

Inhibitory Effect of Roselle Aqueous Extracts-HPMC 6000 Gel on the Growth of *Staphylococcus aureus* ATCC 25923

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INTRODUCTION: Roselle (*Hibiscus sabdariffa* L.) is one of medicinal plants commonly used as beverage herbal medicine. Complexity compounds in the aqueous extracts have provided good antibacterial activity by which growth of Gram negative and positive bacteria are inhibited. The aims of this research were to formulate HPMC 6000 gel containing the extract and investigate inhibitory activity of the extract and its gel formula against *Staphylococcus aureus* ATCC 25923.

METHODS: Thin-layer Chromatography on Silica-gel GF254 was used for analyzing flavonoids and Polyphenols using butanol-acetic acid-water (4: 1: 5) and chloroforms-ethyl acetate-formic acid (0.5: 9: 0.5) as eluent, respectively. The serial dilution of aqueous extracts powder in citrate buffer was made to obtain 0.50, 0.25, 0.10, 0.05 and 0.02 mg/mL solution. The Roselle aqueous extracts (3%) was formulated as a component of gel containing HPMC 6000 in various concentrations (2%, 3%, and 4%). A diffusion agar method on two layers of nutrient agar media was applied using *Staphylococcus aureus* ATCC 25923 and gentamicin 25 ppm as bacterial test and standard respectively. After incubating for 24 hours at 37°C, the inhibitory effect was denoted by clear zone around the hole and the inhibitory activity was measured as MIC.

RESULTS: The aqueous extract of *Hibiscus sabdariffa* L. contained flavonoid and polyphenol compound based on the TLC-chromatogram profile. It was found that the gel formula containing 3% of HPMC 6000 and the aqueous extract 3% gave a good physical characteristic and the lowest MIC (6.0 mg/ml), equivalent to 7.58 ppm of gentamicin standard at 12.0 mg/mL concentration.

DISCUSSION AND CONCLUSION: The HPMC 6000 at 3% (w/w) concentration in Roselle aqueous extracts gel preparation gave good physical characteristics. The gel preparation exhibited inhibitory activity against *Staphylococcus aureus* ATCC 25923 depicted by Minimum Inhibitory Concentration 6.0 mg/mL. The formula 2 is recommended and prospects for further investigated to be implemented as topical preparations.

Keywords: inhibitory effect, *Hibiscus sabdariffa*, HPMC 6000, *Staphylococcus aureus*

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is one of the medicinal plants commonly produced as beverage and herbal medicine. It has multi activities, one of them is antibacterial activity.¹ The aqueous extracts of Roselle calyces contain saponins, alkaloids, tannins, polyphenols, flavonoids and their glycosides. The saponins and flavonoids are the largest content.^{2,3} These compounds indicate synergistic effects. Complexity compounds in the extracts have provided good antibacterial activity.⁴ Proto-catechuic acid is a polyphenolic compound found in Roselle calyces. It inhibited the bacterial growth of *Methicillin Resistance Staphylococcus aureus* (MRSA), *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* at 5 mg/mL.^{5,6,7}

In term of their antimicrobial activities, the Roselle aqueous extract was used at concentration above its minimum inhibitory concentration (MIC, 3%). The low pH values of Roselle aqueous extract (2.42 ± 0.01) lead to consider the HPMC 6000 was chosen as a gelling agent at concentrations of 2 %, 3 %, and 4 %, because this matrix is stable and indicate a good swelling ability on the acid condition. An effort to discover new topical dosage form containing Roselle extract as active ingredient for anti-infectious diseases is the main target of this research.

MATERIALS AND METHODS

Chemicals

The materials were pharmaceutical grade. Dried aqueous extract of Roselle was purchased from PT. ASIMAS, HPMC 6000, citric acid monohydrate, sodium citrate dihydrate, propylene glycol, sodium benzoate, gentamicin sulfate, nutrient agar (Oxoid), sodium chloride (Merck) and distilled water (PD. Surabaya Air suling). Bacterial test *Staphylococcus aureus* ATCC 25923 was obtained from Department of Microbiology Faculty of Medicine, Airlangga University.

Qualitative analysis of Roselle aqueous extracts

Analysis of the extract included visual examination of organoleptic (shape, odor and color) and pH, while chromatogram pattern of flavonoids and polyphenols was analyzed by Thin Layer Chromatography (TLC) on Kiesel Gel GF₂₅₄ plate. The chromatographic profile of flavonoids was evaluated by shaking 1 g of the extract with *n*-hexane repeatedly until it was colorless and the residue was dissolved in 5 mL of ethanol. Then the solution was spotted and developed in butanol-acetic acid-water (4:1:5, v/v). The presence of flavonoids was denoted by intensive yellow spot on the plate after contacted with ammonia fumes. The polyphenols chromatogram pattern of the extracts was obtained by mixing 1 g of extract and 10 mL of hot distilled water at room temperature, the solution was spotted on TLC plate after filtering and developed in chloroforms-ethyl acetate-formic acid (0.5:9:0.5, v/v) and sprayed with FeCl₃ solution for indicating the presence of polyphenols by the appearance of black spot.^{1,2}

