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Original Article

FORMULATION OF NANOEMULGEL CONTAINING EXTRACT OF *IMPATIENTS BALSAMINA* L. AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: This study aimed to optimize the nanoemulgel formulation for balsam leaves (*Impatients balsamina* L.) extract and determine its antibacterial activity.

Methods: Balsam leaves were extracted using the maceration method using ethanol. The nanoemulsion of balsam leaves ethanol extract was prepared with various oil, surfactants, and co-surfactant concentrations. Characterization was conducted on the nanoemulsion formed, including transmittance, droplet size, polydispersity index, and zeta potential. The chosen nanoemulsion formula was then transformed into a gel preparation using various gelling agent concentrations, i.e., Carbopol 940 and chitosan, and optimized using the Design-Expert v13 software with the simplex lattice design method.

Results: The study discovered the optimum nanoemulgel formula with a desirability value of 0.859. The ratio of Carbopol 940 with chitosan was 1.38% and 0.12%w/w, respectively, with an antibacterial activity inhibition zone against *S. epidermidis* of 22±2 mm in diameter.

Conclusion: The observed responses closely matched the predicted values provided by the optimization method. The optimized nanoemulgel formulation has the potential to develop as an antibacterial dosage form.

Keywords: Nanoemulgel, Gelling agent, *Impatients balsamina*, L., Optimization, Formulation

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INTRODUCTION

The antibacterial activity of balsam leaf extract (*Impatiens balsamina* L.) is demonstrated against *Staphylococcus aureus, Propionibacterium acnes, and Staphylococcus* epidermidis bacterias [1, 2]. In addition, this balsam plant showed antifungal activities against dermatophyte fungi and candida albicans [3]. Naphthoquinone, kaempferol, and quercetin are the primary antibacterial compounds found in the balsam leaf. Phenolics and flavonoids are two additional compounds that can potentially possess antibacterial properties [4, 5].

There are some drawbacks associated with applying nanoemulsions to the skin, including poor dispersion and retention. However, nanoemulsion preparations have the benefit of increasing the concentration of drugs that pass through the skin and into the bloodstream [6]. Gel preparations have the disadvantage of being unable to accommodate hydrophobic molecules; however, gel preparations can increase dispersion and the amount of water the skin retains. Therefore, using a nanoemulsion in conjunction with a gel will make up for the deficiencies of each of these different preparations. When nanoemulsion and gel are combined, the resulting substance is referred to as nanoemulgel [7, 8]. In this investigation, the nanoemulgel preparation was chosen because it has the potential to significantly boost the antibacterial action of balsam leaf extract, which has low solubility in water.

A nanoemulgel is a combination of nanoemulsions that have been integrated into a gel matrix. This combination will affect the skin's ability to absorb substances. Nanoemulgel has the potential to improve patient compliance due to its non-greasy, non-irritating, and better drug-release properties than ointments and creams. These properties make nanoemulgel superior to ointments and creams. The homogeneous dosage form and the hydrogel matrix's consistency also contribute to the growing interest in nanoemulgel [9].

When it comes to the formulation of nanoemulgel preparations, the gelling agent component emerges as a critical determinant that has the potential to influence the nanoemulgel's resulting physical properties [10, 11]. Carbopol is a hygroscopic material that does not degrade. Carbopol dispersion can keep its viscosity during storage for an extended period when kept at room temperature. As a gelling agent, the concentration of Carbopol can range anywhere from 0.5 to 2%. Because a gel can be formed with only a relatively low concentration of Carbopol, this substance makes for an excellent gelling agent. Carbopol has a high viscosity in a small concentration, which will be beneficial when combined with chitosan, which has a slight viscosity so that it can produce a gel with suitable viscosity, good flow properties, and an attractive appearance, which makes its use easier and attracts patients to use it [12]. Carbopol's high viscosity in a small concentration will be beneficial when combined with chitosan. We investigated the formulation of nanoemulgel preparations containing balsam leaves extract (*Impatiens balsamina* l.) and its antibacterial activity against *S. epidermidis* as part of this study.

