PREDICTION OF THE SIMPLEX LATTICE DESIGN PROGRAM ON THE HYDROGEL FORMULA IN THE COMBINATION EXTRACT OF PINANG YAKI (ARECA VESTIARY GISEKE) FRUIT STALK, GEDI (ABELMOSCHUS MANIHOT LINN.) LEAF, AND GOROHO (MUSA-EUMUSA-AAB) BANANA PEEL

Hosea Jaya Edy[a]*, Widya Astuty Lolo[b], Weny Indayany Wiyono[c], Edy Parwanto[d]

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Abstract: Background: Optimization is part of the formulation process used in the manufacturing of pharmaceutical preparations to obtain good and quality result, such as determining the number of drug substances. Aims of this study to determine the optimum hydrogel in the extract combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels based on pH, spreadibility, and anti-microorganism. Extraction using ethanol was carried out on Pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels. The extracts were used for hydrogel preparation. Aim: Formula optimization of the hydrogel based on the weight of each extract was carried out using the simplex lattice design (SLD) program. Material and method: The optimization process was carried out on 13 cycles formulas with different extract compositions. The responses tested in the process were pH value, spreadability, antibacterial activity against *Staphylococcus aureus*, and *Escherichia coli*, and antifungal activity against *Candida albicans*. Results: The optimal concentration proposed by the SLD program was 0.50 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.42 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 1.08 g of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract. The desirability value for the optimum formula is 0.706, which means that the response variable of the optimization process is getting closer to the predetermined target value. Conclusion: The values of pH, spreadability, inhibition of *S. aureus*, *E. coli*, and *C. albicans* in the hydrogel formula predicted by the SLD program were: 6.44, 5.66 cm, 2.06 cm, 1.67 cm, and 1.59 cm.

Keywords: Formula optimization, simplex lattice design, pinang Yaki (*Areca vestiary* Giseke), gedi (*Abelmoschus manihot* Linn.), goroho banana (*Musa-Eumusa-AAB*).

[a]. Study Program of Pharmacy, Faculty of Mathematics & Natural Science, Universitas Sam Ratulangi, Manado, Indonesia.
[b]. Study Program of Pharmacy, Faculty of Mathematics & Natural Science, Universitas Sam Ratulangi, Manado, Indonesia.
[c]. Study Program of Pharmacy, Faculty of Mathematics & Natural Science, Universitas Sam Ratulangi, Manado, Indonesia.
[d]. Department of Biology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

***Corresponding Author**

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INTRODUCTION

Formula optimization is included in the formulation process for product manufacturing, especially in the medical field. This step is important to determine the optimum formula composition to achieve the specified parameters. To achieve this goal, the simplex lattice design (SLD) program can be used. In formula optimization, the ingredient composition numbers are different, but the total amount in the formula is the same. The SLD program has been used in previous studies, among others, in deodorant optimization[1]. In addition, the optimal formula recommended in the SLD program is also equipped with statistical analysis calculations, so that it is precise and accurate. The program has been used by researchers for formula optimization[2,3,4].

Gels are soft or semi-solid pharmaceutical preparations. These preparations are made up of inorganic particles that penetrate a liquid solvent[5]. An example of a gel preparation is a hydrogel. Hydrogels use a solvent in the form of water. Appearance of the hydrogel is shiny, elegant, and easy to apply to the skin.

Although the efficacy of hydrogels depends on the type, depth, severity of the wound, and patient profile. Hydrogels are versatile and excel in maintaining the integrity and functionality of the active ingredients, as well as the controlled release of the active ingredients at the wound site. In addition, hydrogels are more suitable for combining different active ingredients. Of course, the choice of hydrogel for therapy depends on the indications and the purpose of therapy according to the patient's condition[6].

Three endemic plant species found in North Sulawesi included of pinang Yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.), and goroho (*Musa-Eumusa-AAB*). Pinang yaki (*Areca vestiary* Giseke) is a type of palm endemic at Sulawesi island. Pinang yaki (*Areca vestiary* Giseke) is one of the important components in a tropical rain forest ecosystem[7]. Previous study demonstrated that the fruit stalk of pinang yaki (*Areca vestiary* Giseke) contains flavonoids, saponins, and triterpenoids. Substances contained in fruit stalk of pinang yaki (*Areca vestiary* Giseke) are proven to strongly inhibit the growth of bacteria[8]. The results of other studies show that in addition to these three substances, fruit extract of pinang-yaki also contains tannins[9]. Regarding the saponin content in pinang yaki (*Areca vestiary* Giseke), it has been proven that bioactive saponins work effectively as antifungals[10].

The gedi (*Abelmoschus manihot* Linn.) is widespread throughout eastern Europe and in Asia. These plants live in subtropical and tropical areas in Asia. In addition, this plant grows in the Pacific Islands. Gedi (*Abelmoschus manihot* Linn.) leaves are edible and consumed as a popular vegetable in eastern Indonesia, the South Pacific Islands, and Papua New Guinea. Nutrients contained in gedi (*Abelmoschus manihot* Linn.) leaves, including minerals, β-carotene, and folate[11]. Gedi (*Abelmoschus manihot* Linn.) leaves and flowers have medicinal effects[12]. An earlier study found that all parts of gedi (*Abelmoschus manihot* Linn.) contain active substances like flavonoids[13,14]. In more detail, it is shown that the parts of the flower contain flavonoids in small quantities relative to other parts of the plant[15]. Traditionally, gedi (*Abelmoschus manihot* Linn.) is used to treat pain, inflammation, chronic bronchitis, and urinary infections. In addition, gedi (*Abelmoschus manihot* Linn.) is also used for antibacterial, antiviral, and wound-healing properties[11,16]. The results of another study showed that plant extracts had antifungal activity[17,18].

