Extraction of *Andrographis paniculata* as Material for Developing Soap and Sanitizer

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**Abstract.** The aim of this work to analyse quantitative study of chemical properties of different *A. paniculata* extracts obtained from different extraction methods which are methanol and ethanol extracts by maceration and Soxhlet extraction. This consists of antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC). This project also aims to study the chemical and physicochemical properties of the developed soap and sanitizer in order to evaluate the quality of the products. The properties of original extracts were compared with the products in order to investigate the improvement of the antioxidant contents of the products upon addition of the *A. paniculata* extract to them. The antioxidant activity is evaluated using 2,2-diphenyl-1-picrylhydrazyl test. TPC is determined by using Folin-Ciocalteu calorimetric method where Folin-Ciocalteu reagent is used. TFC is quantified by following aluminium chloride calorimetric method. All the tests are quantified using spectrophotometric. Simple antimicrobial assay by hand printing method on agar plate media was also done to analyze the efficiency of the sanitizers against humans’ pathogens on hands. The results showed the *A. paniculata* macerated methanol extract exhibited highest antioxidant activity with 76.027% as well as the highest TPC and TFC with 0.222 mg GAE/ml and 7.500 mg QE/ml. The results also showed both products were able to retain similar antioxidant content, higher TPC and lower TFC to that indicated by the original plant extract used to prepare the products. The result showed the improvement of antioxidant contents of soap and sanitizer upon the addition of *A. paniculata* extracts. Therefore, *A. paniculata* extracts are potent natural ingredients for antioxidant properties purpose and can be used to replace the harmful synthetic ingredients on products formulations.

1. **Introduction**

The skin is the largest organ of the human body as it forms barrier against harmful chemical and physical impacts of environment on humans, but hands are generally the primary mode of transmission of microbes and infections. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent the infections [1]. Nowadays, hand hygiene is very important due to the emergence of Coronavirus disease 2019 (COVID-19) pandemic that has risen to be a significant global health concern given its contagious nature. For generations, hand washing with soap and water has been considered a measure of personal hand hygiene [2]. The Centre for Disease Control and Prevention (CDC) United States has promoted and encouraged hand hygiene through handwashing as well as the use of hand sanitizer for this dangers imposed disease. A therapeutic vaccine is not yet on the rise, so preventive measures are the current approach to restraint the transmission of cases by the practice of soap and sanitizer usage on hands [3].

The products of basic saponification reactions are soaps [4] that have been used among people since its discovery by the ancient Babylonians as a cleansing substance [5]. At present, mostly liquid soaps and solid bar soaps commercially available as medicinal soap products. On the other hand, hand sanitizers are agent that are applied to the hands for the purpose of removing common pathogens and they come as liquid, gel or foam. Both sanitizers and soaps has been enriched in order to provide different medicinal properties using various natural ingredients such as
anti-inflammatory, antibacterial, antifungal, and antioxidant to the final product [6]. Additionally, natural ingredients also have an impact on the odor, texture and color of the products. To give the desired bioactivities especially when preparing medicinal products, it is more attractive and important to substitute natural ingredients for harmful synthetic substances. Examples of synthetic substances which have been most commonly used in such consumer products to give antioxidant and antibacterial effects respectively are triclosan and butylated hydroxytoluene (BHT). Because of the recent concern on the detrimental effects of the synthetic antibacterial and antioxidant the natural ingredients attained from common medicinal plants could be used as substitutes for these synthetic substances [7].

In Malaysia, hempedu bumi or _Andrographis paniculata_ (A. paniculata) are listed as a valuable herb to be commercialized because this plant has been effectively used as traditional medicines, in Asia as well as around the world, for centuries. Indeed, many researchers in India, Thailand and other countries keen to study the antioxidant and antibacterial activities of _A. paniculata_ extracts [8] as many previous researchers claimed _A. paniculata_ possesses antioxidant and antimicrobial properties. Ethanol extract of _A. paniculata_ showed potent antibacterial activity [9] while methanol extract from the leaves of _Andrographis paniculata_ has been shown to be antioxidant [10]. However, applicability of _A. paniculata_ extracts in the preparation of herbal products such as soaps and sanitizers has not been reported up yet by any previous researchers. _A. paniculata_ is readily available and an easily cultivated plant. Therefore it can be suitable even for scale application in industries. In this study, extracts from _A. paniculata_ were investigated, and medical values were incorporated to enhance the products. The efficiency of any product depends on the scientific validation that are done on them. Thus, the project aims to study the chemical properties such as antioxidant properties and total phenolics and flavonoids on _A. paniculata_ extracts, as well as the properties on the products developed from the extracts. This is to investigate the improvement of the antioxidant contents of the products upon addition of the _A. paniculata_ extract.