MATERIALS AND METHODS

Materials

Materials used in this research were Balsam leaves (*Impatiens balsamina*, L.) collected from Sukoharjo, Indonesia (-7.547678914660727, 110.73054624485195). The plant was determined at UPT Laboratorium-Setia Budi University using plant determination key books from Steenis and ensured to be *Impatients balsamina* L (No. 115/DET/UPT-LAB/18.01.2021). Citronella oil was purchased from CV Lansida, Yogyakarta, Indonesia. Ethanol, propylene glycol, Span 80, Tween 80, methylparaben, Carbopol 940 (Brataco, Indonesia), triethanolamine (TEA), Muller-Hinton Agar (MHA), chitosan, ampicillin, NaCl, NaOH, DMSO were purchased from Merck. *S. epidermidis* ATCC 12228 bacterial suspension was a collection of the Faculty of Pharmacy, UMS.

Extraction of balsam leaves

The balsam leaves are allowed to dry and then crushed into a fine powder. Maceration was performed on 500 grams of dried samples

with a ratio of 1:10 using ethanol 96%. The maceration process lasted five days, and stirring occurred once every twenty-four hours. The maceration results were then concentrated using a rotary evaporator at a temperature of 40 °C. The process continued to the evaporation stage above the water bath for six days to obtain a concentrated extract.

Antibacterial assay of balsam leaf extract

The well method was used for the antibacterial assay. Mueller-Hilton Agar was used as the medium, 100% DMSO was used as the negative control, and ampicillin concentrations of 1% and 3% were used as the positive controls. Samples were made from two concentrations of balsam leaf extract, 1 and 3%, by weighing 100 mg and 300 mg of the viscous extract and dissolving them in 10 ml of DMSO. The next step was to make streak plates of *S. epidermidis* by smearing one loop on the MHA's surface, then incubating for one day at 37 ºC. Suspension of *S. epidermidis* bacterial culture was prepared by adding one ose of the *S. epidermidis* streak plate with NaCl until it matched the transparency of the 0.5 Mc Farland standard (10^8 CFU/ml). 200 µl of staphylococcus epidermidis bacterial culture suspension was taken, then poured while flattening it over the mueller hinton agar medium, waiting 10 min. After that, wells were made by punching holes in mueller hinton agar media with a diameter of 6 mm. Negative control, positive control, and samples were put into the wells as much as 50 µl, then incubated for 24 h at 37 ºC, and the test was repeated three times [13].

Solubility test of balsam extract

Ten (10) mg of balsam extract was dissolved in various solvents such as citronella oil, ethanol 96%, Span 80, Tween 80, propylene glycol, and water. This process continued until the extract was dissolved, at which point a clear solution was produced.

Pseudo-ternary phase diagram design

Pseudo-ternary phase diagram design was utilized to develop fifteen (15) different formulas for nanoemulsions [14]. Every one of the formulas called for the use of particular carrier components, a combination of surfactants and co-surfactants, distilled water, and an oil phase. The clarity and consistency of each formula were carefully observed (settled for a day to see if there was a separation). The spectrophotometer (Genesys 10S UV-Vis) was set to a wavelength of 650 nm, and the transmittance was determined using that setting. Pseudo-ternary diagrams of formula designs and optimum formulas can be visualized with the triplot software [15]. The droplet size was measured using a Particle Size Analyzer PSA SZ-100 (Horiba Scientific).

Formulation and characterization of nanoemulgel

The nanoemulgel formula was made by varying the ratio of gelling agents Carbopol 940 and chitosan with a total concentration of 1.5% (table 1.). The chitosan was dissolved in 5 ml of 1% glacial acid solution. Carbopol 940 was weighed and crushed in an expanded mortar with warm water. Chitosan solution was added and crushed in a mortar containing Carbopol 940. Methylparaben was dissolved in 3 ml of 96% ethanol, and then TEA was added. Nanoemulgel was formed by adding 90 ml of balsam leaves extract nanoemulsion in the gel and stirring until homogeneous.

pH determination

The pH of the nanoemulgel was measured using a pH meter (Ohaus ST3100) and replicated three times.

Viscosity measurement

The viscosity of the nanoemulgel was measured with a viscometer (Rion VT06 RION CO., LTD) and replicated three times.

Table 1: The design of the nanoemulgel formulation for balsam leaves extract.