Bananas, which in the genus of *Musa*, originate from Southeast Asia and the Western Pacific. Today, bananas are cultivated in tropical and sub-tropical areas as a source of human nutrients[19]. Based on data at the Lembaga Ilmu Pengetahuan Indonesia (LIPI), goroho banana has accession number is LIPI-0264, the name of the accession is goroho banana, the biological status of the cultivar. Scientific name of goroho banana is *Musa acuminata x Musa balbisiana*, the name in the taxonomic classification is *Musa-Eumusa-AAB*, and the area of origin is North Sulawesi[20]. The active ingredients in the goroho (*Musa-Eumusa-AAB*) banana extract include phenol compounds[21,22,23], flavonoids[21,22,24], tannins[21,22], alkaloids[21,24], saponins, steroids, and triterpenoids[21]. Previous studies show that sap of fruit peel goroho (*Musa-Eumusa-AAB*) banana has anti-bacterial activity and anti-inflammatory activity[25]. The results of other studies show that extract of fruit peel goroho banana (*Musa-Eumusa-AAB*) has anti-bacterial activity[26]. Recent research results demonstrate that banana peel can be used to treat infectious diseases caused by *Staphylococcus aureus* and *Escherichia coli*[27]. Previous studies showed that extract of fruit peel barangan banana (*Musa acuminata* Colla.) has anti-fungal activity[28].

The three plants above contain chemicals that are useful as anti-bacterial and anti-fungal. Therefore, the active ingredients in this plant extract can be used for health, including for the wound healing process. The three plant extracts can be mixed in a hydrogel formula as active ingredients. In the application of hydrogel preparations has promising application prospects in medicine, such as for wound healing. The apllication of hydrogel preparations for wound healing, it is also necessary to pay attention of pH, spreadability, and anti microorganism activity. Hydrogel preparations as topical preparations have a neutral pH value or corresponding pH value of the skin. The pH value of of hydrogel prepararion as a topical preparations that are good and do not have adverse effects on the skin. are 6.77 ± 0.06[29], while the spreadability ranged from 5 to 7 cm[30].

The anti-bacterial activity of the plant prevents bacterial infection in the wound area. One of the factors that affect wound healing is bacterial infection[31]. *S. aureus* is an example of bacteria found in dermal wound areas[32]. *S. aureus* and *E. coli* are common pathogens, both of which are often used in testing the inhibition zone of the active substance contained in the preparation[8,33]. Besdie that, a previous studies have shown that *Candida albicans* was found to infect skin wounds[34]. In addition, the content of anti-fungal chemical compounds can be used as an active ingredient in various preparations to treat fungal infections. In this research we intend to hydrogel optimization using SLD program. Based on the description above, the recent study was aimed: 1) to analyze the response of hydrogel formulation in the extract combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels based on pH, spreadability, and anti microorganism such as *S. aureus, E. coli, and C. albicans*, and 2) to determine the optimum composition of hydrogel formulation in the extract combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels based on pH, spreadability, and anti-microorganism (*S. aureus, E. coli, and C. albicans).*

MATERIALS AND METHODS

Plant material

Plants material used for extraction such as pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels. These plants material obtained from the slopes of Mount Mahawu, Tomohon, North Sulawesi Province, Indonesia. The collection of plants material was carried out in October-November 2020. Identification was carried out in the Laboratorium Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sam Ratulangi, Manado, Indonesia.

Preparation of extracts

The equipment for extraction process in this study were blender (Philips), oven (Memmert), macerator (Innova 2100), vacuum rotary evaporator (Buchi), and porcelain plate. Each

plant material was washed, and then dried in an oven at 60 °C. The perfect dry ingredients were mashed using a blender. The dry matter powder was sieved with a mesh size of 12/40. Extraction in this study was carried out on each raw material of these plant species. The extraction process used 96% ethanol as solvent (Brataco Company).

The extraction process uses the repeated maceration method or re-maceration with 96% ethanol as solvent (Brataco Company). Maceration was carried out by soaking each powder with ethanol as a solvent. The process was carried out constantly for 24 hours at the macerator at a speed of 150 rpm. The maceration filtrate collected in a round bottom flask for solvent evaporation. The evaporation process of solvent uses a rotary vacuüm evaporator. The process continues until a thick extract is obtained.

Hydrogel manufacture

The equipment used in the hydrogel preparation process were a glass appliance (Pyrex), a hand mixer (Philips), an autoclave (Hettich), and a pH meter (Testr 30). The substances used in the manufacturing of hydrogel are carbopol 940 (Fagron), triethanolamine (Sigma-Aldrich), glycerine (P&G), propylene glycol (Dow Chemical Co.), and distilled water. In each formula (run), the hydrogel contains 2% active substance, which is a combination of the three extracts from the pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels. The combined weight of the three extracts was 2 grams in 100 grams hydrogel. The hydrogel preparation begins with developing a hydrogel base, specifically carbopol 940 for 24 hours using part of the aquadest. After the base expands, propylene glycol and glycerol was added sequentially and then stirred with a hand mixer until it produces homogeneous mixture and after the formation. The next step is to add TEA slowly while stirring continuously until the mixture reaches pH 7. The final step is to add the aquadest until the hydrogel base weight approaches 98 g, which was made 13 times according to the number of trials recommended by the SLD program. The hydrogel base formed is then sterilized using an autoclave. The extract was weighed (Table 1), which is added into each hydrogel base aseptically and then stirred until a homogeneous hydrogel preparation is formed[29,35].

