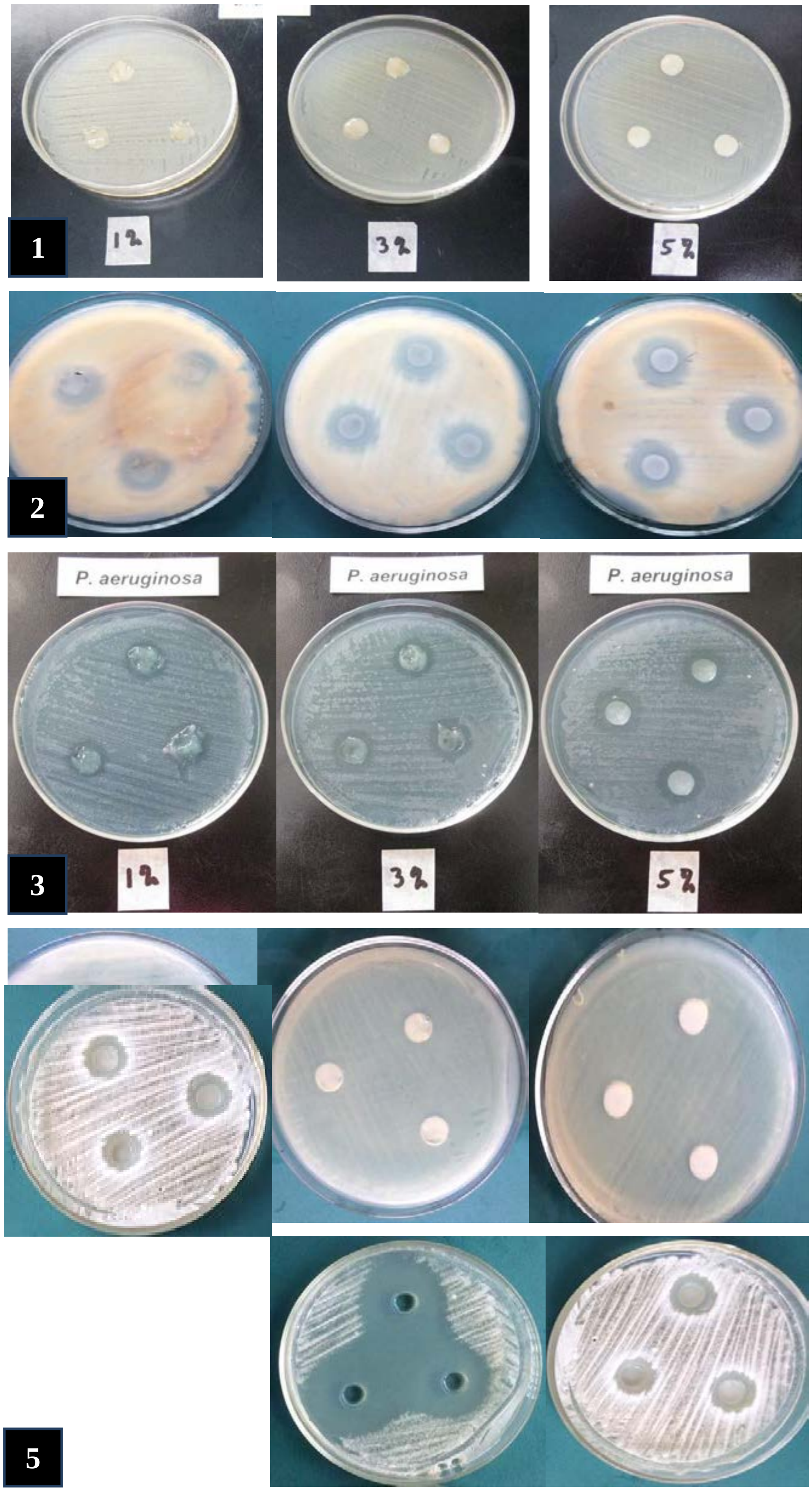

Figure 3. Antibacterial Test of Formulated Hand Sanitizer Samples Against (1) *E. coli* (2) *S. aureus* (3) *P. aeruginosa* (4) *C. albicans* (5) *T. mentagrophytes* (from left to right, F1, F2, and F3)

Nzekwe *et al*. (2021) assessed the effects of various formulation conditions such as pH, electrolyte concentration, and additives such as plant extracts and benzalkonium chloride on the killing rate of ABHS against microorganisms. Addition of plant extracts such as aloe vera, carrots and cucumber led to wider differences in the activities of ethanol and isopropanol against *S. aureus* than *E. coli*. Moreover, inclusion of extracts can confer additional properties in ABHS such as antioxidant activity, skin brightening effects, and anti-inflammatory effects. Addition of plant-based extracts to current formulation was noted as a possible solution to reduce the toxicity problem posed by alcohol based-hand sanitizers (Alghamdi, 2021).

