Trop J Nat Prod Res, November 2023; 7(11):5183-5187
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Optimization of Cream Formulation Containing *Peperomia pellucida* Leaf Extract and Chitosan Nanoparticles

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ARTICLE INFO

Article history:
Received 02 September 2023
Revised 16 October 2023
Accepted 02 November 2023
Published online 01 December 2023

ABSTRACT

Peperomia pellucida and chitosan have several uses in medicine, like an antibacterial agent. Antibacterials can be developed in the form of cosmetic preparations such as creams. This work aims to develop creams using *P. pellucida* leaf extract and chitosan nanoparticles using Tween 80 as an emulsifier and cetyl alcohol as a stiffening agent to assess the physical properties and stability of these creams. *P. pellucida* was extracted by the maceration method using ethanol as a solvent. The ionic gelation technique was used to prepare chitosan nanoparticles. Eight different cream formulas were created using different amounts of cetyl alcohol and Tween 80. The physical characteristics of creams were evaluated including organoleptic, homogeneity, pH, spreadability, adhesiveness, and viscosity. Software Design Expert was used for the optimisation, and the cycle test method was used to test the cream's stability. The extraction resulted in *P. pellucida* leaf extract with a yield of 13.37%. The characterisation of chitosan nanoparticles obtained an average particle size of 200.43±1.64 nm, a PDI of 0.347±0.01, and a zeta potential of –44.53±0.80 mV. The optimal formula of cream was obtained with a concentration of cetyl alcohol and Tween 80 of 3.8% and 6.1%, respectively. The viscosity, adhesion and spreadability of the cream were 9660±104.33 cps, 5.83±0.04 cm and 4.13±0.07 seconds, respectively. The cream provides good stability. Cetyl alcohol and Tween 80 were used to successfully produce a cream that contained *P. pellucida* leaf extract and chitosan nanoparticles and showed good stability.

Keywords: *Peperomia pellucida*, chitosan nanoparticles, Design Expert, cream, optimisation

Introduction

In traditional medicine, *Peperomia pellucida* leaf is a plant that has been widely used in various traditional treatments such as treating seizures, gastrointestinal disorders, dysentery, diarrhoea, indigestion, abscesses and injuries. *P. pellucida* also has phytochemicals that have antibacterial properties.[1] In addition to *P. pellucida* leaf, chitosan, a chitin biopolymer, has many benefits in the pharmaceutical and biomedical industries. For instance, it can be used as an anti-bacterial.[2] Chitosan has several benefits when it is in the form of nanoparticles, especially that it is non-toxic, stable while being used, has a high surface area and may be utilised as a matrix for different kinds of pharmaceuticals and plant extracts.[3]
Cream is a semisolid emulsion with one or more active ingredients dissolved or spread out. It is also a two-phase system, with the active ingredients being finely and uniformly spread out in the continuous or external phase. Cream is a conventional emulsion preparation that is promising as a topical drug delivery agent.[4] Cream production requires an emulsifier that functions as a unifier between the oil phase and the water phase.[5] The emulsifier often used in creams is Tween 80, which can bind hydrophilic groups from water, reducing the water phase's interfacial tension and, consequently, reducing the viscosity.[6]

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Citation: Parwati P and Wikantyasning ER. Optimization of Cream Formulation Containing *Peperomia pellucida* Leaf Extract and Chitosan Nanoparticles. Trop J Nat Prod Res. 2023; 7(11):5183-5187. http://www.doi.org/10.26538/tjnpr/v7i11.22