Hydrogel formula optimization

Optimization of the hydrogel formula based on the weight of each extract was carried out using the SLD program with Design-Expert®7 (DX7) software (Stat-Ease, Inc., Hennepin Square Suite 480, 2021 East Hennepin Ave. Minneapolis, MN 55413-2726). The optimization process begins with performing 13 cycles of hydrogel preparations using a formula established by the SLD program. The test or measurements of the value is carried out on the basis of the predetermined response to be tested. All data from the tested reaponses were entered into the SLD program for analysis. Optimal formula of the hydrogel was determined based on the following criteria: 1) low level (%) of extracts such as pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels, 2) high level (%) of extracts such as pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels. To obtain the formula optimum based on the test pH value, the spreadability diameter, and the inhibition zone against *S. aureus*, E. *coli*, and C. *Albicans*. Bacteria isolates of *Staphylococcus aureus* (ATCC 29737), and *Escherichia coli* (ATCC 8739) were used, while fungal isolate was used in this research is *Candida albicans* (ATCC 10231).

Hydrogel formula reponses analysis

The hydrogel formula responses observed in this study were the pH value, spreadability, and antibacterial. The instrument used to measure the pH of the hydrogel is a pH meter. The tools needed to measure of hydrogel spreadability are glass plates, loads and timers. The tools used in the antibacterial activity assay are petri dishes (Pyrex), loop needles, bunsen lamps, incubators (Ecocell), LAF (Hysc), and autoclaves (Hettich). The antibacterial activity test in this study was carried out using the disc diffusion method. The size of the inhibition zone was measured in *S. aureus, E. coli* and *C. albicans.*

Data entered into the program include pH 6-6.5, spreadability 5.5-6.5 cm, inhibition zone for *S. aureus* 1.1-2.0 cm, *E. coli* 1.1-2.0 cm, and *C. albicans* 1.1-2.0 cm, respectively. Determine of hydrogel optimum formula in the extract combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels based on the desirability value and contour plot generated from the SLD program.

RESULT

Collection of plant materials

These three types of plants have anti-bacterial and anti-fungal abilities in this study presented in figure 1**.**

A. Pinang Yaki (*Areca vestiary* Giseke) B. Gedi (*Abelmoschus manihot* Linn.) C. Goroho (*Musa-Eumusa-AAB*)

Figure 1. Pinang Yaki (Areca vestiary Giseke), gedi (Abelmoschus manihot Linn.), and goroho (Musa-Eumusa-AAB). Photographer: HJE (Time: 03rd October 2020).

Hydrogel formula optimization

Hydrogel formula optimization begins with determine the lowest and highest weights for each extract. The lowest weights of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels extracts were 0.4%, 0.3%, and 0.8%, respectively, while the highest weights for pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels extracts were 0.9%, 0.8%, and 1.3%, respectively. The excipient composition of hydrogel formula processed by the SLD program with 13 runs (Table 1). The response values generated by the 13 tested cycles are shown in table 2. The appearance of the hydrogel formula processed by the SLD program is presented in figure 2.

Table 1. Formulation of hydrogel in the combination extract of pinang yaki (Areca vestiary Giseke) fruit stalk, gedi (Abelmoschus manihot Linn.) leaf, and goroho (Musa-Eumusa-AAB) banana fruit peels by Simplex Lattice Design (SLD).

Run	**Optimized extract**			**Carbopol 940**	**Glyserol**	**Propylen Glykol**	**TEA**	**Extract**	**Aquadest**
	Pinang yaki (*Areca vestiary* Giseke)	**Gedi (*Abelmoschus manihot* Linn.)**	**Goroho (*Musa-Eumusa-AAB*)**						
1	0.40	0.55	1.05	2	12.5	2	3	2.5	q.s
2	0.48	0.63	0.88	2	12.5	2	3	2.5	q.s
3	0.65	0.55	0.80	2	12.5	2	3	2.5	q.s
4	0.73	0.38	0.88	2	12.5	2	3	2.5	q.s
5	0.40	0.30	1.30	2	12.5	2	3	2.5	q.s
6	0.90	0.30	0.80	2	12.5	2	3	2.5	q.s
7	0.48	0.38	1.13	2	12.5	2	3	2.5	q.s
8	0.57	0.47	0.97	2	12.5	2	3	2.5	q.s
9	0.40	0.30	1.30	2	12.5	2	3	2.5	q.s
10	0.90	0.30	0.80	2	12.5	2	3	2.5	q.s
11	0.65	0.30	1.05	2	12.5	2	3	2.5	q.s
12	0.40	0.80	0.80	2	12.5	2	3	2.5	q.s
13	0.40	0.80	0.80	2	12.5	2	3	2.5	q.s

Abbreviation: TEA = tri etanol amine, q.s = quantum sufficit.

Figure 2. The appearance of the hydrogel formula processed by the Simplex Lattice Design (SLD) program.

Table 2. The response value of 13 runs of hydrogel formula in the optimization process

Run	Response				
	pH	Spreadability (cm)	Anti-microorganism Inhibition zone diameter (cm)		
			S. aureus	*E. coli*	*C. albicans*
1	6.35	5.30	1.80	1.45	1.55
2	5.83	4.90	1.80	1.45	1.50
3	6.27	5.05	1.85	1.25	1.45
4	6.35	5.40	2.00	1.35	1.55
5	6.72	6.35	2.20	1.65	1.55
6	6.40	5.40	1.85	1.20	1.35
7	6.50	5.50	2.10	1.70	1.65
8	6.45	5.60	2.05	1.65	1.50
9	6.75	6.20	2.10	1.65	1.60
10	6.40	5.80	1.75	1.20	1.45
11	6.30	5.85	2.00	1.40	1.55
12	5.32	4.70	1.60	1.15	1.30
13	5.35	4.75	1.65	1.20	1.25

Abbreviation: pH = power of hydrogen, cm = centimeter.