4. Conclusion

Passiflora edulis leaf extract was added as an active ingredient to formulate a hand sanitizer. The leaf extracts contained alkaloids, flavonoids, and tannins based on the phytochemical screening analysis. The presence of the said compounds was further confirmed, as shown in the FTIR spectra. The leaf extracts were observed to prevent the growth of *P. aeruginosa*, *S. aureus*, and *T. mentagrophytes*. The formulated hand sanitizer had a greenish tinge and characteristic citrus odor. The addition of varying amounts of *P. edulis* leaf extracts was observed to significantly affect the hand sanitizer's physical properties ($p < 0.05$). It was observed that as the amount of *P. edulis* leaf extract in the hand sanitizer is increased, the density increased while the pH and viscosity decreased. The formulated hand sanitizer can prevent the growth of pathogenic organisms such as *S. aureus*, *P. aeruginosa*, and *T. mentagrophytes.*

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References

Alghamdi, H.A. 2021. A need to combat COVID-19; herbal disinfection techniques, formulations and preparations of human health friendly hand sanitizers. *Saudi J. Biol. Sci*, ***28*(7)**: 3943–3947.

Alsaidan, M.S., Abuyassin, A.H., Alsaeed, Z.H., Alshmmari, S.H., Bindaaj, T.F., &Alhababi, A.A. 2020. The Prevalence and Determinants of Hand and Face Dermatitis during COVID-19 Pandemic: A Population-Based Survey. *Dermatol Res Pract.*, 2020, 6627472, 8 pp.

Ayalew A.A. 2020. Chromatographic and spectroscopic determination of solvent-extracted *Lantana camara* leaf oil. *J. Int Med Res.*, **48(10)**: 1-12.

Ayres, A.S.F.S.J., de Araújo, L.L.S., Soares, T.C., Costa, G.M., Reginatto, F.H., Ramos, F.A., Castellanos, L., Schenkel, E.P., Soares-Rachetti, V.P., Zucolotto, S.M., &Gavioli, E.C. 2015. Comparative central effects of the aqueous leaf extract of two populations of *Passiflora edulis*. *Revista Brasileira de Farmacognosia*, **25**: 499-505.

Binder, L., Mazál, J., Petz, R., Klang, V., &Valenta, C. 2019. The role of viscosity on skin penetration from cellulose ether-based hydrogels.*Skin Res. Technol.*, **25**: 725–734.

Caldwell, J.M., de Lara-Tuprio, E., Teng, T.R., Estuar, M.R.J.E., Sarmiento, R.F.R., Abayawardana, M., Leong, R.N.F., Gray, R.T., Wood, J.G., Le, L.V., McBryde, E.S., Ragonnet, R. &Trauer, J.M. 2021. Understanding COVID-19 dynamics and the effects of interventions in the Philippines: A mathematical modelling study. *Lancet Regl Health West Pac.*, **14**: 100211.

Cazarin, C.B.B., da Silva, J.K., Colomeu, T.C., Batista, Â.G., Meletti, L.M.M., Paschoal, J.A.R., Bogusz Junior , S., Reyes, F.G.R., Augusto, F., de Meirelles, L.R., Zollner, R.L., MarósticaJúnior, M.R. 2015. Intake of *Passiflora edulis* leaf extract improves antioxidant and anti-inflammatory status in rats with 2,4,6-trinitrobenzenesulphonic acid induced colitis. *J. Funct. Foods*, **17**: 575-586.

Chojnacki, M., Dobrotka, C., Osborn, R., Johnson, W., Young, M., Meyer, B., Laskey, E., Wozniak, R., Dewhurst, S., &Dunman, P.M. 2021. Evaluating the Antimicrobial Properties of Commercial Hand Sanitizers. *mSphere*, **6(2)**: e00062-2.

da Silva, J.K., Cazarin, C.B.B., Colomeu, T.C., Batista, Â.G., Meletti, L.M.M., Paschoal, J.A.R., BoguszJúnior, S., Furlan, M.F., Reyes, F.G.R., Augustu, F., MarósticaJúnior, M.R. &Zollner, R.L. 2013. Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: In vitro and in vivo study. *Food Res. Int.*, **53(2)**: 882-890.

Dhawan, K., Dhawan, S., & Sharma, A. 2004. Passiflora: a review update. *J Ethnopharmacol.*, **94(1)**: 1-23.

