The Solvent Effect of Hand sanitizer Formulation from Pecut Kuda (Stachytarpheta jamaicensis [L] Vahl) extract

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Abstract. Staphylococcus aureus and Escherichia coli are a major cause of various humans and animal infections. Washing hands is a simple matter to eliminate and minimize germs on the hands with antiseptic hand sanitizer. Pecut kuda (Stachytarpheta jamaicensis (L) Vahl) contains a flavonoid compound known as antibacterial activity. Flavonoids are generally soluble in polar solvents. The type of solvent used for extraction influences which compounds are isolated. The purpose of the study was to assess the solvent effect of hand sanitizer formulation from pecut kuda extract. METHOD. Extract of pecut kuda was made by used three combination solvent of water and ethanol. There were 0:1, 1:1, and 1:2 for water and ethanol. Every formula was tested antibacterial activity against E.coli and Staph aureus. The highest antibacterial activity was made hand sanitizer gel by used optimization from HPMC K 100M and PEG used Design-Expert software. The antiseptic test was carried out on the best formula. RESULT. The result showed the best antibacterial activity was 0:1 composition of water and ethanol. The best formula of hand sanitizer was made of 11.7% HPMC K 100M and 88.3% PEG. That formula had antiseptic activity as 69.19%.

1. Introduction
Infectious diseases are the leading cause of global morbidity and mortality. Staphylococcus aureus and Escherichia coli are a major cause of various humans and animal infections.1 One of the spreads of microorganisms in humans is the hand, which is a means of transmission of microbes in the respiratory tract and mouth. Washing hands is a simple matter to eliminate and minimize germs on the hands with antiseptic hand sanitizer.2 Flavonoid is well known as antibacterial agents against a wide range of pathogenic microorganism.3 Flavonoids have been reported to be a significant topoisomerases inhibitors, which contributes to their antimicrobial activity. For example, DNA gyrase is an essential enzyme for DNA replication and it is exclusive to prokaryotes, which makes it an attractive target for antibacterial drugs.4 Stachytarpheta jamaicensis [L.] Vahl leaves commonly known they contain flavonoid compounds that have been known to exhibit antimicrobial activity on E. coli, and S. aureus.5

Flavonoids are the most representative polyphenolic compound from plant material. Flavonoids are generally soluble in polar solvents. Conventional extraction (solid–liquid) uses organic solvents to obtain bioactive extracts from vegetable matrices. Commonly these solvents are chosen according to their...
dielectric constant (ε) that is assumed to be correlated with the solvent polarity. However, the ability of solvents to form hydrogen bonds with the metabolite of interest should also be considered when choosing the right solvent, because both properties significantly impact the solvation capacity of solvents. The type of solvent used for extraction influences which compounds are isolated. A water mixture with ethanol are able to form hydrogen bonds. These mixtures have been shown useful to recover a wide range of polyphenols from plant materials. The purpose of the study was to assess the solvent effect of hand sanitizer formulation from pecut kuda extract.

2. Methods

2.1 Extraction

Pecut kuda extraction was extracted by maceration using (a) ethanol-water ratio of 1:0, (b) ethanol-water ratio of 1:1 and (c) ethanol-water ratio of 2:1. During the immersion, the process is stirred three times a day in the morning, afternoon, and evening for 30 minutes using a shaker. Maserat is then filtered and separated from the pulp. The obtained filtrate was evaporated using a rotary evaporator of ± 40°C. The extract produced was calculated each yield and drying shrinkage levels and observed organoleptic, which includes form, odor, color, and taste.

2.2 Antibacterial Activity

The Kirby Bauer disc diffusion method was used. The cell concentration of bacteria which were to be inoculated were standardized to MC Farland 0.5 turbidity standard. Suspension for the respective microbes was pipetted and pour evenly on NA (for E.coli) and MHA (for S.aureus). Sterilized filter paper discs (diameter, 6mm) was impregnated of pecut kuda extract. The discs were subsequently placed on the surface of inoculated agar medium. Disc with 5μg/disc of ciprofloxacin served as positive control and 5% DMSO was used as negative controls. All tests were carried out in triplicate. Zone of inhibition was then measured using a scale.