Ingredients	Formulation				
	I	II	III	IV	V
Carbopol 940	1.5	1.375	1.25	1.125	1
Chitosan	0	0.125	0.25	0.375	0.5
Water	2	2	2	2	2
Stearic acid 1%	5	5	5	5	5
Ethanol 96%	3	3	3	3	3
TEA	0.65	0.65	0.65	0.65	0.65
Methyl Paraben	0.02	0.02	0.02	0.02	0.02
Nanoemulsion	90	90	90	90	90

Adhesion test

Two hundred and fifty (250) mg nanoemulgel was placed on top of a glass object attached to another glass object. One (1) kg is placed on it in 5 min. The object glass is mounted on the test instrument, and the load weighing 80 grams is removed. The time until the object-glass was released was recorded as adhesion ability. This test was replicated three times.

Spreadability test

Five hundred (500) grams of nanoemulgel were placed in the middle of the first petri dish that had been given a millimeter block. The second petri dish is placed on top of the first petri dish, which is the initial load within 1 minute, then the load is added to 50 grams every 1 minute until the load is 300 grams. The gel's diameter spread was measured on four sides and replicated three times.

Antibacterial test of nanoemulgel balsam leaves extract

The well diffusion method was used to test the antibacterial activity of the sample against *S. epidermidis*. Mueller-hilton agar (MHA) was used as the medium. The positive control was ampicillin 0.9%, and the negative control was nanoemulgel without extract. The sample used in this test weighed 300 mg.

Data analysis

The two-way analysis of variance (ANOVA) was used with Design-Expert v13 software to examine the impact of each factor at different levels on the response (Stat-Ease, Inc., USA). The response surface plots were created using the Design-Expert v13 software to graphically determine the effect of each factor on the response (Stat-Ease, Inc., USA).

RESULTS AND DISCUSSION

Extract of balsam leaf

The maceration method yielded a balsam leaf extract of 18.41%, slightly lower than the extraction conducted by the previous study, which obtained a yield of 20.3% [16]. The maceration technique using ethanol was chosen because of the simplicity, and ethanol could dissolve almost all compounds in the balsam leaf; both polar and non-polar compounds, had a low boiling point, safe and non-toxic. Based on this, it is expected that ethanol's maceration process can extract more secondary metabolite from balsam leaves [2].

Antibacterial activity of balsam leaf extract

Antibacterial activity test against *Staphylococcus epidermidis* was carried out on crude balsam leaves extract (fig. 1.). Antibacterial activity test against crude balsam leaves extract at concentrations of 1%, and 3% gave the value of inhibition zones against *Staphylococcus epidermidis* of 24±1 mm and 26.67±3 mm. Statistical analysis showed significant differences between 1% and 3% of balsam leaf extracts compared to control but lower than ampicillin 1% and 3% ($p<0.05$). This result was similar to the previous study [2]. The ethanolic extract of balsam leaf has been proven to contain naphthoquinones and flavonoids responsible for antimicrobial activity [4, 17].

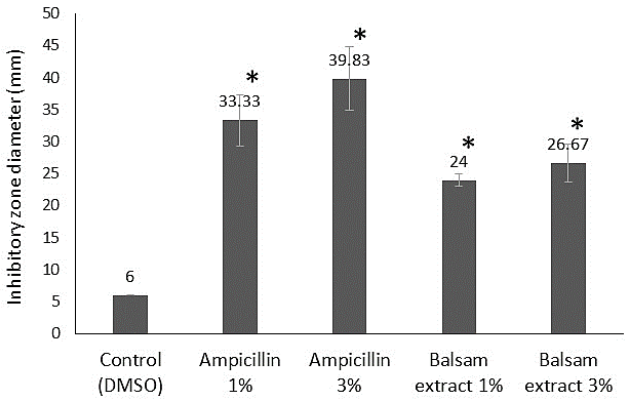

Fig. 1: Preliminary test results antibacterial activity of balsam leaf extract against *Staphylococcus epidermidis* ATCC 12228. Each bar represents the mean±SD of three replication. *Denote the significance levels compared with control (DMSO) group (p<0.05)

Solubility of balsam leaf extract

A solubility test is critical to know and determine the appropriate solubility extracts to facilitate the application for subsequent use. An extract solubility test was carried out on all solvents used in the formula (fig. 2). The results obtained Tween 80, propylene glycol and citronella oil can dissolve a large amount of samples. Thus, Tween 80 and propylene glycol were selected as surfactants and co-surfactants; and citronella oil was used as the oil phase of the nanoemulsion. Tween 80 and propylene glycol were commonly used in a nanoemulsion preparation, while citronella oil was an essential oil used in nanoemulsion and has some activities, including antibacterial [10, 18].