Doss, A., Doss, P.A., &Dhanabalan, R. 2008. In-Vitro Antimicrobial Activity of Extracts of *Passiflora edulis* (Passifloraceae) and *Sphaeranthusindicus* (Asteraceae).*Ethnobotanical Leaflets*, **12**: 728-733.

Dzotam, J.K., Touani, F.K. &Kuete, V. 2015. Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosomamafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria. *BMC Complement Altern Med*., **16(9)**:1-8.

Fallica, F., Leonardi, C., Toscano, V., Santonocito, D., Leonardi, P., & Puglia, C. 2021. Assessment of Alcohol-Based Hand Sanitizers for Long-Term Use, Formulated with Addition of Natural Ingredients in Comparison to WHO Formulation 1. *Pharmaceutics*, **13(4)**: 571.

Farag, M. A., Otify, A., Porzel, A., Michel, C. G., Elsayed, A., &Wessjohann, L. A. 2016. Comparative metabolite profiling and fingerprinting of genus Passiflora leaves using a multiplex approach of UPLC-MS and NMR analyzed by chemometric tools. *Anal Bioanal Chem.*, **408(12)**: 3125–3143.

Ferreres, F., Sousa, C., Valentão, P., Andrade, P.B., Seabra, R.M. & Gil-Izquierdo, Á. 2007. New *C*-Deoxyhexosyl Flavones and Antioxidant Properties of *Passiflora edulis* Leaf Extract. *J. Agric. Food Chem.*, **55(25)**: 10187–10193.

Filipe, H.A.L., Fiuza, S.M., Henriques, C.A., & Antunes, F.E. 2021. Antiviral and antibacterial activity of hand sanitizer and surface disinfectant formulations. *Int J. Pharm.*, **609:** 121139.

Golin, A.P., Choi, D., &Ghahary, A. 2020. Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. *Am. J. Infect Control*, **48(9)**: 1062-1067.

Guevara, B.Q. (Ed.), Quinto A., & Santos M.A. 2005. Microbiology Section, **A guidebook to Plant Screening: Phytochemical and Biological, Revised Edition**, Manila: UST Publishing House.

Hans, M., Lugani, Y., Chandel, A.K., Rai, R., Kumar,S. 2021. Production of first- and second-generation ethanol for use in alcohol-based hand sanitizers and disinfectants in India. *Biomass Convers Biorefin*., **27**: 1-18.

He, X., Luan, F., Yang, Y., Wang, Z., Zhao, Z., Fang, J., Wang, M., Zuo, M., & Li, Y. 2020. *Passiflora edulis*: An Insight Into Current Researches on Phytochemistry and Pharmacology. *Front pharmacol.*, **11**: 617.

Hoang, H.T., Van Tran, V., Bui, V.K.H., Kwon O.H., Moon, J.Y., & Lee, Y.C. 2021. Novel moisturized and antimicrobial hand gel based on zinc-amino clay and *Opuntia humifusa* extract. *Sci Rep*, **11**: 17821.

Jain, V. M., Karibasappa, G. N., Dodamani, A. S., Prashanth, V. K., & Mali, G. V. 2016. Comparative assessment of antimicrobial efficacy of different hand sanitizers: An *in vitro*study. *Dent Res J (Isfahan)*, **13(5)**: 424–431.

Johnson, M., Maridass, M., & Irudayaraj, V. 2008. Preliminary Phytochemical and Antibacterial Studies on *Passiflora edulis*. *Ethnobotanical Leaflets*, **2008(1):** Art. 51, 425-432.

Kannan, S., Devi, B.P., &Jayakar, B. 2011. Antibacterial evaluation of the methanolic extract of *Passiflora edulis*. *Hygeia-J. D. Med.*, **3(1)**, 46–49.

Kanu, A.M., Okorie, A.C., Uche, C., & Awa, U.N. 2017. Phytochemical Screening and Antimicrobial Activity of Ethanoic Extract of *Passiflora edulis var. flavicarpa*Seed on Selected Pathogens**.** *Univ J.Microbiol Res.*, **5(3)**: 35-39.

Klunda, T., Machová, E., Čížová, A., Horák, R., Poláková, M., &Bystrický, S. 2016. Alkyl glycosides as potentialanti-*Candida albicans* growth agents. *Chem. Pap.*, **70(9)**: 1166-1170

Manaye, G., Muleta, D., Henok, A., Asres, A., Mamo, Y., Feyissa, D., Ejeta, F., &Niguse, W. 2021. Evaluation of the Efficacy of Alcohol-Based Hand Sanitizers Sold in Southwest Ethiopia. *Infect Drug Resist.*, **14**, 547–554.