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In addition to the emulsifier, a stiffening agent is needed to increase the viscosity of the cream preparation. One of the commonly used stiffening agents used is cetyl alcohol. Stability can be increased by mixing a non-ionic emulsifier like Tween 80 with a stiffening agent like cetyl alcohol.[7]
In the dosage formulation process, optimisation needs to be carried out to determine the best formula. One method for obtaining the optimal formula is using Design Experts. Such software can determine the relative proportions of ingredients used in a formula to produce the best formula according to the specified criteria.[8] The simplex lattice design method is another method used to optimise formulas for various amounts of material composition whose total amount is the same. This method can identify the optimum formula using a smaller number of experiments to minimise the use of materials.[9]
The combined cetyl alcohol with Tween 80 in topical cosmetic preparations may affect the physical properties of the cream. Up to date, there are no studies on the formulation of creams containing *P. pellucida* and chitosan nanoparticles using a combination of cetyl alcohol and Tween 80. Hence, this study aimed to produce creams using *P. pellucida* leaf extract and chitosan nanoparticles with Tween 80 as an emulsifier and cetyl alcohol as a stiffening agent. The software Design Expert 13 and the Simplex Lattice Design (SLD) method were utilized to assess the physical properties and stability of the formulated creams.

Materials and Methods

Material

P. pellucida was collected from *Paal Merah District (*9MP8+F5X), West Sumatera, Indonesia, in March 2023. The plant was authenticated at the Faculty of Science and Mathematics of Diponegoro University (Voucher number V100210011). Chitosan (CDH Fine Chemical, Indonesia), 70% Ethanol, distilled water, sodium tripolyphosphate (NaTPP), cetyl alcohol, Tween 80, Span 80, stearic acid, glycerine, triethanolamine (TEA), methyl paraben and propyl paraben were obtained from Bratachem (Indonesia).

Preparation of P. pellucida Leaf Extract

P. pellucida leaf extract was extracted using the maceration method, employing 70% ethanol as the solvent. A quantity of 300 grams of *P. pellucida* leaf powder was poured into a solution containing 3000 millilitres of 70% ethanol. The mixture was stirred for 5 minutes and was put to rest for 24 hours with intermittently stirring. The maceration process utilised a Buchner funnel for filtration. Subsequently, the residual powder undergoes a second round of maceration. Re-maceration was conducted on two separate occasions. The macerate was collected, and the solvent was removed using a rotary evaporator (Heidolph Hei–VAP). Subsequently, the extract was further concentrated by evaporating it using a water bath (Memmert, USA) until a dense extract was obtained.

Chitosan Nanoparticle Preparation

Chitosan nanoparticles were prepared using the ionic gelation method, which incorporated *Sodium tripolyphosphate* (NaTPP). Chitosan was dissolved in 1% acetic acid and the pH of the solution was adjusted using NaOH 1 N to pH 5, then added slowly, or dropwise, to the NaTPP solution with a ratio of 5:1 using a magnetic stirrer (Thermo Scientific CIMAREC) at room temperature. Stirring was continued for 1 hour so that the crosslinking process was perfect until a suspension of nanoparticles was formed.[10] The formed particles were then characterised using a Particle Size Analyzer (PSA) (Horiba SZ100-Z). The parameters analysed included the mean particle diameter (ZAve), polydispersity index (PI), and Zeta potential.

Cream Formulation

Optimisation was carried out using the Design Expert software version 13 of the Simplex Lattice Design (SLD) method with response parameters in the form of viscosity, spreadability, and adhesion. The cream is formulated through emulsification. Briefly, the oil phase (stearate acid, cetyl alcohol, Span 80, and Propylparaben) and the water phase (aquadest, methylparaben, glycerin, Tween 80, and TEA) were melted separately in the water bath (Table 1). After that, the water phase was gradually converted to the oil phase in the hot mortar with constant stirring until a cream mass was formed. *P. pellucida* leaf extract and chitosan nanoparticles were added to the cream little by little, stirring until the mixture is homogeneous.[11,12]