### 2. Methodology

#### 2.1. Extraction using cold maceration and Soxhlet extraction

Maceration was carried out using methanol and ethanol as solvents. First, 10% percent concentration of extracts were prepared by adding 20 grams of _A. paniculata_ dry powder into a 200ml conical flask of solvents. The conical flasks were wrapped with cotton and aluminium foil and were transferred into an incubator shaker. They were kept for 1 day at 37°C with gentle shaking at 120 rpm. Then, the extraction was filtered using Whatman No. 1 filter paper in a filter funnel and the filtrate were collected. The filtrate was evaporated using rotary evaporator at 50°C for methanol extract and 70°C for ethanol extract. The concentrated extract was then transferred to glass petri dishes and dried in drying oven at 40°C. The weight of extract was then measured after solvent evaporation and kept into air tight containers [12,13].

Soxhlet extraction was carried out using ethanol and methanol as solvents. _A. paniculata_ dry powder was extracted using Soxhlet apparatus. The extraction process was done for 6 hours at constant temperature of 60°C [14]. The crude extract of _A. paniculata_ was evaporated using a rotary evaporator. The weight of extract was then measured after solvent evaporation and kept into air tight container.

#### 2.2. Develop soap from _A. paniculata_ extracts

First, 10 g of solidified basic glycerine soap was broken down to smaller pieces and melted on water bath. Then, 1.5 g of the macerated _A. paniculata_ extract of ethanol solvent was added to the melted soap along with 5 ml of ethanol. About 0.033 g of stearic acid, 1 ml each of citronella oil and cinnamon oil were added to the melted soap. The melted soap was gently mixed for about 30 minutes and moulded on moulds. The soap was allowed to solidify at room temperature until set and kept under physical observation for any characteristic changes. These steps were repeated to develop soap from macerated _A. paniculata_ extract using methanol solvent [15].
2.3. Develop liquid-gel sanitizer from A. paniculata extracts
To develop liquid-gel sanitizer from A. paniculata extracts, Carbopol was used as it acts as gelling agent because it creates good viscosity for gel dosage. A total of 1 g Carbopol was added into 30 ml hot distilled water then stirred by using a stirrer to homogenize the gel. Then 7.5 ml A. paniculata extract of ethanol solvent was added followed by 0.1 g methyl paraben that acts as preservative, and 5 ml of glycerin which acts as humectants that aims to protect the hand skin against dryness. Then, 40 ml 99% isopropyl alcohol was added into the solutions [15]. The isopropyl alcohol used as the solvent in an amount that does not affect the antibacterial activity of the extract [16]. A few drops of triethanolamine (TEA) was added gradually along with few drops of perfume and green colourant (Star Brand, Malaysia). The ingredients were stirred well until liquid-gel sanitizer solution formed. These steps were repeated to develop sanitizer from macerated A. paniculata extract of methanol solvent.

2.4. Chemical properties analyses of A. paniculata extracts and products
The properties of A. paniculata extracts and products were determined by the use of antioxidant assay (DPPH), total phenolic content (TPC) and total flavonoid content (TFC) methods.

2.4.1. Antioxidant activity assay. 2,2-diphenyl-1-picrylhydrazl (DPPH) was used to evaluate the antioxidant activity of A. paniculata extracts and products based on the capacity of the antioxidant compounds to scavenge the DPPH free radicals in vitro [17]. The test sample was prepared by mixing 1 ml of the A. paniculata extract with 2 ml of 0.1 mM DPPH methanolic solution and the mixture was allowed to react and incubated in the dark for 30 minutes. A small amount of the solution was transferred into cuvette. The absorbance of the reaction mixture was measured at 517 nm by using a spectrophotometer (Thermo Fisher Scientific) [18]. The control was prepared by adding 2 ml of 0.1 mM DPPH methanol solution with 1 ml of methanol. Three replicates absorbance readings were taken for each extract. These steps were repeated for products of A. paniculata extracts. The optical density obtained was converted into percentage (% inhibition) of free radical scavenging activity and was calculated using the following relation [19].