2.3 Optimization of Formula Hand sanitizer Gel Pecut Kuda Extract

The concentration of HPMC K100M and Propylenglikol chosen as optimized factors. Based on Table I, the 100% HPMC K100M concentration value set for the gel formula is 5%, and the importance of 0% is 0.25%. The 100% propylene glycol concentration determined in the gel formula is 10%, and the amount of 0% is 5%. The draft formula was made based on Simplex Lattice Design predictions using Design Expert 7.1.5 software and obtained eight (8) formulas. The projection of the software formula design has shown in Table 1.

| Formula | Software Prediction Concentrations used in preparations |
|---------|--------------------------------------------------------|
|         | HPMCK100M (%) | Propilenglikol (%) | HPMCK100M (%) | Propilenglikol (%) |
| 1       | 100           | 0                 | 5             | 5                 |
| 2       | 75            | 25                | 3,8125        | 6,25              |
| 3       | 50            | 50                | 2,625         | 7,5               |
| 4       | 25            | 75                | 1,4375        | 8,75              |
| 5       | 0             | 100               | 0,25          | 10                |
| 6       | 100           | 0                 | 5             | 5                 |
| 7       | 50            | 50                | 2,625         | 7,5               |
| 8       | 0             | 100               | 0,25          | 10                |

2.4 Antiseptic Activity

The hand sanitizer gel of pecut kuda extract which showed activity against test organisms in the agar diffusion test were further evaluated for their efficacy in reducing baseline bacterial counts of resident flora on the hands of the subject. The subject was asked to apply the hand sanitizer gel to their palms.
Before the application of the hand sanitizer gel, a swab sample from the palm was obtained and inoculated into a growth medium. After 1 – 2 minutes of wearing the hand sanitizer gel, the palms were again swabbed to obtain an inoculum that was inoculated into a growth medium. The plate was incubated at 37°C for 24 hours and the number of colonies was counted. The negative control gel was formulated using the exact same formulation but without the extract. Whereas the positive control which is used in hand sanitizer gel dosage is a commercial hand sanitizer gel.

2.5 Analysis Data

The data inhibition zone (mm) obtained was submitted to the analysis of variance using Kruskal Wallis. Kruskal-Wallis used to detect if there is any significant difference variables (water-ethanol ratio) were used. The significances were ranked using Kruskal-Wallis analysis with 95% confidence intervals.

3. Result and Discussion

Pecut kuda extract produced in various water-ethanol solvent compositions has the same form, smell, and taste but different colors. The color of pecut kuda extract with water-ethanol compositions (0:1) has a blackish-green color while the water-ethanol composition (1:1) and (1:2) has a blackish-brown color. The difference in the shade of pecut kuda extract produced is due to the composition (1: 1) and (1: 2) containing water. Water has a higher boiling point than ethanol, so the addition of water to the composition of the solvent used will result in a longer evaporation process, and the risk of oxidation of compounds that cannot stand on high temperatures. The extraction yield range to 11,7% to 26,2% and water-ethanol composition (1:2) give the highest yielding of extractable solids. Phytochemical screening results with various compositions of pecut kuda extract solvent showed positive effects of phenolic and flavonoid compounds. The quality control of pecut kuda extract with various water-ethanol composition as solvents as shown in table 2.

Table 2. Quality Control of Pecut Kuda Extract with Various Water-Ethanol Compositions as Solvents

| Parameter         | Pecut kuda extract with various water-ethanol composition as solvents |
|-------------------|------------------------------------------------------------------------|
|                   | 0:1  | 1:1  | 1:2  |
| Organoleptic      | Form | Thick extract |
|                   | Color | Blackish-green | Blackish-brown | Blackish-brown |
|                   | Smell | Specific smell of a pecut kuda |
|                   | Taste | Specific taste of a pecut kuda |
| Yield (%) b/b     | 11.27 | 15.24 | 26.2  |
| Lost on Drying (%)| 4.00  | 5.70  | 4.05  |
| Phenolic Qualitative test | FeCl₃ | Blackish-green (+) |
| Flavonoid Qualitative test | HCl + Magnesium | Orange (+) |

Antibacterial activity of pecut kuda extract was carried out by the Kirby-Bauer disk diffusion method. Bacterial inoculation is carried out by the pour method so that the bacteria will be spread evenly on the media, not only growing on the surface but also growing up to the inside of the media. Clear zone around the disc indicates the inhibition of microorganism growth by antimicrobial agents on the surface of the agar media.

Pecut kuda extract with various water-ethanol compositions as a solvent can inhibit the growth of Staphylococcus aureus and Escherichia coli. The highest antibacterial activity had shown in the composition (1:2) and (1:1), but the composition (1:1) is not substantially different from (1:2). These also showed that the antibacterial...
activity obtained in this study varied according to the extraction solvent used. The variation may probably be due to the type of bioactive compounds present in the different extraction solvents.