Qualitative analysis of HPMC 6000

The qualitative examination of HPMC 6000 included pH value and viscosity was analyzed using pH-meter and Brook field viscometer respectively.^{6,8}

Viscosity was measured according to the Brookfield viscometer manual. The spindle was lowered and centered in the test material (600 mL in beaker) to meet the "meniscus" of the fluid was at the center position of the immersion groove. The viscosity measurement was performed by turning of the switch "ON". Allow time was need for the indicated reading to stabilize. The reading was noted and multiplied by the factor appropriate to the viscometer model/spindle/speed combination being used. Refer to the available table or to the FACTOR FINDER for calculating viscosity. Readings below 10.0% torque (dial reading) should be avoided.

Determining the MIC of Roselle aqueous extracts

The MIC of Roselle aqueous extracts was performed by agar diffusion method and molding hole against *Staphylococcus aureus* ATCC 25923. The bacterial test was cultured on the slant nutrient agar media in glass tubes and incubated for 24 hours at 37°C. The inoculum suspension was prepared by adding sterile 0.9% NaCl solution to fresh culture, shaking and measuring the optical density at 580 nm, adjusted until 25% transmittance of inoculum obtained. The extracts were weighed 100 mg and dissolved in citrate buffer until 10 mL. The solution was diluted to 0.50, 0.25, 0.10, 0.05 and 0.02 mg/mL to obtain the concentration higher than the MIC. Two layers of test media prepared and applied. The medium agar was perforated with 6 sterile holders. Samples and positive control (gentamicin 25 ppm) were put into each of the holes, incubated at 37°C for 24 hours and observed. The growth inhibitory zone diameter was measured and the smallest concentration that still inhibits the growth of the test bacteria (MIC) was determined.

Formulation of Roselle aqueous extracts gel

Based on the MIC of the Roselle extract, the gel formula was performed using the extract at higher concentration than the MIC. The 7.5 g of the extracts and 250 mg of sodium benzoate were dissolved with warmed citrate buffer (70-80°C), then poured into HPMC 6000 dispersion with 10 g propylene glycol. The solution was stirred until gel mass formed and stopped at 35°C. Composition of the gel formulation was shown in Table 1.

Physical examination of the gel preparation

Physical examination of the gel preparation included viscosity, pH and dispersive analysis. The analysis of the dispersive power was carried out by two calibration slides. Approximately 1 g gel put in the middle of the slide and covered with the other slide. Weights were orderly added starting from 5 g on the upper slide. The weight was continuously added until the preparation no longer spreads (approximately 5 minutes) and the diameter was recorded. Afterwards, a curve of the relationship between the dispersion diameter (cm) and the weight (g) was observed. The dispersion ability was determined from the slope of the regression equation of the dispersion diameter and the weight.^{9,10,11} The experiment was replicated three times.

Determining the MIC of the selected formula

The gel solution 12.0 mg/mL was diluted to obtain solution at 6.0, 3.0, 1.5, 0.8, 0.4, 0.2, 0.1 and 0.05 mg/mL concentration. The determination of MIC was carried out same as the extract. The medium agar was perforated with 19 sterile holders. Approximately 50 μ L of the positive control (gentamicin), negative control (gel base) and sample were put into each hole. The disk was incubated at 37°C for 24 hours, the growth inhibitory zone and its diameter (mm) were observed and measured. The gentamicin solution at 100 ppm was made and diluted to obtain solution at 25, 20, 15, 10 and 5 ppm concentration. Logarithmic of gentamicin concentration vs. the inhibitory zone diameter (mm) curve was made and the regression equation obtained was used to calculate the inhibitory activity of the sample solution equally to the gentamicin standard by plotting inhibitory diameter.

Statistical analysis

The significant difference of inhibitory activity among Roselle aqueous extract formulas was determined by one-way variance analysis (ANOVA) method. Furthermore, the significant differences were determined by the Honestly Significant Difference (HSD) test with the reliability value of 0.95 ($\alpha = 0.05$). If the value is > 0.05 then there is no significant difference between the tested formulas.

RESULTS

Screening of the extract contents was carried out according to Marliana¹² and Viliani.¹³ Based on the profile of the TLC chromatogram, Roselle aqueous extracts contained polyphenols and flavonoids as reported in the previous research.¹⁴ The pH value of the extract was 2.54 ± 0.004 , close to the literature (2.42 ± 0.01).^{2,3}

Regardless HPMC qualification, its viscosity > 100 cPss at 2% concentration.^{15,16} The viscosity criteria accepted if the measured result is not less than 75.0% and not more than 140.0%.