\[
\% \text{ inhibition} = \frac{(Ab - Ae)}{Ab} \times 100
\]

2.4.2. Determination of total phenolic content (TPC). The total phenolic content of A. paniculata extracts and products was determined by using Folin-Ciocalteu calorimetric method. Folin-Ciocalteu reagent was used to determine the total phenolic compounds in the A. paniculata extracts. For preparing the test sample, 1 ml of A. paniculata extract was pipetted and transferred to a test tube with addition of 2 ml of 15% sodium carbonate solution, 0.5 ml of Folin-Ciocalteu reagent and made up the volume to 10 ml by adding with distilled water. The solution was left for 15 minutes in the dark before the absorbance was read. A small amount of the solution was transferred to the cuvette. These steps were repeated for products of A. paniculata extracts. For preparing standard curve of gallic acid, gallic acid standard solutions in the range of 0.01-0.08 mg/ml were prepared from 1 mg/ml stock gallic acid in ethanol. The absorbance of the sample extract as well as the gallic acid standard solutions was determined at wavelength of 718 nm against a blank solution using a spectrophotometer [20]. Three replicate absorbance readings will be taken for each extract. The obtained absorbance value for the test sample was referred to the standard gallic acid calibration curve and the TPC was calculated by using the following equation. The amount of phenolic content in extract compared with the standardized gallic acid in miligrams equivalent of gallic acid per gram of extract (mgGAE/g extract) from the formula. Where TPC is the total phenolic content, GAE is the equivalent value of gallic acid obtained from substitution in a linear equation (mg/ml), V is the sample volume (ml), D is the dilution factor and W is the sample weight (g).
2.4.3. Determination of total flavonoid content (TFC). Total flavonoid content of A. paniculata extract and products was quantified by following aluminium chloride colorimetric method using spectrophotometric technique. For the preparation of the test sample, 0.05 ml of A. paniculata extract was pipetted and transferred to a test tube. 0.3 ml of 5% sodium nitrate solution and 4 ml of distilled water was added into the test tube. After five minutes, 0.3 ml of 10% of aluminum chloride was added into the test tube. At the sixth minutes, 2 ml of 1 M sodium hydroxide was added into the test tube and the volume was made up to 10 ml by addition of distilled water. The solution was left in the dark for 15 minutes before the absorbance was read. Orange yellowish color appears from transparent. These steps were repeated for products of A. paniculata extracts. The TFC of the sanitizer and soap were compared to that of control sanitizer and soap. The sanitizer and soap as control test samples were made without the addition of A. paniculata extracts.

For preparing standard curve of quercetin, quercetin standard solutions in the range of 0.01-0.10 mg/ml were prepared from 1 mg/ml stock quercetin in ethanol. The absorbance of the sample extract as well as the standard solution were determined at wavelength of 415 nm against the blank solution using a spectrophotometer [21]. The obtained absorbance value for the test sample was referred to the standard quercetin calibration curve and the total content of flavonoid compounds was calculated using following relation.

\[
\text{TFC} = \frac{\text{GAE} \times V \times D}{W}
\]  

2.4.4. Statistical analysis. The results obtained in the determination of antioxidant activity, total phenolic content and total flavonoid content of A. paniculata were represented as the values of three individual replicates as mean ± standard error and the results were analyzed by ANOVA.

2.5. Evaluation of physicochemical parameters of products from A. paniculata extracts

2.5.1. Ph. The pH of the prepared products were determined by using Digital pH meter (pH Meter Hanna Instruments). The soaps were dissolved in 100 ml of distilled water and stored for 2 hours while pH for sanitizers were directly determined [15][22].

2.5.2. Moisture content. Two grams of sample products were placed in a weighed porcelain crucible. The samples were left in an oven at 105 °C for three hours and then transferred to a desiccator for one hour [15][22]. The moisture content was calculated using following relation.

\[
\text{Moisture content} = \frac{\text{Difference in weight (g)}}{\text{Sample weight (g)}} \times 100\%
\]

2.5.3. Foam height
About 0.5 g of the sample was taken, dispersed in 25 ml of distilled water. After that, it was transferred to a 100 ml measuring cylinder. The volume was made up to 50ml with water. 25 strokes were given and stand till aqueous volume measured up to 50ml and the foam height was measured, above the aqueous volume [15][22].