The antibacterial activity of pecut kuda extract was suspected due to the presence of flavonoid and phenolic secondary metabolites. The mechanism of flavonoids as an antibacterial is by denaturing bacterial cell proteins and damaging cell membranes without being irreparable. While the phenolic mechanism is interacting with bacterial cells through an adsorption process involving hydrogen bonds, at low levels, protein-phenol complexes are formed with weak bonds and decompose, followed by phenol penetration into cells, causing precipitation and protein denaturation. The antibacterial activity of pecut kuda extract with various water-ethanol compositions as solvents is shown in Table 3.

**Table 3.** Antibacterial activity pecut kuda extract with various water-ethanol compositions as solvents

| Pecut kuda extract with Various Water-Ethanol Compositions as Solvents | Antibacterial Activity (mm) |
|---|---|
| 0:1 | 11.67<sup>a</sup> | 11.6<sup>c</sup> |
| 1:1 | 7<sup>b</sup> | 6.16<sup>d</sup> |
| 1:2 | 5.8<sup>b</sup> | 6.66<sup>d</sup> |

Fig. 1. *different superscript represent significant difference among various water-ethanol compositions as solvents

Pecut kuda was extracted with a water-ethanol (0:1) which provides the highest antibacterial activity to *Staphylococcus aureus* and *Escherichia coli* is formulated in the form of a hand sanitizer gel. Characteristics of Eight (8) hand sanitizer gel formula of pecut kuda extract has a homogeneous semi-solid dosage form, the specific odor of pecut kuda, greenish-brown color, and taste on cold skin. The hand sanitizer gel of pecut kuda extract has a pH of 6. The acidity level of gel dosage must conform to the skin’s pH which is 4.5 – 6.5. The pH value of gel that does not conform with the skin’s pH will cause irritation to the skin. If it is too acidic, it will cause skin irritation and if too alkaline, it can cause scaly skin. It happens due to the damaged of the acidic mantle on the stratum corneum layer of the skin. The characteristics of hand sanitizer gel from *S. jamaicensis* extract can be seen in Table 4.

**Table 4.** Characteristic of hand sanitizer gel from pecut kuda extract

| Characteristic | Formula | Equation |
|---|---|---|
| Viscosity (cp) | 1 2 3 4 5 6 7 8 | Y = 5.10 x 10<sup>4</sup> (A) + 2.55 x 10<sup>3</sup> (B) – 3.60 x 10<sup>4</sup> (AB) |
| Spread Power (cm) | 7.54 10.74 16.97 24.47 32.66 7.71 17.22 31.82 | Y = 6.83 (A) + 30.34 (B) – 13.38 (AB) |
| Stickiness Power (second) | 6.63 5.14 4.20 2.28 1.05 6.30 4.46 1.01 | Y = 6.60 (A) + 1.14 (B) |
| Protection Power (second) | 13.98 11.17 7.09 3.79 1.45 14.67 6.79 1.42 | Y = 14.45 (A) + 1.36 (B) – 3.28 (AB) |

The characteristics of viscosity, spreadability, and protective power showed that the use of HPMC K100M and Propylenglycol alone gave positive values, but the use of a combination of both showed negative values. This indicates that the use of HPMC K100M and Propilenglikol together can reduce the
value of viscosity, dispersion, and protection power. The coefficient value of HPMC K100M in testing the viscosity, dispersion, adhesion, and protective power shows the coefficient value that is greater than the value of the coefficient of propylene glycol. This shows that the use of HPMC K100M has a more significant influence in increasing the viscosity, spreadability, adhesion, and protective power of hand sanitizer gel pecut kuda extract. Optimum formula were developed based on desired response (viscosity, spreadability, adhesion and protection power), then optimization technique using desirability approach was employed. The highest desirability point were choosed as optimum formula. The optimum formula was obtained in 11.7% HPMC K100M and 88.3% Propylenglycol with the desirability value is 0.948.

The preparation of pecut kuda extract in the optimum formula hand sanitizer gel has a thick liquid form, blackish-brown color, distinctive aroma of a pecut kuda, and cause a sensation of coldness to the skin. Gel preparations are similar preparations and have a pH of 6. Verification was employed with a one-sample t-test between the predicted and the actual values. Statistic analysis shows that Sig. (2-tailed) off all parameter >0.05, so we can conclude that there is no significantly different between predicted and actual results, then all equation obtained can be used to compose formula which results in optimum physical characteristics of hand sanitizer gel of pecut kuda extract. One sample T-Test between software predicted ant the actual values can be seen in Table 5.