pH value response of hydrogel formula

The pH response of hydrogel formula showed the lowest pH value 5.32 at run 12, while the highest pH value was 6.75 at run 9. Based on the normal plot of residual curves, it was discovered that all the data of the pH value were normally distributed. ANOVA analysis showed the p-value was 0.0001 ($p<0.05$) at the 95% confidence level, which means that there was a significant difference in the pH values of the 13 hydrogel preparations tested. A mathematical equation describing the effect of each extract on the pH value has followed a quadratic equation model (equation 1).

$$Y= 6.40(A)+5.31(B)+6.47(C)+1.52(AB)–1.00(AC)+1.18(BC) \quad (1)$$

Description: Y=pH value, A=pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, B=gedi (*Abelmoschus manihot* Linn.) leaf extract, C=Goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the equations 1, demonstrated that all components in the hydrogel formula play importance roles in the increase in pH value. The goroho (*Musa-Eumusa-AAB*) extract had the greatest impact on increasing the pH value, because the content of these extract is the most among other components in the hydrogel formula. The hydrogel formula run 9 has the highest pH value, specifically 6.75, followed by run 5 with a pH value of 6.72, both preparations had the highest content of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract, namely 1.30 g. The contour plot diagram demonstrated of the three extracts interaction in the hydrogel formula on pH value response (Figure 3).

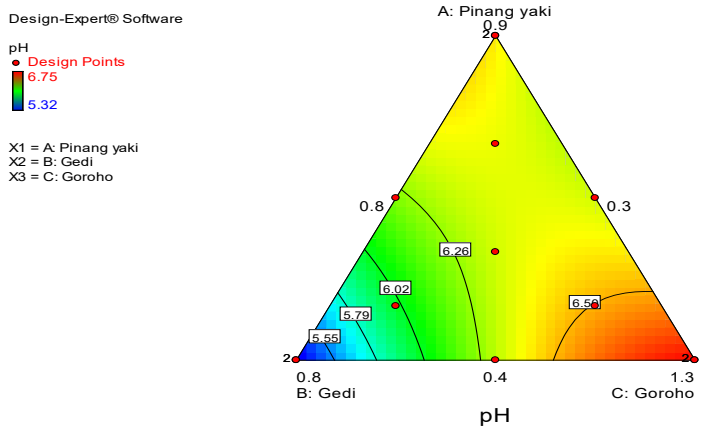

Figure 3. Contour plot for pH value response of hydrogel formulation in the extracts combination of pinang yaki (Areca vestiary Giseke) fruit stalk, gedi (Abelmoschus manihot Linn.) leaf, and goroho (Musa-Eumusa-AAB) banana fruit peels.

Based on the figure 3, the red area shows that goroho (*Musa-Eumusa-AAB*) banana fruit peels extract has the greatest impact on increasing the pH value. On the other hand, the blue area indicates that gedi (*Abelmoschus manihot* Linn.) leaf extract has the lowest impact on increasing the pH value.

Spreadability response of hydrogel formula

The spreadability respons of hydrogel fromula showed the lowest spreadability value 4.70 cm at run 12, while the highest spreadability value was 6.35 cm at run 5. Based on the normal plot of residual curves, it was discovered that all the data of the spreadability value were normally distributed. ANOVA analysis showed the $p<0.0001$ at the 95% confidence level, which means that there was a significant difference in the spreadability of the 13 hydrogel preparations tested. The P-value based on the lack of fit analysis was 0.5470 at the 95% confidence level. Based on the fact was concluded that there is no significant difference between the spreadability data based on the experimental results compared with the spreadability data predicted by SLD program. A mathematical equation describing the effect of each extract on the spreadability value has followed a linier equation model (equation 2).

$$Y= 5.55(A)+4.64(B)+6.16(C) \quad (2)$$

Description: Y=spreadability, A=pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, B=gedi (*Abelmoschus manihot* Linn.) leaf extract, C=goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the equations demonstrated that all components in the hydrogel formula play importance roles in the increase in spreadability. Such as the pH response, the goroho (*Musa-Eumusa-AAB*) banana fruit peels extract had the greatest impact on increasing the spreadability, because the content of these extract is the most among other components in the hydrogel formula. The hydrogel formula run 5 has the highest spreadability, specifically 6.35 cm, followed by run 9 with a spreadability of 6.20, both preparations had the highest content of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract, namely 1.30 g. On the other hand, hydrogel formula run 12 had the lowest spreadability, namely 4.70 cm, followed by run 13 with a spreadability of 4.75 cm, both preparations had the lowest content of gedi (*Abelmoschus manihot* Linn.) leaf extract 0.80 g.

The contour plot diagram demonstrated of the three extracts interaction in the hydrogel formula on spreadability response (Figure 4).

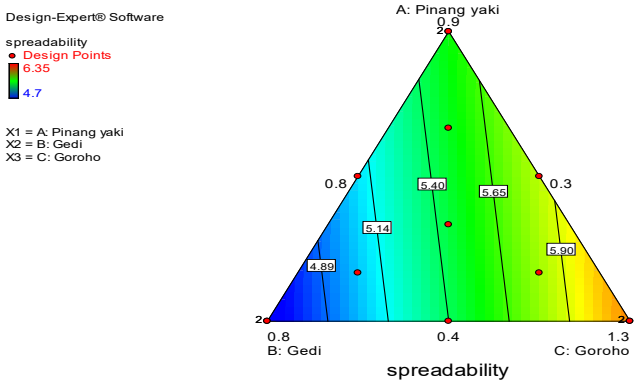

Figure 4. Contour plot for spreadability response of hydrogel formula in the extracts combination of pinang yaki (Areca vestiary Giseke) fruit stalk, gedi (Abelmoschus manihot Linn.) leaf, and goroho (Musa-Eumusa-AAB) banana fruit peels.