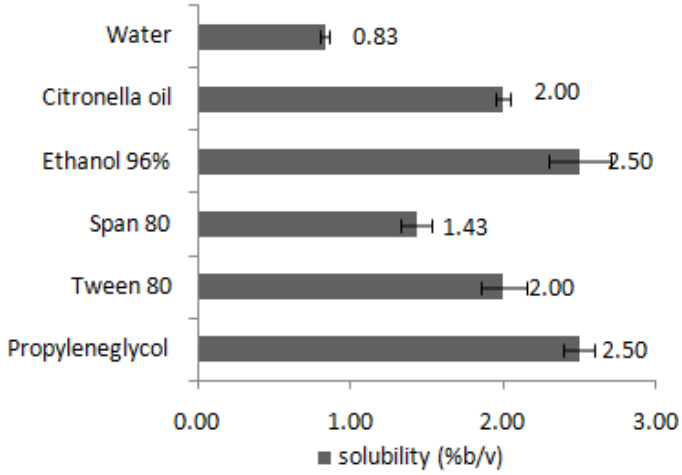

Fig. 2: Solubility of ethanol extract of balsam leaf in several solvents (Each bar or point represents the mean±SD of three replication)

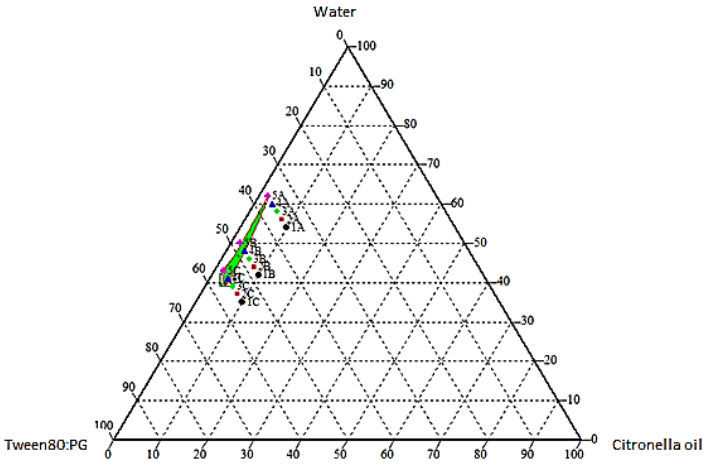

Fig. 3: A pseudo-ternary diagram of nanoemulsion containing water, Tween 80, propylene glycol, and citronella oil. The green area of the phase plot displays the nanoemulsion area

Construction of nanoemulsion pseudo-ternary phase diagram

Tween 80, propylene glycol, and citronella oil were mixed in 15 formulas with various concentrations and visualized with a pseudo-ternary diagram to determine whether the formula design was included in the formation region of a clear and stable nanoemulsion (fig. 3.). The shaded area of the phase plot displays the nanoemulsion area, and the unshaded area displays the emulsion area. One formula from the nanoemulsion area was selected (table 2.), which had a % transmittance of 90.2±0.96, a droplet size of 60.9±17.598 nm, and a polydispersity index of 0.463±0.079.

Table 2: Composition of nanoemulsion selected

Materials	Composition
Balsam leaves extract	1 gram
Citronella oil	2 ml
Tween 80	27.5 ml
Polyethylene glycol	27.5 ml
Water ad	100 ml

The criteria for a good nanoemulsion include having a transmittance value ranging from 90% to 100%. This is because, in that value range, the nanoemulsion appears to have a clear and transparent visual appearance. The small droplet size is responsible for the high percent transmittance value. Because its HLB value is 13.3, the emulsion produced is of the oil-in-water (w/w) type. The critical micelle concentration (CMC) value, which helps determine the critical limit of the concentration at which micelles can form, is an indicator of essential properties in manufacturing nanoemulsions. The surface tension will decrease as the concentration of the surfactant increases, and it will continue to do so until it reaches a constant interfacial tension. The CMC value represents the sweet spot for the amount of surfactant that should be used [19].