The MIC of the Roselle aqueous extracts was 0.1 mg/mL against *Staphylococcus aureus* ATCC 25923 (Table 2, Figures 1 and 2). This value is higher than the previous research, the MIC of the aqueous extracts of

Roselle calyces against *Staphylococcus aureus* and *Streptococcus faecalis* was reported 0.5 mg/mL. Furthermore, *Eschericia coli*, *Klebsiella pneumoniae* and *Salmonella typhii* were inhibited by the MIC value of 1.0 mg/mL.^{7,16} Despite these effects, Roselle extracts have therapeutic effect for gastrointestinal infection, diarrhea, and skin diseases.⁷

The viscosity and pH value of the gel base its preparation were depicted in Figure 3 and Figure 4. It was found that viscosity of the gel preparation containing aqueous Roselle extracts was higher than the gel base (without the Roselle extract). On the other hand, the pH value of the gel preparation was lower than the gel base.

The dispersive power analysis of the gel base and gel preparation depicted in Figure 5 showed that, both the gel base and preparation of 1st and 2nd formula reached the maximum dispersion capacity at 10 g and 35 g loading load (the weight of the load placed on the gel base and gel preparation), respectively, while the 3th formula reached maximum dispersion capacity at 65 g loading load.

The bacterial inhibitory activity of the gel preparations indicated that the greater concentration of HPMC, the lower inhibition activity was obtained (Figure 6 and Table 3). The greater the viscosity of the gel preparation, the lower capacity of active material to be released.^{9,10,11}

Based on the physical evaluation, formula 2 was chosen, because of its viscosity was close to the specification (30000 cPs). The result of the MIC determination of formula 2 (Figure 7), the inhibitory diameter (Table 4) and the inhibitory graph of formula 2 (Figure 8) were analyzed statistically. Gentamicin 25 ppm was chosen as positive control to ensure that the bacterial test used in this research was sensitive against the antibiotic. A serial concentration of the gentamicin was used as the standard curve for evaluation the extract potency relative to the standard.

Based on the one-way ANOVA analysis, there was a significant difference between the inhibitory activity of 12.0 and 6.0 mg/mL, and there was no significant difference between the 3.0, 1.5 and 0.8 mg/mL of the gel preparation. In conclusion, formula 2 exhibited MIC at 6.0 mg/mL against the *Staphylococcus aureus* ATCC 25923.

The inhibitory activity of the gentamicin at serial dilution against the bacterial test was evaluated by the regression equation, where Y and X were the diameter of inhibitory zone (mm) and log of concentrations (ppm), respectively. The log concentration of formula 2 with diameter of inhibitory zone 9.75 mm was calculated by the regression equation. Equivalent to this growth inhibitory diameter (x), 7.58 ppm of the gentamicin concentration was obtained. Furthermore, the inhibitory potency of the Roselle aqueous extracts gel at 12.0 mg/mL (Roselle concentration in gel 3% w/w) against the *Staphylococcus aureus* ATCC 25923 was equal to 7.58 ppm of the gentamicin sulfate standard solution.

DISCUSSION

Identification of polyphenols and flavonoids chromatogram pattern showed a positive result by which they play an important role toward the antibacterial activity.^{4,12,14} The pH of 1% solution of Roselle aqueous extracts was high acidity due to a lot of organic acid contents, such as malic acid and ascorbic acid. The acidity of Roselle also play an important role toward its antibacterial activity.^{1,2,14} The qualification of the HPMC 6000 indicated that the matrix had viscosity satisfactory for gelling agent. The pH value of 2% w/w solution of HPMC 6000 in the water was 4.445 ± 0.053 stabilized by the acidic properties of the extract. The pH value was different from the literature (5.0-8.0)¹⁵ might be caused by the different producers, the quality and the storage condition of raw materials.

It was found that MIC of the Roselle aqueous extracts against *Staphylococcus aureus* ATCC 25923 was

at 0.1 mg/mL. This value was suggested to choose the concentration of the formula, to which 3% w/w was set as the extract concentration based on the preliminary optimization.

The gel formula was performed by three concentrations of HPMC 6000 (2%, 3%, and 4%, w/w). The ingredients of the preparation formula were propylene glycol as a humectant, Roselle aqueous extracts as an active material, sodium benzoate as a preservative, citrate acid and sodium citrate as buffer. The gel base preparation without the extracts was formulated to identify the effect of Roselle aqueous extracts on the physical characteristics of the gel preparation. The gel preparation was made of 250 g with citrate buffer dissolved with pH of 4.505 and each formula was made for one dosage. Replication was not performed due to the limited number of Roselle aqueous extracts. It was found that the viscosity of formula 1, 2, and 3 were 7600, 69200 and 277200 cPs, respectively. On the other hands, the viscosity of the gel base of formula 1, 2, and 3 were 7080, 63800 and 261600 cPs, respectively. The presence of Roselle aqueous extracts 3% w/w increased the viscosity.