Based on the figure 4, the red area shows that goroho (*Musa-Eumusa-AAB*) banana fruit peels extract has the greatest impact on increasing the spreadability. On the other hand, the blue area indicates that gedi (*Abelmoschus manihot* Linn.) leaf extract has the lowest impact on increasing the spreadability.

Anti microorganism response of hydrogel formula

a). Anti *S. aureus* respons of hydrogel fromula

Anti *S. aureus* respons of hydrogel fromula showed the lowest inhibition zone diameter 1.60 cm at run 12, while the highest inhibition zone diameter was 2.20 cm at run 5. The lowest inhibition zone diameter is discavered in the hydrogel formula run 12 contains 0.40 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.80 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 0.80 g of goroho *(Musa-Eumusa-AAB)* banana fruit peels extract. The largest inhibition zone diameter is discovered in the hydrogel formula run 5, contains 0.40 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.30 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 1.30 g of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the normal plot of the residuals curve, data of inhibition zone diameter of *S. aureus* were normally distributed. ANOVA analysis showed the p-value was 0.0004 at the 95% confidence level, which means that there was a significant difference in the inhibition zone diameter of *S. aureus* of the 13 hydrogel preparations tested. The P-value based on the lack of fit analysis was 0.8330 at the 95% confidence level. Based on these results, there is no significant difference between the inhibition zone diameter of *S. aureus* data based on the experimental results compared with the data predicted by SLD program. A mathematical equation describing the effect of each extract on the spreadability value has followed a special cubic equation model (equation 3).

$$Y=1.80(A)+1.62(B)+2.15(C)+0.53(AB)+0.15(AC)–0.57(BC)+4.34\ (ABC) \quad (3)$$

Description: Y=Anti *S. aureus*, A=pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, B=gedi (*Abelmoschus manihot* Linn.) leaf extract, C=goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the above equation (equation 3) demonstrated that all components in the hydrogel formula play an important role

in increasing the inhibition zone diameter against the growth of *S. aureus*. Therefore, preparations containing the three extracts of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels have a positive contribution to antibacterial activity. The combination had the greatest impact and have the ability to kill *S. aureus* bacteria with a value in the mathematical equation of +4.34. Based on the inhibition zone diameter data (Table 2) hydrogel formula run 5, 7, 8, and 9 have an inhibition zone diameter of more than 2.00 cm. The contour plot diagram demonstrated of the three extracts interaction in the hydrogel formula on inhibition zone diameter response to *S.aureus* (Figure 5). The hydrogel preparation with the greatest antibacterial activity against *S. aureus* was due to the equivalent composition of the three extracts.

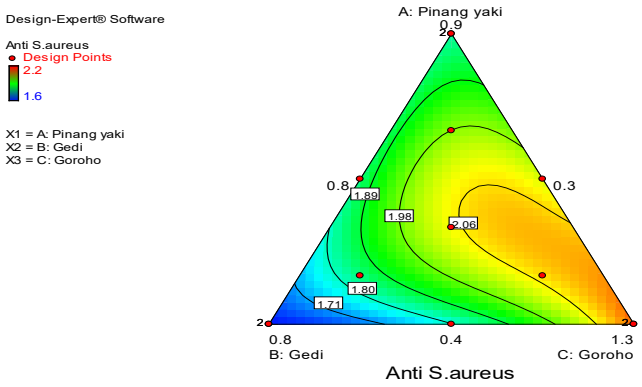

Figure 5. Contour plot for inhibition zone diameter response to S.aureus of hydrogel formula in the extracts combination of pinang yaki (Areca vestiary Giseke) fruit stalk, gedi (Abelmoschus manihot Linn.) leaf, and goroho (Musa-Eumusa-AAB) banana fruit peels.

b). Anti *E. coli* respons of hydrogel fromula

Anti *E. coli* respons of hydrogel fromula showed the lowest inhibition zone diameter 1.15 cm at run 12, while the highest inhibition zone diameter was 1.70 cm at run 7. The lowest inhibition zone diameter is discavered in the hydrogel formula run 12 contains 0.40 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.80 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 0.80 g of goroho *(Musa-Eumusa-AAB)* banana fruit peels extract. The largest inhibition zone diameter is discovered in the hydrogel formula run 7, contains 0.48 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.38 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 1.13 g of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the normal plot of the residuals curve, data of inhibition zone diameter of *E. coli* were normally distributed. ANOVA analysis showed the $p<0.0001$ at the 95% confidence level, which means that there was a significant difference in the inhibition zone diameter of *E. coli* of the 13 hydrogel preparations tested. The P-value based on the lack of fit analysis was 0.0808 at the 95% confidence level. Based on these results, there is no significant difference between the inhibition zone diameter of *E. coli* data based on the experimental results compared with the data predicted by SLD program. A mathematical equation describing the effect of each extract on the spreadability value has followed a special cubic equation model (equation 4).

$$Y=1.19(A)+1.18(B)+1.16(C)+0.20(AB)-0.12(AC)+0.21(BC)+7.36(ABC) \quad (4)$$

Description: Y=Anti *E. coli*, A=Pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, B=Gedi (*Abelmoschus manihot* Linn.) leaf extract, C=Goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on mathematical equations (4), and counterplots (Figure 6), it was concluded that each extract has a positive coefficient of antibacterial activity. The combination of the three extracts using mathematical equations has the greatest ability to increase the antibacterial activity against *E. coli* with a value of +7.36. Based on figure 5, there is a red area in the middle of the triangle. The red color in the counterplot area indicates the greatest effect of the extract combination on the inhibition zone diameter response to *E. coli*. The most important data for the diameter of the inhibition zone was 1.70 cm by hydrogel cycle run 7 containing 0.48% extract of pinang yaki (*Areca vestiary* Giseke) fruit stalk, 0.38% gedi (*Abelmoschus manihot* Linn.) leaf extract, and 1.13% extract of goroho (*Musa-Eumusa-AAB*) banana fruit peels.