Optimization of balsam leaf nanoemulgel formulation

Before testing the nanoemulgel's physical properties, the nanoemulgel formula was put through an organoleptic evaluation, which involved analyzing its outward appearance, including its smell, color, and consistency. Formula one has the thickest consistency because it is the only one of the five that contains Carbopol 940, without any combination with chitosan. The four different formulations all smell and look the same; specifically, they all have the aroma of citronella oil, are uniformly yellow, and have a consistent appearance.

The physical characteristics of the nanoemulgel formulated are displayed in table 3. The response of viscosity, pH, spreadability and adhesiveness of the nanoemulgel was analyzed using Design-Expert software (fig. 4), and the equation of the responses can be seen in table 4.

Table 3: Physical characteristics of balsam leaves extract nanoemulgel

Formula	pH	Adhesiveness (s)	Viscosity (dPas)	Spreadability (cm)
F1	5.19±0.02	12.25±0.26	506.67±20.82	3.97±0.06
F2	5.30±0.03	11.23±0.12	413.33±15.28	5.07±0.21
F3	5.39±0.03	8.96±0.19	296.67±20.82	5.63±0.06
F4	5.47±0.03	5.12±0.27	280.00±17.32	6.03±0.06
F5	5.49±0.04	5.49±0.04	243.33±11.55	6.40±0.10

*Data was represented as mean±SD (n=3)

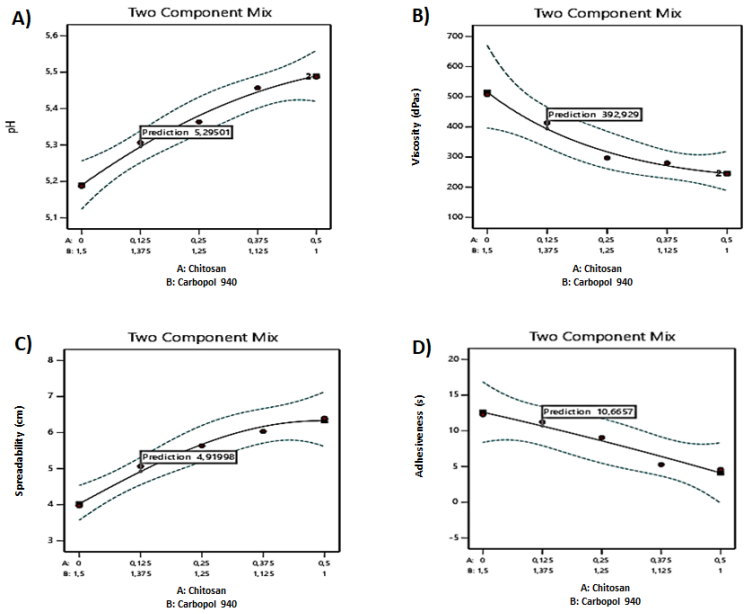

Fig. 4: Graph of response surface of pH (A), viscosity (B), spreadability (C), adhesiveness (D) of the nanoemulgel using simplex lattice design

Table 4: Equation of response balsam leaves extract nanoemulgel using simplex lattice design

Responses	Equations
pH	Y= 0.74A+0.71B+0.014AB
Adhesiveness (s)	Y= 4.10A+12.61B+1.01AB
Viscosity (dPas)	Y= 2.39A+2.71B-0.199AB
Spreadability (cm)	Y = 0.80A+0.60B+0.203AB

The gel preparation was put through the pH test to guarantee it was safe. Table 3, which displays the pH response values of the five formulas, is suitable for use on the skin because it has a pH that falls within the range of 4.5-6.5. This indicates that it will not cause the user's skin irritation when applied [20]. The equation that can be found in table 4 shows that the chitosan component has a more significant influence on the pH value response, which might be

caused by the pH of Carbopol being lower than that of chitosan. chitosan has a pH ranging from 3.0 to 3.5, whereas Carbopol 940 is between 2.5 and 2.8. According to fig. 3, a higher pH response value can be achieved by increasing the levels of chitosan while simultaneously decreasing the levels of Carbopol 940.