Characterisation of creams

This test was conducted by observing the texture, smell and colour of the cream prepared. After preparing the cream, approximately 0.5 grams of cream was applied to the glass surface to test the cream's homogeneity. This test was designed to assess the cream shape's physical uniformity across the entire object. If there are no lumps or large grains in any component of the cream mixture, the mixture is considered homogenous. The pH was determined using a pH meter. The pH meter electrode was dipped into a prepared solution for evaluation. One gram of cream was diluted with 10 mL of distilled water to create the solution. The viscosity of the cream using a Brookfield viscometer, the container containing a sample of the cream preparation was fastened, the spindle was fastened and the rotor was turned on. The viscosity measurements were recorded immediately after the display. A spherical glass surface with a diameter of 15 cm was placed with the cream, which weighed around 1 gram. The cream was then covered with another glass, which was left in place for a minute. To evaluate the cream spread's spreadability under load, its diameter was measured.[13,14] A total of 0.5 g of preparation was spread on a glass disc, and another glass object was placed on top of it and put under load for one minute. Following the discharge of the load, a disc of glass mounted on test equipment was timed up until a second object of the glass fell off.[15]

Stability

The stability test was carried out using the cycling test method. The cream was stored at ± 4°C for 24 hours and then at ± 40°C for 24 hours. The test was carried out for 6 cycles, and then the physical changes in the cream were observed including organoleptic, homogeneity, pH, viscosity, spreadability, and adhesion.[16]

Statistical analysis

To compare the mean values of each test group, statistical analysis was carried out using the IBM SPSS One-sample T-test program with a 95% confidence level. In this study, all experimental data were measured thrice, and the means and standard deviations were computed.

Table 1: Formulation composition of cream containing *P. pellucida* leaf extract and chitosan nanoparticles

Material	Formulas (%)							
	I	II	III	IV	V	VI	VII	VIII
P. pelllucida Leaf Extract	1	1	1	1	1	1	1	1
Chitosan Nanoparticles	1	1	1	1	1	1	1	1
Cetyl Alcohol	8	3.5	5	2	8	5	2	6.5
Tweens 80	2	6.5	5	8	2	5	8	3.5
Span 80	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Stearic acid	12	12	12	12	12	12	12	12
Glycerin	7	7	7	7	7	7	7	7
TEA	2	2	2	2	2	2	2	2
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Aquadest ad	100	100	100	100	100	100	100	100

Results and Discussion

The extraction resulted in *P. pellucida* leaf extract with a yield of 13.37% and a weight of 40.13 g. On the characterisation of chitosan nanoparticles done using a particle size analyzer (PSA) obtained an average particle size of 200.43 ± 1.64 nm. Particles are nanosized if they have 10 nm–1000 nm sized particles. The smaller the particle size, the better; the smaller the particle is, the easier it is to disperse.[17] The Polydispersity Index (PDI) of chitosan nanoparticles was 0.347 ± 0.01, indicating that the chitosan nanoparticles showed a uniform or heterogeneous particle size distribution. This is because if the PDI value is closer to 0.0, the nanoparticles were considered uniform, but, if it was close to the value of 1.0, the sample is not uniform and has an extensive distribution of particle sizes.[18] The next characterisation is the zeta potential, which shows the surface charge on the particles and is measured to determine the stability of the nanoparticles in suspension.[19] In the study, the zeta potential value obtained was -44.53

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

± 0.80 mV, implying that the colloid system formed is stable. This is because particles with a zeta potential more positive than +30 mV or more negative than −30 mV have good colloidal stability.[20,21]

Eight formulations based on the Design Expert software were prepared and evaluated with parameters including organoleptic, homogeneity, pH, viscosity, spreadability and adhesion. Table 2 enumerates the evaluation results. The results showed all cream preparations were successfully made with a greenish-yellow physical appearance due to a mixture of *P. pellucida* leaf extract, a soft and semi-solid texture, and a distinctive odour of *P. pellucida* leaf extract, and all formulations produced homogeneous preparations without visible coarse particles. The pH value of all formulations meets the standard pH range (4.5–6.5),[12] which is safe and does not irritate the skin.[16,22] The eight formulations have the viscosity value required by standard reference, ranging from 2000 cps to 50,000 cps.[23] Positive results were obtained from the spreadability test since the output adheres to the requirement of good spreadability, which is between 5 cm–7 cm.[24] The better the spread of the cream, the better the contact between the skin and the cream is and the more even the distribution of the active ingredients will be.[25] The results of the eight formulas also showed that only Formulas I and III did not meet the requirements for good adhesion because they had an adhesion time of less than 4 seconds.