Table 5. One Sample T-test between software predicted and the actual values

| Parameter                  | Prediction Software | Optimal Formula | Significance | Conclusion       |
|----------------------------|---------------------|-----------------|--------------|------------------|
| Viscosity (cP)             | 4999.9              | 3.760           | 0.057        | Not Significant  |
| Spread activity (cm²)      | 26.2089             | 22.190          | 0.053        | Not Significant  |
| Stickiness (detik)         | 1.77735             | 1.370           | 0.082        | Not Significant  |
| Protection activity (detik)| 2.54813             | 2.003           | 0.076        | Not Significant  |

Antiseptic activity the optimum formula hand sanitizer gel of pecut kuda extract was carried out by replica method. The hand sanitizer gel of pecut kuda extract can reduce the number of bacterial colonies on the correspondent’s hand by 69.91%, the positive control was 86.88% and the the negative control was 3.62%. The negative control showed antiseptic activity because the gel base formula contained 0.1% methylparaben preservative. The results of aseptic activity testing can be seen in Table 6.

Table 6. Antiseptic activity of hand sanitizer gel pecut kuda extract

| Sampel                             | Antiseptic Activity (%) |
|------------------------------------|-------------------------|
| Control negative                   | 3.62<sup>a</sup>        |
| Control positive                   | 86.88<sup>b</sup>       |
| Hand sanitizer gel of Pecut Kuda extract | 69.91<sup>c</sup>       |

Fig. 2. different superscript represent significant difference among various water-ethanol compositions as solvents

4. Conclusion
The use of solvents in the extraction of pecut kuda has a significant influence on the antibacterial activity, the optimum formula for handsaniter gel of pecut kuda extract are HPMC K100M 11.7% - Propylenglycol 88.3% and the handanitizer gel of pecut kuda extract has antiseptic activity.

References
[1] Raho B G and Abouni B, 2015, Escherichia coli and Staphylococcus aureus most common source of infection, The Battle Against Microbial Pathogen: Basic Science, Technological Advances and Educational Programs
[2] Ningsih D R, Zusfahair, Kartika D and Fatoni A, 2017, Formulation of hand sanitizer with antibacterials substance from n-hexane extract of soursop leaves (Annona muricata Linn), Malaysian Journal of Fundamental and Applied Sciences, 13 (1), 1-5

[3] Xie Y, Yang W, Tang F, Chen X, and Ren L, 2015, Antibacterial activities of flavonoids: structure – activity relationship and mechanism, Curr Med Chem, 22(1), 132-49

[4] Gorniak I, Bartoszewski R, Krolczewski J, 2019, Comprehensive review of antimicrobial activities of plant flavonoids, Phytochem Rev, 18, 241-272

[5] Liew P M, Yong Y K, 2016, Stachytarpheta jamaicensis (L.) Vahl: From Traditional Usage to Pharmacological Evidence, Evidence-Based Complementaty and Alternative Medicine, Hindawi Publishing Corporation

[6] Huaman-Castilla N L, Martinez-Cifuentes M, Camilo C, Pedreschi F, Mariotti-Celis M, and Perez-Correa J, 2019, The Impact of Temperature and Ethanol Concentration on the Global Recovery of Specific Polyphenols in an Integrated HPLE/RP Process on Carmenere Pomace Extracts. Molecules, 24(17), 3145

[7] Radji, M., Suryadi, H., Ariyanti, D., 2007, Uji Efektivitas Antimikroba Beberapa Merek Dagang Pembersih Tangan Antiseptik, Majalah Ilmu Kefarmasian, IV(1) ISSN 1693-9883

[8] Harley, J P and Prescott, L M, 2002, Laboratory Exercises in Microbiology. 5th Edition, The McGraw-Hill Companies.

[9] Gberikon, G M, Adeoti, I I and Aondoackaa, A D, 2015, Effect of Ethanol and Aqueous Solutions as Extraction Solvents on Phytochemical Screening and Antibacterial Activity of Fruit and Stem Bark Extract of Tetrapleura tetraptera Strep tococcus salivarus and Streptococcus mutans, International Journal of Current Microbiology and Applied Sciences, 4(5), 404-410

[10] Pelczar, M J and Chan, E C S, 1988, Dasar-Dasar Mikrobiologi, translated by Hadioetomo, R. S., Penerbit Universitas Indonesia, Jakarta

[11] Siswando dan Soekardjo, B, 2000, Kimia Medisinal, Edisi 2, Airlangga University Press, Surabaya