The pH value of formula 1, 2, and 3 were 3.199 ± 0.003 , 3.165 ± 0.002 and 3.153 ± 0.006 , respectively. Then pH value of the base gel formula 1, 2, and 3 were 4.556 ± 0.006 , 4.564 ± 0.006 and 4.570 ± 0.006 , respectively. It can be concluded that the pH of preparation was much lower than the pH of gel base even though they were treated by citrate with 0.02 of buffer capacity. This condition was happen because of the buffer capacity failed hold the pH of the preparation containing 3% (w/w) quite acidic extract of Roselle aqueous extracts. Statistical test using one-way ANOVA ($p = 0.05$) showed that there was a significant difference among the pH of formula 1, 2, and 3, as well as the pH of the gel base formula 1, 2, and 3.

The slope calculation of the regression equation of the dispersion diameter vs. weight of loads to evaluate dispersal ability of the gel preparation and the base gel of formula 1, 2, and 3 as depicted in Figure 5 was performed statistically by one-way ANOVA ($p = 0.05$). It was found that there was no significant difference in the slope between formula 1 and 2; but significant difference was found between formula 1 and 3; formula 2 and 3. The significant difference in the slope no found between formula 1 and formula gel base; formula 2 and gel base 2; but no significant difference between formula 3 and gel base 3. The capacity of dispersion was denoted by the diameter of maximum dispersion on the adding of certain loads, by which the gel preparation was not disperse anymore.

According to the slope value and the loads to reach maximum dispersion capacity, it can be concluded that the gel preparation formula dispersed more easily than the gel base, because viscosity of the gel base is lower than the one of preparation. Since the pH value of the gel preparation was close to 3 and the analysis of the dispersive power was conducted in the 30th day after the preparation was made, this might caused unstable the gel preparation. The viscosity of the HPMC solution was stable at pH 3-11, but the stability might be disturbed if there was an active material that possessed strong acidity.¹⁵ In this research, the active material was acid solution of the Roselle aqueous extracts.

The inhibitory activity test of the gel base was performed to minimized effects of the gel component. The activity test was aimed to ensure that the growth inhibitory responses derived from the gel preparation. The bioassay indicated that the gel preparation exhibited higher inhibitory activity than the gel base activity. The gel preparation of formula 1, 2, and 3 exhibited growth inhibitory diameter of 10.80 ± 0.17 mm; 9.80 ± 0.30 mm; and 9.38 ± 0.36 mm, respectively. The one-way ANOVA ($p = 0.05$) showed that there was a significant difference between formula 1 and 2, as well as the formula 1 and 3. There was no significant difference between formula 2 and 3. The viscosity of the gel preparation might affect release of the active materials. The higher the viscosity, the more difficult the active materials released, because of difficult mobility of the active materials.^{9,10,11}

Base on the physical characterization, the selected gel preparation was formula 2, the one containing

HPMC 6000 concentration of 3% (w/w) with specification of acid gel preparation with viscosity of 30000 cPs. The three formulas had pH value; which did not meet the specification. Therefore, the formula was selected in accordance with the viscosity value that was close to the specification, namely formula 2. Then MIC of formula 2 was determined. The preparation was diluted until it reached a concentration of 0.05 mg/mL. The growth inhibitory activity was appeared at the dilution of 12.0-0.8 mg/mL. However, the zone was higher than the gel base. Statistical test using the one-way ANOVA indicated that there was a significant difference between the activity of the gel preparation at 12.0 and 6.0 mg/mL and the gel base. In addition, significant difference was not found between the inhibitory activity of the gel preparation with concentration of 3.0; 1.5; and 0.8 mg/mL and the gel base. The non-significant difference between the gel preparation and the gel base indicated that the inhibitory activity was not caused by Roselle aqueous extracts, but affected by other components in the formula, such as propylene glycol and sodium benzoate. The smallest concentration showed the existence of a significant difference between the inhibitory activity of the preparation and the gel base was 6.0 mg/mL. In conclusion, the concentration of the Roselle aqueous extracts of formula 2 might be recommended as an antibacterial activity toward *Staphylococcus aureus* ATCC 25923. The MIC of the gel preparation was higher than the Roselle aqueous extracts, because the gelling agent/polymer of the gel preparation might affected the release of the Roselle aqueous extracts from three preparation formulas.

The potential ratio of the formula 2 inhibited the test bacterial was determined using gentamicin sulfate standard. Correlation between growth inhibitory diameter of the gentamicin solution at 5-25 ppm against *Staphylococcus aureus* ATCC 25923 and concentration log of the gentamicin standard used to determine the potency of the gel preparation through the regression equation: $y = 10.2584x + 0.5479$ with $r = 0.9837$. The formula 2 exhibited growth inhibitory activity against *Staphylococcus aureus* ATCC 25923 equal to gentamicin sulfate standard solution of 7.58 ppm.