2.5.4. Foam retention. About 25 ml of the 1% soap solution was taken into 100 ml graduated measuring cylinder. The cylinder was covered with hand and shaken for 10 times. The volume of the foam at 1-minute interval for 4 minutes was recorded [15, 22].

2.5.5. High temperature stability
The product samples were dissolved in 50 ml distilled water and were allowed to stand at 50 °C for one week. The stability of the samples were observed each day during this period. The sample which was
homogenous and stable liquid after standing was indicated as stable. The sample in which the crystals were roughened and precipitated, indicated as unstable [15][22].

2.6. Antimicrobial assay by hand printing method on agar plate media

2.6.1 Medium preparation of bacteria. For nutrient agar making, 11.5 g of nutrient agar powder was mixed with 500 ml of distilled water. This mixture was then heated on a hot plate with frequent stirring to prevent agar from over-cooking at the bottom of the flask. Then, the agar was sterilized for 2 hours and 30 minutes in an autoclave at 121 °C. Then, the agar was left to cool and poured into petri dishes carefully in the laminar chamber. After the agar solidified, they were stored in a chiller [23].

2.6.2. Hand printing method. Hand printing method on sterile agar plate media was used to test the effectiveness of the developed sanitizer from A. paniculata extracts. There are 3 categories of hand print which are unsanitized hand, sanitized hand using ethanol and methanol A. paniculata sanitizers, and sanitized hand using sanitizer that are commercialized in market. Each category was carried out in triplicate with different 3 individual’s hand to fairly check the antibacterial activity of the formulated antibacterial sanitizer [24]. Hand printing of bare hands without sanitation (exposed to the environment) were made on agar plate media. Then, the hand printings also were done after the individuals’ hands were sanitized with the developed sanitizers and commercial sanitizers. The plates were then incubated at 37 °C for 24 hours and the pattern of microbial growth on the areas of the hand prints was observed.

3. Results and Discussion

3.1. Chemical properties of A. paniculata extracts

The properties of A. paniculata extracts were determined by the use of 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay method, total phenolic content using Folin-Ciocalteu procedure and total flavonoid content using aluminium chloride colorimetric method.

3.1.1. Antioxidant activity assay of A. paniculata extracts. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) assay was used to evaluate antioxidant activity for methanol and ethanol extracts of A. paniculata in scavenging the free radicals. It is based on discoloration of violet colour for stable DPPH free radical solution change to yellow when it is reduced by antioxidant compound [25]. It is a simple, fast and sensitive assay for this antioxidant activity. The DPPH scavenging activity of the different A. paniculata extract indicates that A. paniculata extracts possess the antioxidant activity properties which have potential to scavenge the DPPH free radicals. Table 1 demonstrates the DPPH scavenging activity of ethanol and methanol extract solvents using cold maceration and Soxhlet extraction. It was measured at 517 nm wavelength because it has maximum absorption at this wavelength [26].

| Method of Extraction | Type of Solvent | DPPH scavenging activity (%) |
|----------------------|----------------|-----------------------------|
| Cold maceration      | Ethanol        | 70.673 ± 2.316              |
|                      | Methanol       | 76.027 ± 0.294              |
| Soxhlet extraction   | Ethanol        | 68.036 ± 0.533              |
|                      | Methanol       | 71.023 ± 0.533              |

Data represent the mean ± standard deviation (n=3).

As reflected in the Table 1, methanol extract by cold maceration displayed the highest DPPH scavenging activity of 76.027 ± 0.294 % followed closely by methanol extract by Soxhlet extraction with 71.023 ± 0.533 %. Ethanol extract by cold maceration displayed DPPH scavenging activity of 70.673 ± 2.316 % and the lowest was ethanol extract by Soxhlet extraction with 68.036 ± 0.533 %. For both methods, there is no significant difference of DPPH scavenging activity between methanol and ethanol solvents.
The result was in agreement with the previous researches. Using maceration, it is reported DPPH scavenging activity of \textit{A. paniculata} extracts, those prepared from methanol or ethanol are slightly different with 32.7\% and 29.9\% DPPH, respectively [12]. Another study reported DPPH scavenging activity of \textit{A. paniculata} extracts using methanol as solvent was higher than ethanol with 93.174\% and 80.887\%, DPPH respectively [27]. By comparison of these two studies, DPPH scavenging activity of \textit{A. paniculata} extracts using methanol as solvent was higher than ethanol but with no significant difference. By comparing the results from present study and previous researchers, macerated methanol extract exhibits the highest antioxidant study.