Based on data analysis and processing with Design Expert software version 13, the SLD equation for each response can be determined following a linear model.

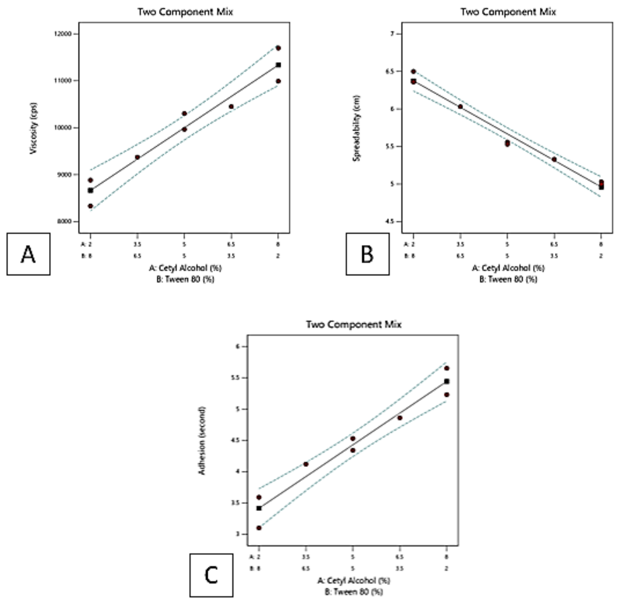

Figure 1: Plot of cetyl alcohol and Tween 80 concentration on the (A) viscosity, (B) spreadability and (C) adhesion

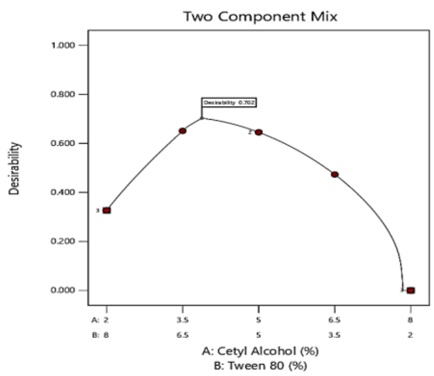

Figure 2: Contour plot of the optimised formula using SLD approach

Based on the equation (Table 3a), the relationship between the response of the viscosity test to cetyl alcohol and Tween 80 shows a positive effect on increasing viscosity. Cetyl alcohol has a greater effect on increasing the viscosity (+1134.14) compared to Tween 80 (+8664.36). The linear graph (Figure 1a) illustrates that higher cetyl alcohol composition added to the formula with a small ratio of the Tween 80 composition will result in more viscous cream. This happens because cetyl alcohol is a thickening agent that can increase the viscosity.[26]So, if the concentration of cetyl alcohol is greater, the viscosity will increase.

The response graph for the relationship between the amount of cetyl alcohol and Tween 80 on the spreadability of the cream (Figure 1b) with the coefficient of Tween 80 being higher than that of cetyl alcohol indicates that the concentration of Tween 80 increases the spreadability. According to the equation (Table 3b), Tween 80 (+6.37) significantly increased the spreadability compared to cetyl alcohol (+4.96) because Tween 80 is hydrophilic,[27]so it binds more water, increasing the cream's spreadability.

The relationship between the adhesiveness test response to cetyl alcohol and Tween 80 significantly increased adhesion. Cetyl alcohol has a greater effect on increasing adhesion (+5.44) compared to Tween 80 (+3.41). The linear graph (Figure 1c) reveals that a concentration of cetyl alcohol higher than Tween 80 can increase adhesion because the higher the viscosity, the longer the adhesion time.