CONCLUSION

The HPMC 6000 at 3% (w/w) concentration in Roselle aqueous extracts gel preparation gave good physical characteristics. The gel preparation exhibited inhibitory activity against *Staphylococcus aureus* ATCC 25923 depicted by Minimum Inhibitory Concentration 6.0 mg/mL. The formula 2 is recommended and prospects for further investigated to be implemented as topical preparations.

ACKNOWLEDGMENTS

The authors thank Faculty of Pharmacy, Airlangga University for supporting facilities and materials.

REFERENCES

1. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L.–A Phytochemical and pharmacological review. Food Chemistry 2014; 300 165:424–443.
2. Jung EK, Kim YJ, Joo N. Physicochemical properties and antimicrobial activity of Roselle (*Hibiscus sabdariffa* L.). Journal of Science Food Agriculture 2013; 93: 3769–3776.
3. Alaga TO, Edema MO, Atayese AO, Bankole MO. Phytochemical and *in vitro* 305 antibacterial properties of *Hibiscus sabdariffa* L (Roselle) Juice. Journal of Medicinal Plant Research 2014; 8(6): 339–344.
4. Borrás-Linares I, Fernández-Arroyo S, Arraez-Roman D, Palmeros-Suarez PA, Del Val-Díaz R, Andrade-Gonzales I, Fernández-Gutiérrez A, Gómez-Leyva JF, Segura-Carretero A. Characterization of phenolic compounds, anthocyanidin, antioxidant and 310 antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). Industrial Crops and Products 2015; 69 385–394.

5. Liu K, Tsao S, Yin M. *In vitro* antibacterial activity of Roselle calyx and protocatechuic acid. *Phytotherapy Research* 2005;19: 942–945.
6. Navarro VM, Rojas G, Zepeda LG, Aviles M, Fuentes M, Herrera A, Jimenez E. Antifungal and antibacterial activity of four selected Mexican medicinal plants. *Pharmaceutical Biology* 2006; 44 (4): 297–300.
7. Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *Journal of Medicinal Plants Research* 2007; 1(1): 009–013.
8. Brookfield Engineering Laboratories, Inc. 11 Commerce Boulevard, Middleboro, MA 02346-1031 USA
9. Gendy AM, Jun HW, Kassem AA. *In vitro* release studies of flur biprofen from 320 different topical formulations. *Drug Development and Industrial Pharmacy* 2002; 28(7): 823–831.
10. Ueda, C. T., Shah, V. P., Derdzinski, K., Ewing, G., Flynn, G., Maibach, H., Marques, M., Rytting, H., Shaw, S., Thakker, K., Yacobi, A. 2009. Topical and Transdermal Drug Products. *Pharmaceutical Forum*, 35 (2009), pp. 750-764.
11. Olejnik A, Goscianska J, Nowak I. Active compounds release from semisolid dosage forms. *Journal of Pharmaceutical Sciences* 2012; 101 (11).
12. Marlina SD, Suryanti V, Suyono. Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah labu siam (*Sechium edule* Jacq. Swartz.) dalam ekstrak etanol. *Biofarmasi* 2015; 3(1): 26–31. 330
13. Villani T, Juliani HR, Simon JE, Wu QL. *Hibiscus sabdariffa*: Phytochemistry, quality control, and health properties: African Natural Plant Products 2013; 2: 210–215.
14. Al-Hashimi AG. Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts. *African Journal of Food Science* 2012; 6(21): 506–511.
15. Rowe RC, Sheskey PJ, Quinn ME. *Handbook of Pharmaceutical Excipients*, 6th ed. 335 Washington: American Pharmacists Association 2009; 326–328.
16. Ford JL. Design and evaluation of hydroxypropyl methyl cellulose matrix tablets for oral controlled release: A historical perspective in: *Hydrophilic Matrix Tablets for Oral Controlled Release*. New York: Springer; 2014: 18. 340

Table 1: Gel formula of Roselle aqueous extracts

Materials	Formula					
	Preparation (%)			Base (%)		
	1	2	3	1	2	3
HPMC 6000	2	3	4	2	3	4
Roselle aqueous extracts	3	3	3	-	-	-
Propylene glycol	5	5	5	5	5	5
Sodium benzoate	0.1	0.1	0.1	0.1	0.1	0.1
Citrate buffer (pH 4.505)	89.9	88.9	87.9	92.9	91.9	90.9

Table 2. The result of MIC determination of Roselle aqueous extracts

Conc. (mg/mL)	Rep	Inhibitory Diameter (mm)			Average
		1	2	3	
0.50		8.80	8.65	8.00	8.48 ± 0.42
0.25		8.20	7.70	7.55	7.82 ± 0.34
0.12		7.70	7.35	7.20	7.42 ± 0.26
0.10		6.65	-	6.60	6.62 ± 0.05
0.05		-	-	-	-
0.02		-	-	-	-