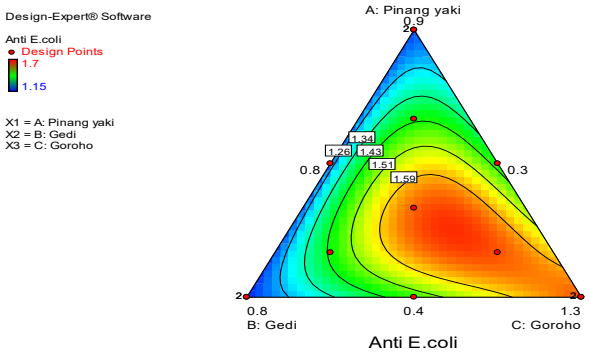

Figure 6. Contour plot for inhibition zone diameter response to E. coli of hydrogel formula in the extracts combination of pinang yaki (Areca vestiary Giseke), gedi (Abelmoschus manihot Linn.), and goroho (Musa-Eumusa-AAB).

c). Anti *C. albicans* respons of hydrogel fromula

Anti *C. albicans* respons of hydrogel fromula showed the lowest inhibition zone diameter 1.25 cm at run 13, while the highest inhibition zone diameter was 1.65 cm at run 7. The lowest inhibition zone diameter is discavered in the hydrogel formula run 13 contains 0.40 g of pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, 0.80 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 0.80 g of goroho *(Musa-Eumusa-AAB)* banana fruit peels extract. The largest inhibition zone diameter is discovered in the hydrogel formula run 7, contains 0.48 g of pinang yaki (*Areca vestiary Giseke*) fruit stalk extract, 0.38 g of gedi (*Abelmoschus manihot* Linn.) leaf extract, and 1.13 g of goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on the normal plot of the residuals curve, data of inhibition zone diameter of *C. albicans* were normally distributed. ANOVA analysis showed the p-value was 0.021 at the 95% confidence level, which means that there was a significant difference in the inhibition zone diameter of *C. albicans* of the 13 hydrogel preparations tested. The p-value based on the lack of fit analysis was 0.05515 at the 95% confidence level. Based on these results, there is no significant difference between the inhibition zone diameter of *C. albicans* data based on the experimental results compared with the data predicted by SLD program. A mathematical equation describing the effect of each extract on the spreadability value has followed a quadratic equation model (equation 5).

$$Y=1.40(A)+1.28(B)+1.58(C)+0.47(AB)+0.29(AC)+0.53(BC) \quad (5)$$

Description: Y=Anti *C. albicans*, A=Pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, B=Gedi (*Abelmoschus manihot* Linn.) leaf extract, C=Goroho (*Musa-Eumusa-AAB*) banana fruit peels extract.

Based on mathematical equations (5) it was concluded that each extract has a positive coefficient of antifungal activity. Goroho (*Musa-Eumusa-AAB*) banana fruit peels extract has the most roles in antifungal activity with a value of +1.58. Pinang yaki (*Areca vestiary* Giseke) fruit stalk extract also plays a significant role as an antifungal with a value of +1.40 followed by gedi (*Abelmoschus manihot* Linn.) leaf extract with a value of +1.28. Based on the contour plot (Figure 7), the red area shows that goroho (*Musa-Eumusa-AAB*) banana fruit peels extract has the greatest impact on increasing the anti *C. albicans*. On the other hand, the blue area indicates that gedi (*Abelmoschus manihot* Linn.) leaf extract has the lowest impact on increasing the anti *C. albicans*.

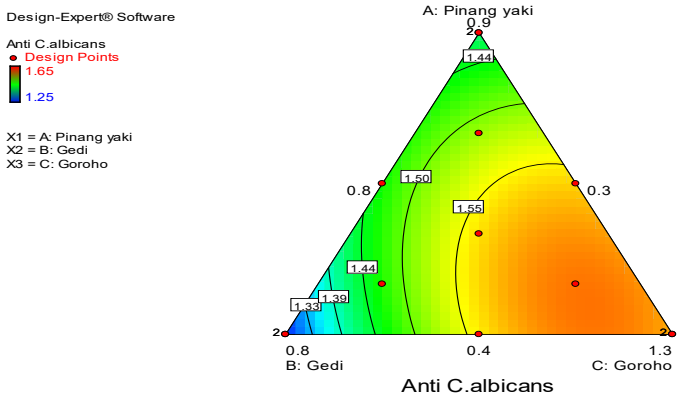

Figure 7. Contour plot for inhibition zone diameter response to C. albicans of hydrogel formula in the extracts combination of pinang yaki (Areca vestiary Giseke), gedi (Abelmoschus manihot Linn.), and goroho (Musa-Eumusa-AAB).

Optimum hydrogel formula

The results of optimum weight composition in the extracts combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels analyzed by the Design Expert 7.00 software using the SLD program are 2 composition solutions (Figure 8).