3.1.2. Determination of total phenolic content (TPC). The phenolic content of the \textit{A. paniculata} extracts was measured by using the calorimetric Folin Ciocalteu method. A blue colour complex formed because of the reaction between the Folin Ciocalteu reagent and phenolic compounds under alkaline condition which absorbs radiation and allows quantification [28] that are quantified spectrophotometrically at the wavelength of 718 nm. t is calculated by referring to the gallic acid standard curve with equation $y = 3.185x + 0.049$, $R^2 = 0.996$ where $x$ is the concentration of gallic acid (mg/ml) and $y$ is the absorbance value. The result of the total phenolic content of the \textit{A. paniculata} extracts are expressed in mg GAE/ml as shown in Table 2.

| Method of Extraction     | Type of Solvent | Total Phenolic Content (mg GAE/ml) |
|--------------------------|-----------------|-----------------------------------|
| Cold maceration          | Methanol        | 0.222 ± 0.039                     |
|                          | Ethanol         | 0.140 ± 0.027                     |
| Soxhlet extraction       | Methanol        | 0.180 ± 0.017                     |
|                          | Ethanol         | 0.062 ± 0.010                     |

Data represent the mean ± standard deviation (n=3).

As can be seen in Table 2, the result obtained for TPC reported that methanol extract by cold maceration possessed the highest phenolic content with 0.222 ± 0.039 mg GAE/ml followed by methanol extract using Soxhlet extraction. The least total phenolic content is recorded by ethanol extract using Soxhlet extraction with 0.062 ± 0.010 mg GAE/ml. By comparing both extraction methods, cold maceration is better as it shows higher reading values of total phenolic contents than Soxhlet extraction. There was significant difference between total phenolic content in methanol and ethanol extracts. The result was in agreement with a study conducted by [12] who found that methanolic had higher value n their phenolic contents than ethanolic extracts at 264.82 mg/100g GAE and 262.57 mg/100g GAE respectively. This result also in agreement with a statement reported by [29] that stated among the pure solvents, methanol was the most efficient solvent for extraction of phenolic compounds, followed by ethanol [30] also reported the methanol and ethanol extracts of \textit{A. paniculata} recorded the highest phenolic content compared to other solvents.

This study is in accordance with a study conducted on TPC comparing between cold maceration and Soxhlet extraction for \textit{Thymelaea hirsute L.} [31] which reported the macerated methanol extracts were richer in total phenolic content than the methanol extracts obtained by Soxhlet method with 259.63 ± 3.17 mg GAE/ml and 206.70 ± 2.15 mg GAE/ml respectively. Thus, it supported the result of TPC obtained which indicated methanol extract of \textit{A. paniculata} using cold maceration is higher than methanol extract of \textit{A. paniculata} using Soxhlet extraction.

3.1.3. Determination of Total Flavonoid Content (TFC). The Total Flavonoid Content in the \textit{A. paniculata} extracts were determined by following the aluminium chloride calorimetric method. The TFC was referred to the Quercetin standard curve of $y = 0.1252x + 0.002$, $R^2 = 0.9917$ where $x$ is the
concentration of quercetin (mg/ml) and y is the absorbance of the samples were measured at the wavelength of 510 nm. The result of the TFC was presented in the Table 3.

Table 3: Total flavonoid content (mg QE/ml) in A. paniculata extracts for two different extraction methods.

| Method of Extraction | Type of Solvent | Total flavonoid content (mg QE/ml) |
|----------------------|-----------------|------------------------------------|
| Cold maceration      | Ethanol         | 7.274 ± 0.012                      |
|                      | Methanol        | 7.500 ± 0.016                      |
| Soxhlet extraction   | Ethanol         | 2.569 ± 0.331                      |
|                      | Methanol        | 2.929 ± 0.428                      |

Data represent the mean ± standard deviation (n=3)

As reflected in the Table 3, methanol extract by cold maceration recorded the highest total flavonoid content of 7.500 ± 0.016 mg QE/ml followed closely by ethanol extract by cold maceration with 7.274 ± 0.012 mg QE/ml. Ethanol extract by Soxhlet had the lowest total flavonoid content with 2.569 ± 0.331 mg QE/ml. For both methods, there was no significant difference of total flavonoid content between methanol and ethanol solvents but exhibited significant TFC values between both extraction methods. A study conducted on TFC comparing between cold maceration and Soxhlet extraction for Thymelaea hirsute L. [31] reported the macerated extracts were richer in total flavonoid content than the extracts obtained by Soxhlet method. However, this result disagreed with previous study reported by Pushpanathan and Nithyanandam [27] which reported A. paniculata extracts with ethanol solvent had higher TFC with 92.870 mg QE/100 g compared to methanol with 74.959 mg QE/100 g.