Note: diameter of reservoir: 6.00 mm

Table 3. Inhibitory diameter of formula 1, 2 and 3 gel preparation of Roselle aqueous extracts

Replication	Inhibitory Diameter (mm)					
	Formula			Formula base		
	1	2	3	1	2	3
1	10.70	10.10	9.45	7.30	7.05	6.80
2	11.00	9.80	9.70	6.70	6.20	6.05
3	10.70	9.50	9.00	6.50	6.05	6.10
Average	10.80 ± 0.17	9.80 ± 0.30	9.38 ± 0.36	6.83 ± 0.42	6.43 ± 0.54	6.32 ± 0.42

Note: diameter of reservoir: 6.00 mm

Table 4. The result of the MIC determination of formula 2 gel preparation of Roselle aqueous extracts

Conc. (mg/mL)	Rep	Inhibitory Diameter (mm)							
		Preparation				Base			
		1	2	3	Average	1	2	3	Average
12.00		9.10	9.90	10.25	9.75 ± 0.59	8.45	8.60	9.00	8.68 ± 0.28

6.00	8.60	8.70	9.10	8.80 ± 0.26	8.40	-	8.55	8.47 ± 0.15
3.00	8.35	8.35	8.30	8.33 ± 0.03	8.30	8.30	8.00	8.20 ± 0.17
1.50	8.30	8.25	7.70	8.08 ± 0.33	8.20	8.25	7.60	8.02 ± 0.36
0.80	8.10	8.00	7.50	7.87 ± 0.32	8.00	8.00	7.30	7.77 ± 0.40
0.40	-	-	-	-	-	-	-	-
0.20	-	-	-	-	-	-	-	-
0.10	-	-	-	-	-	-	-	-
0.05	-	-	-	-	-	-	-	-

Note: diameter of reservoir: 6.00 mm



Figure 1: The result of MIC determination of Roselle aqueous extracts (1, 2, 3 = triple replication; I = 0.50 mg/mL; II = 0.25 mg/mL; III = 0.12 mg/mL; IV = 0.10 mg/mL; V = 0.05 mg/mL; VI = 0.02 mg/mL; G = gentamicin 25 ppm)