The optimum formula criteria parameters provide a formula solution that fits the desired optimization target with a desirability value of 0.702. The desirability value close to number one is the best formula solution. The optimum formula prediction results are presented in Figure 2.

The results of the One-sample T-test (Table 4) with a 95% level of confidence present no significant difference between the predictions and the experimental results for each response with a p-value > 0.05.

A cycling test is carried out to test the product against the possibility of crystallisation and as an emulsion test on cream as an indicator of emulsion stability.[28] Table 5 details the stability test results using the cycling test method with six cycles. Based on the results obtained, the cream formula can stable under extreme conditions with changes in temperature because it remains homogeneous, and the colour, smell, and texture do not change. The test results using the SPSS One-sample T-test program with a 95% confidence level demonstrated that it did not experience a significant shift in pH value, viscosity, spreadability and adhesion (p > 0.05) before and after the cycling test.

Conclusion

The study's findings suggest that cetyl alcohol and Tween 80 affect the physical characteristics of the cream preparations. Using the simplex lattice design method, the ratio of cetyl alcohol and Tween 80 concentrations at 3.8% and 6.1% produced optimised cream containing *P. pellucida* leaf extract and chitosan nanoparticles which exhibited good stability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors thank the financial support from the Ministry of Education, Culture, Research and Technology Republic Indonesia for funding this research (Grant No. 006/LL6/PB/AI.04/2023.170.38/C.1-III/LRI/VI/2023).

Table 2: Creams formulation containing *P. pellucida* leaf extract and chitosan nanoparticles

Parameter	FI	FII	FIII	FIV	FV	FVI	FVII	FVIII
pH	6.5	6.5	6.5	6.5	6.4	6.5	6.4	6.4
Viscosity	8884.33 ± 156.68	9372 ± 114.05	8334.33 ± 60.97	9966.67 ± 62.36	11696.7 ± 142.90	10300 ± 97.97	10990 ± 173.78	10450 ± 85.24
Spreadability	6.36 ± 0.04	6.03 ± 0.04	6.5 ± 0.08	5.56 ± 0.12	5 ± 0.08	5.53 ± 0.09	5.03 ± 0.04	5.33 ± 0.12
Adhesion	3.59 ± 0.05	4.12 ± 0.08	3.10 ± 0.09	4.34 ± 0.04	5.65 ± 0.08	4.53 ± 0.08	5.23 ± 0.06	4.86 ± 0.07

Table 3: Predicted model equations of the creams' responses using the SLD approach

Chart	Parameter	Equality	Model
A	Viscosity	Y = 11334.14 (A) + 8664.36 (B)	Linear
B	Spreadability	Y = 4.96 (A) + 6.37 (B)	Linear
C	Adhesion	Y = 5.44(A) + 3.41(B)	Linear

Table 4: Optimum formula verification

Parameter	predictions	Optimum Formulas	Sig. 2-tailed	Interpretation
Viscosity	9500.019	9660 ± 104.33	0.165	Not significantly different
Spreadability	5.932	5.83 ± 0.04	0.119	Not significantly different
Adhesion	4.049	4.13 ± 0.07	0.251	Not significantly different

Table 5: The results of the stability of the preparation using the cycling test method

Parameter	Cycle		Sig. 2-tailed	Interpretation
	Cycle – 0	Cycle – 6		
pH	6.4 ± 0.00	6.4 ± 0.00	1.000	Not significantly different
Viscosity	9660 ± 104.33	9488.66 ± 34.12	0.069	Not significantly different
Spreadability	5.83 ± 0.04	5.9 ± 0.08	0.322	Not significantly different
Adhesion	4.13 ± 0.07	4 ± 0.09	0.203	Not significantly different

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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