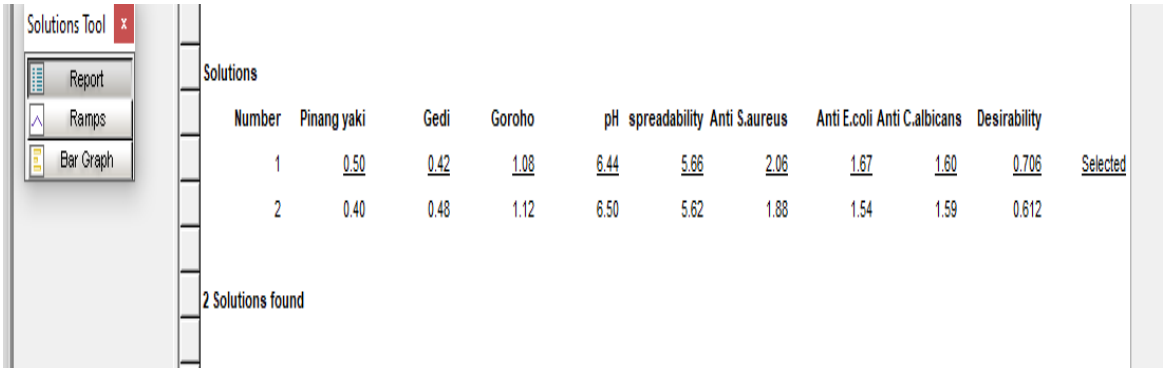

Figure 8. Optimum weight composition in the extracts combination of pinang yaki (Areca vestiary Giseke), gedi (Abelmoschus manihot Linn.), and goroho (Musa-Eumusa-AAB) analyzed by the Design Expert 7.00 software using the SLD program.

The first solution, the optimum formula has a desirability value of 0.706, while the second has a value of 0.612. The desirability value is greater than or close to 1, meaning that each predicted response value is closer to the expected value in the system. The optimization results present not only 2 solutions to the optimum formula, but also equipped with a prediction of the value of each response to be tested in the formula. The optimum hydrogel formula was determined based on the pH value, spreadability, and the inhibition zone diameter against microorganisms such as *S. aureus, E. coli,* and *C. Albicans.* Each tested response is given quality parameters that must be observed by the preparation being tested and the optimum formula recommended. The quality parameter of the specified pH value is between 6 - 6.5 with the target being within the range. The pH value defined by the formulator refers to the pH value of the skin in order to prevent irritation. The spreadability of the hydrogel formula was 5.5 - 6.5 cm, according to the target range.

Based on the contour plot for optimum hydrogel formula in the extracts combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels **(Figure 9)** suggested as solutions.

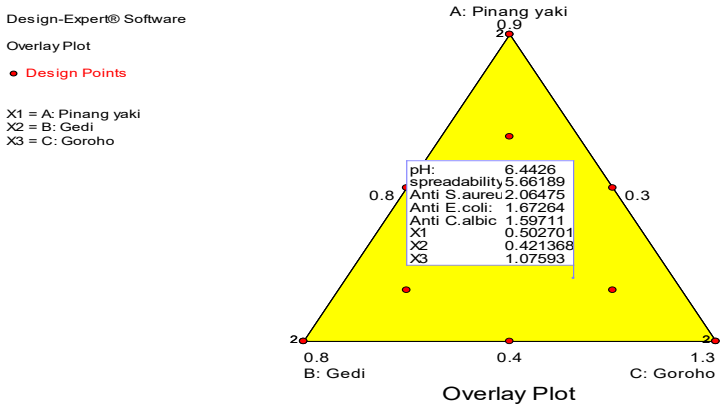

Figure 9. Contour plot for optimum hydrogel formula in the extracts combination of pinang yaki (Areca vestiary Giseke), gedi (Abelmoschus manihot Linn.), and goroho (Musa-Eumusa-AAB).

The yellow color in the contour plot shows the relevant areas with optimal response to the composition of the hydrogel formula. The recommended weight composition in the extracts combination of pinang yaki (*Areca vestiary* Giseke) fruit stalk, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels is 0.50 g, 0.42 g, and 1.08 g, respectively, with a desirability value of 0.706. The relevant contour plot also provides a prediction of quality responses value to be tested (pH, spreadability, and anti microorganism). Prediction of quality response value such as pH 6.44, spreadability 5.66 cm, inhibition zone diameter against *S.aureus, E. coli,* and *C. albicans* are 2.06 cm, 1.67 cm, and 1.60 cm, respectively.

DISCUSSION

The pH response test for demonstrated the acidic or basic nature of the hydrogel formula. Hydrogel preparations as

topical preparations have a neutral pH value or according to the pH value of the skin. Topical preparations that are acidic can irritate and cause erythema, as well as edema of the skin. Previous studies have shown that topical preparations with alkaline properties cause the skin to become dry, rough, and cracked. A good topical preparation has a pH of 6.15±0.04 to 6.98±0.03. In addition, the use of skin topical preparations should not cause side effects[36]. The pH value of the skin is used as a reference for pH optimization of hydrogels and other topical preparations [37,38,39]. The other research results showed that the hydrogel was carried out at pH 6.5-7.1[40]. These results correspond to the pH value of human skin.

The therapeutic effect of the active substance in the preparation affected by spreadability. Good topical preparation has a high spreadability, or vice versa. A good topical preparation facilitates its application, especially in the wound area. On the other hand, preparations with poor spreadability complicate the active substance distribution, thereby hindering the therapeutic process[41]. In this study demosntrated that spreadability of hydrogel formula according with a good spreadability for hydrogel preparations, namely 5-7 cm[30].