Testing for viscosity was carried out so that the viscosity of the nanoemulgel preparation could be determined. If the viscosity of the gel is too high, the active ingredient will not be distributed throughout the gel. Suitable gel viscosity is between 50 and 1000 dPas. Table 6 presents the findings of the viscosity tests conducted on the five different nanoemulgel formulations. The equation in table 4 demonstrates that Carbopol 940 significantly impacts the viscosity response more than chitosan [21]. The viscosity response value is shown to be reduced in fig. 4b when an increased amount of chitosan is present alongside a decreased amount of Carbopol 940. Because Carbopol 940 has a high viscosity even in small amounts, the viscosity value will increase as the amount of Carbopol 940 in the solution decreases. The $p<0.05$ was calculated to be 0.0231. This indicates that Carbopol 940 and chitosan concentration affects the viscosity response value.

The ability of the gel to quickly spread and apply itself to the skin's surface is evaluated using a spreadability test. When applied, gel formulations with a spreadability range of 5-7 centimeters have effective dispersion and a dosage consistency that is comfortable to use. There is a direct correlation between the viscosity and the degree of dispersion. Table 3 presents the findings obtained from the distribution of five different formulas. It can be seen from the equation in table 4 that chitosan has a more significant impact on the response of the preparation's dispersive power value than Carbopol 940 does—according to fig. 4c, increasing the amount of chitosan while simultaneously decreasing the amount of Carbopol 940 results in increased dispersion. The opposite result stated that Carbopol 934 1% produced the highest spreadability of nanoemulgel, compared with xanthan gum and NaCMC 2% [20].

It was determined by testing the adhesiveness of nanoemulgel whether or not it had the ability to adhere to the skin. Nanoemulgel preparations can clog skin pores if they remain adhered to the skin for an extended time and have a powerful effect on the skin. On the other hand, the therapeutic effect will be nullified if the nanoemulgel preparations, after a short period, become only loosely attached [22]. It is recommended that topical preparations have an adhesion time longer than one second. Table 4's equation illustrates that Carbopol has a more significant influence on the response of the adhesion value. This is demonstrated by the fact that the equation contains an equal sign. Fig. 4d demonstrates that a shorter length of adhesion can be achieved by lowering the amount of Carbopol 940 while simultaneously raising the amount of chitosan. According to the calculation results using the simplex lattice design, the p-value of 0.05 is 0.0461, indicating that the concentration of Carbopol 940 and chitosan affects the adhesiveness response value.

For the purpose of optimizing the nanoemulgel formulation, a desirability-based numerical optimization method was used [23]. The investigation employed the Simplex Lattice Design (SLD) produced a desirability value of 0.859 (fig. 5.). Carbopol 940 at a concentration of 1.38% w/w and chitosan at a concentration of 0.12% w/w were determined to be the optimal gelling agent concentrations. There were no significant differences ($p>0.05$) between the predicted and the observed values of responses (pH, viscosity, spreadability, and adhesiveness).

Antibacterial activity of nanoemulgel

An antibacterial test of nanoemulgel preparations was conducted against *Staphylococcus epidermidis*, and the optimal formula was used (fig. 6.). Compared to the crude extract, there were no significant differences in the activity of the nanoemulgel against *S. epidermidis*. Although the gel base may inhibit the diffusion of the active substance, the antibacterial activity might be increased by the content of the citronella oil in the nanoemulgel preparation. The citronella oil contained citronellal, geraniol, and citronellol, which was proven to be an antibacterial agent against Gram-positive or negative bacteria [24].

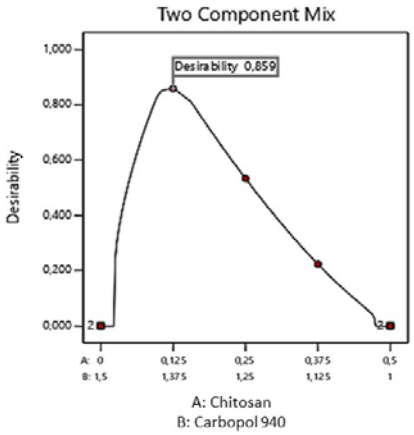

Fig.5: Desirability graph for the optimization of balsam leaf extract nanoemulgel formulation