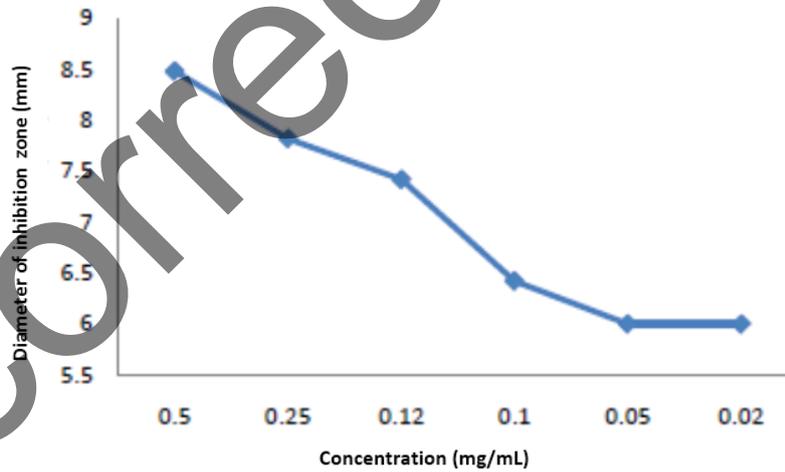


Figure 2: Graph of inhibitory activity of Roselle aqueous extracts

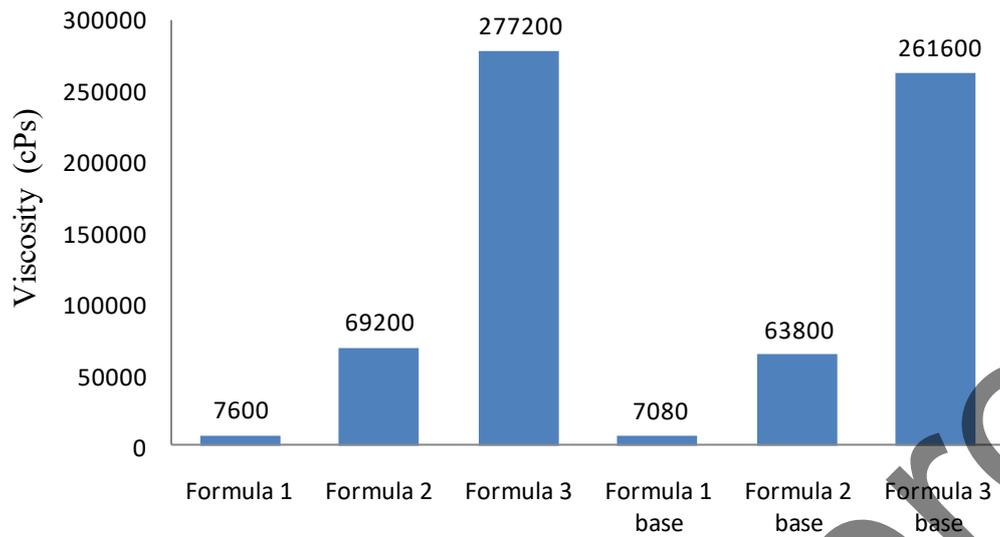


Figure 3; Graph of the viscosity of the gel preparation of Roselle aqueous extracts and gel/formula base

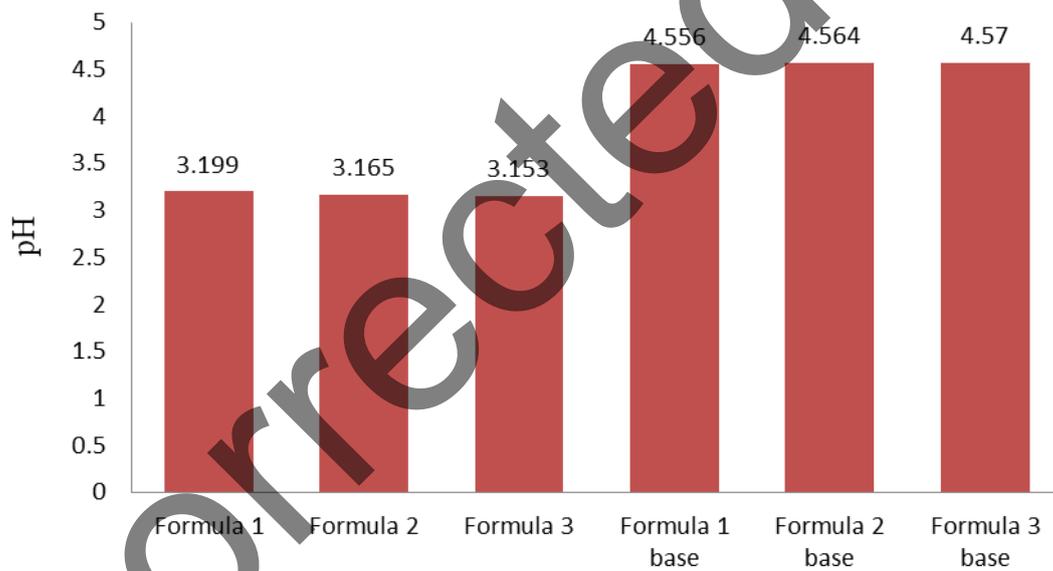


Figure 4: Graph of the pH of the gel preparation and gel/formula base of Roselle aqueous extracts.