Previous research showed that the ethanol extract of pinang yaki (*Areca vestiary* Giseke) with concentrations of 1%, 2%, 3%, 4% and 5% had inhibition zone diameter against *S. aureus* of 0.76 cm, 1.53 cm, 1.33 cm, 1.44 cm, and 1.59 cm, respectively[8]. It has been demonstrated that a 100-500 µg of gedi (*Abelmoschus manihot* Linn.) leaf extract had inhibition zone against to *S. aureus* of 1.2-1.4 cm. The inhibition zone was associated with the flavonoid content of gedi (*Abelmoschus manihot* Linn.) leaf extract[42]. Another study showed that leaf extract of gedi (*Abelmoschus manihot* Linn.) had an inhibition effect on the growth of *S. epidermidis*. The inhibition effect of the extract of gedi leaves (*Abelmoschus manihot* Linn.) against these bacteria was highest at 3% with an inhibition zone diameter of 1.48 cm[43]. Previous study demonstrated that the minimum inhibition concentration of goroho *(Musa-Musa-AAB)* fruit peels saps against *S. aureus* is 50%[25]. The three types of plants are proven to contain flavonoids. Previous studies have demonstrated that flavonoids inhibit bacterial growth. This fact, in accordance with the statement that flavonoids are anti-bacterial. Flavonoids are antibacterial, partly because they can inactivate enzymes in bacteria[27]. These data reinforce that the ethanol extract of pinang yaki (*Areca vestiary* Giseke) has inhibition activity against the growth of *S. aureus.*

The other research showed that the ethanol extract of pinang yaki (*Areca vestiary* Giseke) with concentrations of 1%, 2%, 3%, 4% and 5% had inhibition zone diameter against *E. coli* of 0.66 cm, 0.75 cm, 0.85 cm, 1.12 cm, and 1.33 cm, respectively[8]. In addition, it has been demonstrated that a 100-500 µg of gedi (*Abelmoschus manihot* Linn.) leaf extract had inhibition zone against to *S. aureus* and *E. coli* of 1.2-1.4 cm. The inhibition zone was associated with the flavonoid content of gedi (*Abelmoschus manihot* Linn.) leaf extract[42]. Gedi (*Abelmoschus manihot* Linn.) extract contains saponins[44] that have anti-bacterial activity, including *E. coli.*[45]. Moreover that less polar saponins interact more easily with bacterial cell membranes and damage membrane integrity, and reduce membrane potential[46]. Previous research has shown that the ethyl acetate extract on milk banana peels (*Musa x paradisiaca* Linn.) had an average inhibition zone diameter of *E. coli* 2.13 cm. On the other hand, n-butanol extract on the milk banana peels (*Musa x paradisiaca* Linn.) had an average inhibition zone diameter of *E. coli* 0.97 cm[27]. The hydrogel formula containing a combination of plant extracts in this study has anti-bacterial properties, especially *E.coli.* This is because the three types of plant extracts are proven to contain active substances, including flavonoids, tannins, and saponins.

A previous study showed that plant extracts containing flavonoids had the ability to inhibit fungal growth. It has been demonstrated that *Curcuma longa, Alpinia galanga,* and *Zingiber officinale* extract contain flavonoids and showed antifungal activity against *C. albicans* and *Trichophyton rubrum* with inhibition zone diameters 1.02-2.71 cm and 2.73-4.43 cm, respectively[47]. On the other hand, has demonstrated the effect of gedi (*A. manihot* Linn.) leaf extract 20%, 30%, 40%, 50%, and 60% inhibit the growth of *Aspergillus flavus* 20.9250±9.31714%, 37.0750±8.43460%, 58.0000±2.59101%, 71/0500±4.44410%, 73.4750±3.74288%, respectively[18]. The hydrogel formula containing a combination of plant extracts in this study has anti-fungal properties, especially *C. albicans.* This is because the three types of plant extracts are proven to contain active substances, including flavonoids, and saponins. It has been stated that medicinal plants extract containing flavonoids association with antifungal activities and can be promising for the growth inhibition of fungal infections. The action of flavonoids as antifungals with the mechanism of plasma membrane disruption, mitochondrial dysfunction, inhibition of cell wall formation, cell division, protein synthesis, and efflux-mediated pumping system[48]. Beside that, saponin content in pinang yaki (*Areca vestiary* Giseke) work effectively as antifungals[10]. In addition to flavonoids and saponins, alkaloids as active substances also have antifungal activity. Previous study showed that alkaloids have antifungal activity[49].

The results of hydrogel formula optimization showed that the pH, and spreadability meets to the requirements, therefore its viscosity is very influential in the ease of application of the preparation to the injured skin area. The antimicrobial activity test against *S.aureus*, *E.coli*, and *C.albicans* determined that the diameter of the inhibition zone as a quality parameter was 1.1-2.0 cm. The quality parameter value of the inhibition zone diameter in this study showed the moderate inhibition classification[35]. The targets set for antimicrobial activity are maximized by systems within the program. The optimization of hydrogel formula showed that inhibition zone is large, which means good microorganism-killing ability.

CONCLUSION

The optimum weight composition of the hydrogel formula recommended by the SLD program are pinang yaki (*Areca vestiary* Giseke) fruit stalk extract, gedi (*Abelmoschus manihot* Linn.) leaf, and goroho (*Musa-Eumusa-AAB*) banana fruit peels extracts of 0.50 g, 0.42 g, and 1.08 g, respectively. The desirability value for the optimum formula is 0.706, which means that the response variable of the optimization process is getting closer to the predetermined target value. The values of pH, spreadability, inhibition of *S. aureus*, *E. coli*, and *C. albicans* in the hydrogel formula predicted by the SLD program were: 6.44, 5.66 cm, 2.06 cm, 1.67 cm, and 1.59 cm, respectively. Next, we carried out laboratory tests according to

the predictions of the SLD program on the variables in this study.

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ETHICAL APPROVAL:

This study involved programming with a computer, and did not involve in vitro or in vivo experiments, so it did not require approval from the ethics committee.

AUTHOR CONTRIBUTIONS

Conception: HJE, and EP. Data collection: HJE, WAL, and WIW. Writing of the first draft: EP, HJE, and WAL. Final approval: all author's.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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