However, methanol is the most efficient solvents for the extraction due to the better solvation of flavonoid compounds present in the medicinal plant as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant molecules and the solvent. Ethanol was less efficient in the extraction of antioxidant compounds than methanol, regardless of the fact that their polarities were comparative [32]. This may be due to the low solvation gave by ethanol, probably because of the presence of the ethyl radical that is longer than the methyl radical present in methanol, resulting in a lower solvation of antioxidant molecules [29]. This supports the TFC result from current study which indicated A. paniculata extracts with methanol solvent using the cold maceration method had the highest total flavonoid content.

3.2. Chemical properties of products from A. paniculata extracts

The properties of products from A. paniculata extracts were determined by the use of 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay method, total phenolic content using Folin-Ciocalteu procedure and total flavonoid content using aluminium chloride colorimetric method.

3.2.1. Antioxidant activity assay. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) assay was used to determine antioxidant content in the sanitizer and soap extracts, DPPH was used to access the products’ antioxidant activity potential. Table 4 shows DPPH scavenging activity at wavelength of 517 nm in sanitizer and soap from A. paniculata extracts using cold maceration extraction method. Experiments were carried in triplicate and the antioxidant content of the sanitizer and soap were compared to that of control sanitizer and soap. The control sanitizer and soap were made without the addition of A. paniculata extracts.
Table 4: DPPH scavenging activity in sanitizer and soap from *A. paniculata* extracts using cold maceration extraction method.

| Type of Product | Type of *A. paniculata* extraction solvent | DPPH scavenging activity (%) |
|-----------------|------------------------------------------|------------------------------|
| Sanitizer       | Control                                  | 48.381 ± 1.853               |
|                 | Ethanol                                  | 73.745 ± 2.349               |
|                 | Methanol                                 | 77.509 ± 1.035               |
| Soap            | Control                                  | 50.449 ± 4.582               |
|                 | Ethanol                                  | 81.566 ± 0.252               |
|                 | Methanol                                 | 80.304 ± 2.229               |

Data represent the mean ± standard deviation (n=3).

As reflected from Table 4, the soaps and sanitizers were about 1.5 times and 1.6 times respectively better than the control sanitizer and soap in their antioxidant content. Both products were able to retain similar antioxidant content to that indicated by the original plant extract used to prepare the products [24]. The products upon addition of the *A. paniculata* extract clearly indicate the improvement of the antioxidant content results.

3.2.2. Determination of total phenolic content (TPC). The amount of phenolic content in the products of *A. paniculata* extracts were determined using Folin-Ciocalteu method. It is calculated by referring to the gallic acid standard curve with equation $y = 3.185x +0.049$, $R^2 = 0.996$ where $x$ is the concentration of gallic acid (mg/ml) and $y$ is the absorbance value at the wavelength of 718 nm. TPC in sanitizer and soap from *A. paniculata* extracts using cold maceration are expressed in mg GAE/ml as shown in Table 5.

Table 5. Total Phenolic Content (mg GAE/ml) in sanitizer and soap from *A. paniculata* extracts using cold maceration extraction method.

| Type of Product | Type of *A. paniculata* extraction solvent | Total phenolic content (mg GAE/ml) |
|-----------------|------------------------------------------|-----------------------------------|
| Sanitizer       | Control                                  | 0.132 ± 0.002                     |
|                 | Ethanol                                  | 0.259 ± 0.001                     |
|                 | Methanol                                 | 0.286 ± 0.000                     |
| Soap            | Control                                  | 0.204 ± 0.028                     |
|                 | Ethanol                                  | 0.312 ± 0.015                     |
|                 | Methanol                                 | 0.437 ± 0.006                     |

Data represent the mean ± standard deviation (n=3).

From Table 5, as expected, the control sanitizer shows a minimum TPC of 0.132 ± 0.002 followed by the control soap with 0.204 ± 0.028 mg GAE/ml. Both products exhibited higher phenolic content compared to TPC of the original plant extract used to prepare the products. The improvement of the phenolic content of each product upon addition of the *A. paniculata* extract.