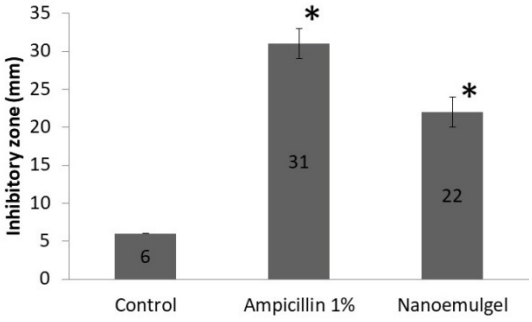

Fig. 6: Antibacterial activity of nanoemulgel leaves extract of balsam water (*Impatiens balsamina* l.) against *S. epidermidis* (Each bar or point represents the mean±SD of three replication. *Denote the significance levels compared with control group ($p<0.05$)

CONCLUSION

According to the findings, chitosan and Carbopol 940 have the potential to affect the nanoemulgel preparation of balsam leaves extract (*Impatiens balsamina* L.), particularly the pH, viscosity, spreadability, and adhesion of the substance. When there is a lower concentration of Carbopol 940, the pH response is more robust, the viscosity is higher, the length of adhesion is shorter, and the spreadability is better. The results of the simplex lattice design (SLD) formula showed that the optimal amounts of Carbopol 940 and chitosan were 1.38%w/w and 0.12%w/w, respectively. The optimized nanoemulgel formulation has the potential to develop as an antibacterial dosage form.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

REFERENCES

1. Su BL, Zeng R, Chen JY, Chen CY, Guo JH, Huang CG. Antioxidant and antimicrobial properties of various solvent extracts from

impatiens balsamina l. stems. J Food Sci. 2012;77(6):C614-9. doi: 10.1111/j.1750-3841.2012.02709.x, PMID 22582943.