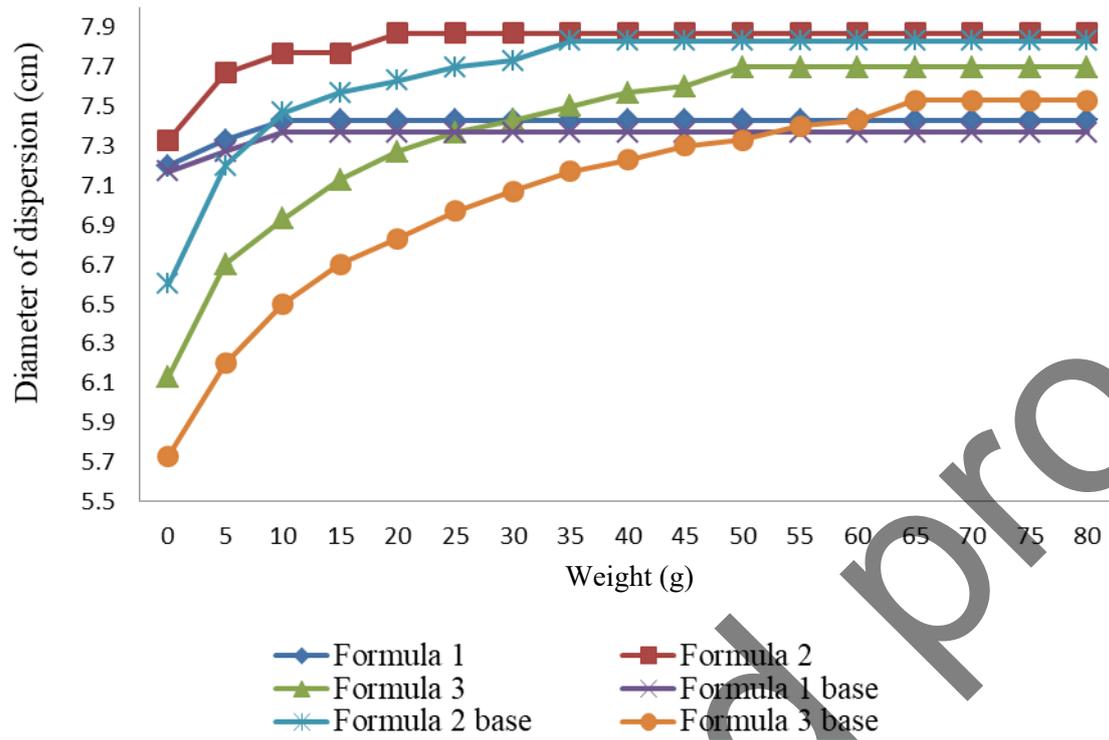


Figure 5: Graph of dispersive power of the gel preparation and gel/formula base of Roselle aqueous extracts



Figure 6. The antibacterial activities of formula 1, 2 and 3 at 12.0 mg/mL (I, II and III = replication; F1 = formula 1; F2 = formula 2; F3 = formula 3; K1 = formula base 1; K2 = formula base 2; K3 = formula base 3; G = gentamicin 25 ppm)

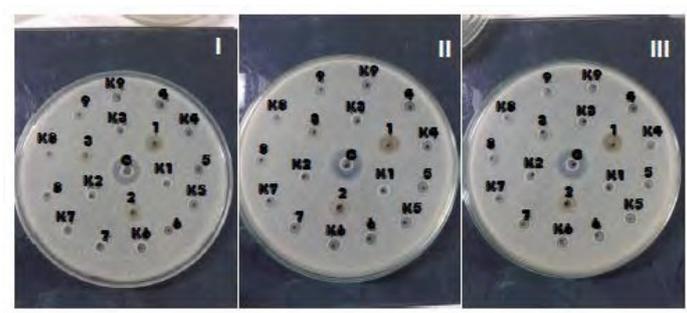


Figure 7. The result of the MIC determination of formula 2 (I, II, III = replication 3; 1 = 12.0 mg/mL; 2 = 6.0 mg/mL; 3 = 3.0 mg/mL; 4 = 1.5 mg/mL; 5 = 0.8 mg/mL; 6 = 0.4 mg/mL; 7 = 0.2 mg/mL; 8 = 0.1 mg/mL; 9 = 0.05 mg/mL; K1 = dilution base 1; K2 = dilution base 2; K3 = dilution base 3; K4 =

dilution base 4; K5 = dilution base 5; K6 = dilution base 6; K7 = dilution base 7; K8 = dilution base 8; K9 = dilution base 9; G = gentamicin at 25 ppm)

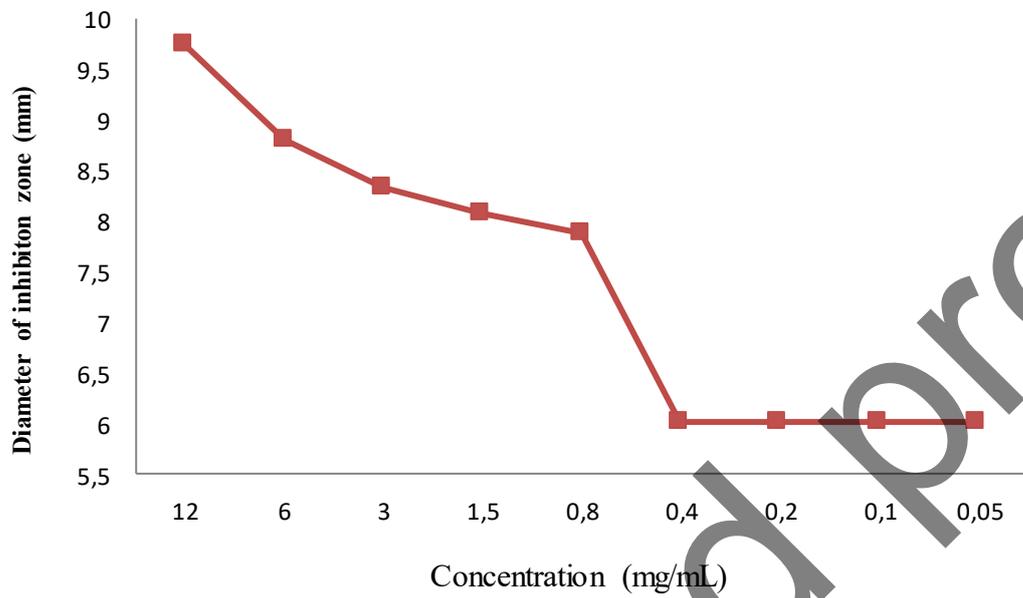


Figure 8. Inhibitory activity of the formula 2 gel preparation of Roselle aqueous extracts