3.2.3. Determination of total flavonoid content (TFC). The amount of flavonoid content in the products of *A. paniculata* extracts were determined using the aluminium chloride calorimetric method. It is calculated based on quercetin standard curve of $y = 0.1252x + 0.002$, $R^2 = 0.9917$ where $x$ is the concentration of quercetin (mg/ml) and $y$ is the absorbance of the samples that were measured at the wavelength of 510 nm. Total flavonoid content in sanitizer and soap from *A. paniculata* extracts using cold maceration are expressed in mg QE/ml as shown in Table 6.
Table 6. Total flavonoid content (mg QE/ml) in sanitizer and soap from *A. paniculata* extracts using cold maceration extraction method.

| Type of Product | Type of *A. paniculata* extraction solvents | Total flavonoid content (mg QE/ml) |
|-----------------|-------------------------------------------|----------------------------------|
| Sanitizer       | Control                                   | 0.150 ± 0.017                    |
|                 | Ethanol                                   | 1.526 ± 0.016                    |
|                 | Methanol                                  | 0.684 ± 0.016                    |
| Soap            | Control                                   | 0.173 ± 0.010                    |
|                 | Ethanol                                   | 6.028 ± 0.005                    |
|                 | Methanol                                  | 1.502 ± 0.008                    |

Data represent the mean ± standard deviation (n=3).

From Table 6, as expected, the control sanitizer shows a minimum TFC of 0.150 ± 0.017 followed by the control soap with 0.173 ± 0.010 mg GAE/ml. Both products exhibited much lower phenolic content compared to TFC of the original plant extract used to prepare the products. These results indicate the deterioration of the flavonoid compounds in the products upon addition of *A. paniculata* extract to it.

### 3.3. Physicochemical parameters of products from *A. paniculata* extracts

The physicochemical properties of sanitizer and soap was assessed by determining the pH, moisture content, foam height and high temperature stability.

#### 3.3.1. pH

The aims of pH test are to analyze whether the gel suitable with the skin and has the appropriate pH value. It is very important to keep optimum pH in the production of products that involved skin usage. This also implied to sanitizer and soap because both products involved skin usage. Optimum pH helps to avoid dry, itchy and sensitive skin from occurring. Table 7 shows the pH of the products.

Table 7. pH of sanitizer and soap.

| Products                  | pH  |
|---------------------------|-----|
| Methanol extract sanitizer| 7.1 |
| Ethanol extract sanitizer | 6.2 |
| Methanol extract soap     | 9.3 |
| Ethanol extract soap      | 9.3 |
| OPAC soap                 | 9.2 |

From Table 7, both products displayed compatible pH ranged from 6.2 to 9.3. Topical dosage should be in the skin’s pH range of 4.5 – 6.5. If it is too acidic, it will cause skin irritation and if too alkaline, it can cause scaly skin [33]. However, for cold process soap, the normal pH is 9 to 10 which is in alkaline condition. Although it is in alkaline pH, it will not give side effect for normal skin since the usage is for wash off soap [23]. Both types of soaps show the reading of pH 9.3 which are equivalent to OPAC soap that have been commercialized in market that shows the reading of 9.2.

#### 3.3.2. Moisture content

The content of moisture affects a product’s processability, shelf life, usability and quality. The moisture content of sanitizer and soap was determined by using oven drying method at 105°C for three hours. Table 8 shows the products’ moisture content.
Table 8. Moisture content of sanitizer and soap.

| Products                | Moisture content (%) |
|-------------------------|----------------------|
| Methanol extract sanitizer | 9.047               |
| Ethanol extract sanitizer | 9.327               |
| Methanol extract soap    | 6.850               |
| Ethanol extract soap     | 7.352               |

Ethanol extract sanitizer had the highest moisture content with 9.327% while methanol soap had the lowest with 6.850%. The moisture content obtained were still accepted as the optimum moisture content to prevent the growth of bacteria, mold and yeast which was usually below 12% [34].

3.3.3. Foam height and foam retention. The stability of foam produced from A. paniculata is evaluated from their foam height and foam retention, which is the time taken for foam destruction. Table 9 shows the products’ foam height measured for sanitizer and soap of A. paniculata extracts.