2. Kang SN, Goo YM, Yang MR, Ibrahim RI, Cho JH, Kim IS. Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of impatiens balsamina L. (Balsaminaceae) at different harvest times. Molecules. 2013;18(6):6356-65. doi: 10.3390/molecules18066356, PMID 23760032.
3. Qin H, Jia L, Yan J, Li D, Sun L, Wang H. *In vitro* and *in vivo* antibacterial activities of impatiens balsamina water extraction. J Jilin Univ (Med Ed). 2013;39:60-4. doi: 10.7694/jldxyxb20130115.
4. Sakunphueak A, Panichayupakaranant P. Comparison of antimicrobial activities of naphthoquinones from impatiens balsamina. Nat Prod Res. 2012;26(12):1119-24. doi: 10.1080/14786419.2010.551297, PMID 21895457.
5. Wang YC, Li WY, Wu DC, Wang JJ, Wu CH, Liao JJ. *In vitro* activity of 2-methoxy-1,4-naphthoquinone and stigmasta-7,22-diene-3β-ol from impatiens balsamina L. against multiple antibiotic-resistant helicobacter pylori. Evid Based Complement Alternat Med. 2011;2011:704721. doi: 10.1093/ecam/nep147. PMID 19773391.
6. Clares B, Calpena AC, Parra A, Abrego G, Alvarado H, Fangueiro JF. Nanoemulsions (NEs), liposomes (LPs) and solid lipid nanoparticles (SLNs) for retinyl palmitate: effect on skin permeation. Int J Pharm. 2014;473(1-2):591-8. doi: 10.1016/j.ijpharm.2014.08.001, PMID 25102113.
7. Prajapati B. "Nanoemulgel" Innovative approach for topical gel based formulation. RRHOAJ 2018;1(2). doi: 10.32474/RRHOAJ.2018.01.000107.
8. Choudhury H, Gorain B, Pandey M, Chatterjee LA, Sengupta P, Das A. Recent update on nanoemulgel as topical drug delivery system. J Pharm Sci. 2017;106(7):1736-51. doi: 10.1016/j.xphs.2017.03.042, PMID 28412398.
9. Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharm. 2017;526(1-2):353-65. doi: 10.1016/j.ijpharm.2017.04.068, PMID 28461261.
10. Imanto T, Prasetiawan R, Wikantyasning ER. Formulasi dan karakterisasi sediaan nanoemulgel serbuk lidah buaya (Aloe Vera L.). Pharmacon J Farmasi Indones 2019;16(1):28-37. doi: 10.23917/pharmacon.v16i1.8114.
11. Bashir M, Ahmad J, Asif M, Khan SUD, Irfan M, Y Ibrahim AY. Nanoemulgel, an innovative carrier for diflunisal topical delivery with profound anti-inflammatory effect: *in vitro* and *in vivo* evaluation. Int J Nanomedicine. 2021;16:1457-72. doi: 10.2147/IJN.S294653. PMID 33654396.
12. Putriana NA, Rusdiana T, Prakoso M. Formulation and physical stability testing of chitosan gel from vaname shrimp shell (litopenaeus vannamei) with Carbopol 940 gelling agent. Indonesian J Pharm. 2019;1:62-6. doi: 10.24198/idjp.v1i3.21556.
13. Munawaroh R. Optimum conditions for extraction of antibacterial compounds from citrus aurantifolia fruit peel waste. Pharmacon J Farmasi Indones. 2017;14(1):34-9. doi: 10.23917/pharmacon.v14i1.5779.
14. Aparna C, Srinivas P, Patnaik KSKR. Enhanced transdermal permeability of telmisartan by a novel nanoemulsion gel. Int J Pharm Pharm Sci. 2015;7(4):335-42.
15. Salvia Trujillo L, Rojas Graü MA, Soliva Fortuny R, Martin Belloso O. Formulation of antimicrobial edible nanoemulsions with pseudoternary phase experimental design. Food Bioprocess Technol. 2014;7(10):3022-32. doi: 10.1007/s11947-014-1314-x.
16. Ih H, Fajriaty I, Wijaya T, Hafizh M. The potential ethnomedicine plant of impatiens balsamina leaves from pontianak, West Kalimantan, Indonesia for wound healing. Nusantara Biosci. 2018;10(1):58-64. doi: 10.13057/nusbiosci/n100109.
17. Qian H, Wang B, Ma J, Li C, Zhang Q, Zhao Y. Impatiens balsamina: an updated review on the ethnobotanical uses, phytochemistry, and pharmacological activity. J Ethnopharmacol. 2023;303:115956. doi: 10.1016/j.jep.2022.115956. PMID 36436713.
18. Agrawal N, Maddikeri GL, Pandit AB. Sustained release formulations of citronella oil nanoemulsion using cavitational techniques. Ultrason Sonochem. 2017;36:367-74. doi: 10.1016/j.ultsonch.2016.11.037, PMID 28069223.
19. Singh RD, Kapila S, Ganesan NG, Rangarajan V. A review on green nanoemulsions for cosmetic applications with special emphasis on microbial surfactants as impending emulsifying agents. J Surfact & Detergents. 2022;25(3):303-19. doi: 10.1002/JSDE.12571.
20. El-Leithy ES, Makky AM, Khattab AM, Hussein DG. Nanoemulsion gel of nutraceutical co-enzyme Q10 as an alternative to the conventional topical delivery system to enhance skin permeability and anti-wrinkle efficiency. Int J Pharm Pharm Sci. 2017;9(10):207-17. doi: 10.22159/IJPPS.2017V9I11.21751.
21. Eid AM, Istateyeh I, Salhi N, Istateyeh T. Antibacterial activity of fusidic acid and sodium fusidate nanoparticles incorporated in pine oil nanoemulgel. Int J Nanomedicine. 2019;14:9411-21. doi: 10.2147/IJN.S229557. PMID 31819440.
22. Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug Dev Ind Pharm. 2016;42(9):1434-45. doi: 10.3109/03639045.2016.1141931, PMID 26821208.
23. Sopyan I, Gozali D, Sriwidodo GRK, Guntina RK. Design-expert software (DoE): an application tool for optimization in pharmaceutical preparations formulation. Int J App Pharm. 2022;14:55-63. doi: 10.22159/IJAP.2022V14I4.45144.
24. Timung R, Barik CR, Purohit S, Goud VV. Composition and anti-bacterial activity analysis of citronella oil obtained by hydrodistillation: a process optimization study. Ind Crops Prod. 2016;94:178-88. doi: 10.1016/j.indcrop.2016.08.021.