Manuel, J.F., Balangcod, T.D., Laruan, L.A., Patacsil, M.C., Martin, S.P. 2011. Suppression of growth of some medically important bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) with plant extracts of selected indigenous semi-temperate medicinal plants in the Philippines. *Tangkoyob Journal*, **6(1)**:1-12.

Mathur P. 2011. Hand hygiene: back to the basics of infection control. *Indian J Med Res.*, **134(5)**, 611–620.

Nzekwa, I.T., Agwuka, O.I., Okezie, M.U., Fasheun, D.O., Nnamani, P.O. &Agubata, C.O. 2021. Designing an ideal alcohol-based hand sanitizer: in vitro antibacterial responses of ethanol and isopropyl alcohol solutions to changing composition. *AAPS Open*, **7(5)**.

Othman, L., Sleiman, A., & Abdel-Massih, R.M. 2019. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Front Microbiol.*, **10(911)**: 1-28.

Park, C., Woo, E.-R., & Lee, D. G. 2010. Anti-*Candida* property of a lignan glycoside derived from *Styrax japonica* S. et Z. via membrane-active mechanisms. *Mol Cells*, **29(6)**: 581–584.

Penecilla G.L., &Magno C.P. 2011. Antibacterial activity of extracts of twelve common medicinal plants from the Philippines, *J. Med. Plant. Res.*, **5(6)**: 3975-3981.

Pizzi, A. 2021. Tannins medical/pharmacological and related applications: A critical review. *Sustain. Chem. Pharm.*, **22**: 100481.

Ramaiya, S.D., Bujang, J.S., &Zakaria, M.H. 2014. Assessment of Total Phenolic, Antioxidant and Antibacterial Activities of Passiflora Species. *ScientificWorldJournal*, **Vol. 2014**, 167309, 10 p.

Ricci, A. Olejar, K.J., Parpinello, G.P., Kilmartin, P.A., &Versari, A. (2015). Application of Fourier Transform Infrared (FTIR) Spectroscopy in the Characterization of Tannins. *Applied Spectroscopy Reviews*, **50(5)**, 407-442.

Ripa, F.A., Haque, M., Nahar, L., & Islam, M.M. 2009. Antibacterial, Cytotoxic and Antioxidant Activity of *Passiflora edulis* Sims. *European Journal of Scientific Research*, **31(4)**: 592-598.

San Luis, G. D., Balangcod, T.D., Abucay, Jose Jr. B., Wong, F.M., Balangcod K. D., Afifi, N.G., & Apostol, O.G. (2014). Phytochemical and antibacterial screening of indigenous species that have potential for revegetation of landslides in Atok, Benguet, Philippines. *Indian Journal of Traditional Knowledge*, **13(1**): 56-62.

Suurbaar, J., Mosobil, R. & Donkor, AM. 2017. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens, *BMC Res Notes*, **10:** 660.

Thombare, M.A., Udugade, B.V., Hol, T.P., Mulik, M.B., &Pawade, D.A. 2015. Formulation and Evaluation of Novel Herbal Hand Sanitizer. *Indo American Journal of Pharmaceutical Research*, **5(1):** 483-488.

Tura, G.T., Eshete, W.B. &Tucho, G.T. 2017. Antibacterial efficacy of local plants and their contribution to public health in rural Ethiopia. *Antimicrob Resist Infect Control*, **6(76)**.

Valle, D. L., Andrade, J. I., Puzon, J. J. M., Cabrera, E. C., & Rivera, W. L. 2015. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pac J Trop Biomed.*, **5(7)**: 532–540.

Wang C, Tee M, Roy AE, Fardin MA, Srichokchatchawan W, Habib HA, et al. 2021. The impact of COVID-19 pandemic on physical and mental health of Asians: A study of seven middle-income countries in Asia. *PLoS ONE*, **16(2)**: e0246824.

World Health Organization, WHO guidelines on hand hygiene in health care: first global patient safety challenge clean care is safer care. 2009.World Health Organization, Geneva

Wu, Y. C., Chen, C. S., & Chan, Y. J. (2020). The outbreak of COVID-19: An overview. *J Chin Med Assoc: JCMA*, **83(3)**: 217–220.

Xu, F., Wang, C., Yang, L., Luo, H., Fan, W., Zi, C., Dong, F., Hu, J. & Zhou, J. (2013). *C*-dideoxyhexosyl flavones from the stems and leaves of *Passiflora edulis* Sims. *Food Chem.*, **136**: 94-99.

Yuan, T. Z., Kao, C. L., Li, W. J., Li, H. T., & Chen, C.Y. (2017). Chemical Constituents of Leaves of *Passiflora edulis. Chem Nat Compd.*, **53(6)**:1165–1166.