Table 9. Foam height of sanitizer and soap.

| Products                | Foam height (cm) | Foam retention (min) |
|-------------------------|------------------|----------------------|
| Methanol extract sanitizer | 2.1              | 1.3                  |
| Ethanol extract sanitizer  | 2.5              | 1.9                  |
| Methanol extract soap    | 10.4             | 6                    |
| Ethanol extract soap     | 12.0             | 8.7                  |

From Table 9, ethanol soap of A. paniculata extracts exhibited the highest foam height with 12.0 cm and foam retention time of 8.7 minutes. The retention time is directly proportional to the foam height.

3.3.4. High temperature stability. The sanitizer and soap of A. paniculata extracts were left to stand at 50 °C for one week to test these products durability against high temperature. Table 10 shows the evaluation on the products towards high temperature.

Table 10. High temperature stability of sanitizer and soap.

| Products                | High temperature stability |
|-------------------------|----------------------------|
| Methanol extract sanitizer | Stable                    |
| Ethanol extract sanitizer | Stable                    |
| Methanol extract soap    | Stable                    |
| Ethanol extract soap     | Stable                    |

As reflected from Table 10, all four products had good stability against high temperature. The samples that were left were observed as homogenous and stable liquid without rough texture.

3.5 Antimicrobial assay by hand printing method on agar plate media
Hand printing method used to determine antibacterial sanitizer effectiveness. The microbial growth area for hand printing was monitor on a sterile agar plate of the hand print. There are 3 categories of hand print which are unsanitized hand, sanitized hand using the formulated A. paniculata sanitizer and sanitized hand using sanitizer that are already commercialized in market. Each category was carried out in triplicate with different 3 individual’s hand to fairly check the antibacterial activity of the formulated antibacterial sanitizer and to be compared to the commercialized hand sanitizer in the market. The results of antimicrobial activity of hand sanitizer are shown in Figure 1.
Figure 1. Antimicrobial activity of hand sanitizer using (A) exposed hands, (B) hand cleaned using ethanol extract sanitizer (C) hand cleaned using methanol extract sanitizer (D) hand cleaned using commercial sanitizer.

From Figure 1(A), as expected from exposed hand, the number of the bacterial colonies grown can be observed on the hand prints are the highest in number and the largest in size. In comparison, for the commercialized sanitizers, it can be observed that in Figure 1(D1) using *Piper betle* leaf extract sanitizer from Watson and Figure 1(D3) using hand sanitizer in Bioprocess school laboratory, there is no bacterial colonies can be seen which indicated no bacterial growth in the relevant areas of the fingers after applying these sanitizers. However, for Figure 1(D2) using sanitizer from MR DIY store, the bacterial colonies were quite significant in number and intermediate in size compared to exposed hand. According to the observation made between Figure 1(B) and(Figure 1(C), ethanol extract sanitizer against removing the bacteria is more effective which could be found on one’s hands compared to methanol extract sanitizer. The results from the hand printing that was done reveals that *A. paniculata* ethanol extract sanitizer has promising antibacterial properties.

4. Conclusion

Results from present study and previous researchers showed that methanol is more efficient solvent in the extraction of antioxidant compounds like phenolics and flavonoids if compared to ethanol even their polarity are high and comparative. Results also showed that cold maceration has higher potential to maximize the extraction of antioxidant compounds from *A. paniculata* plants if compared to Soxhlet extraction method. Cold maceration technique is one of the most used method as it is a simple, easy and low-cost method while Soxhlet extraction method needed a high temperature to extract the bioactive compounds like phenolics and flavonoids. This could influence on the quality of the extracts causing thermal degradation. Thus, methanol *A. paniculata* extract by cold maceration is the best as the results obtained is the highest for total antioxidant, phenolic and flavonoid contents. Then, the macerated methanol and ethanol extracts were used to develop soap and sanitizer. The quality of the soap and sanitizer from *A. paniculata* extracts were evaluated in term of their physicochemical properties. Antibacterial assay by hand printing method on agar plate media was also done to evaluate the
effectiveness of herbal antibacterial sanitizer against the bacteria on our hands. This test was done on the products to prove that the developed products are successful and can be commercialized as well as to promote A. paniculata for its wide potential due to its various properties especially antioxidant. From the hand-printing method on agar plate media, A. paniculata does have antibacterial properties. As polyphenols and flavonoids are also known to exhibit antibacterial activity, the antibacterial activity of ethanol extract can be said to be due to the presence of these compounds. Thus, A. paniculata has the potential to be commercialized into antioxidant soap and sanitizer with both antioxidant and antimicrobial properties.

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