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ENHANCEMENT AND EVALUATION OF SOURSOP (ANNONA MURICATA L.) LEAF EXTRACT IN NANOEMULGEL: A COMPREHENSIVE STUDY INVESTIGATING ITS OPTIMIZED FORMULATION AND ANTI-ACNE POTENTIAL AGAINST PROPIONIBACTERIUM ACNES, STAPHYLOCOCCUS AUREUS, AND STAPHYLOCOCCUS EPIDERMIDIS BACTERIA

Mahaveer Singh [1], T. Naga Aparna [2], Vasanthi. S [3], Suraj Mandal [4], Lalita Shashikant Nemade [5], Sharang Bali [6] and Dr. Nihar Ranjan Kar [7]*

[1] Associate Professor, Dr. Karigowda College of Pharmacy, Hassan.
[2] Associate Professor, Sri Indu Institute of Pharmacy, Sheriguda, Ibrahimpatnam.
[3] Assistant Professor, Department of Microbiology, ACS Medical College and Hospital, Chennai.
[4] Assistant Professor, IIMT College of Medical Sciences, IIMT University, O-Pockect, Ganganagar, Meerut, U.P., India.
[5] Associate Professor, Govindrao Nikam College of Pharmacy Sawarde. University of Mumbai.
[6] Assistant Professor, School of Pharmacy, Chouksey Engineering College, Lal Khadan, Masturi Road Bilaspur, Chhattisgarh.
[7] Assistant Professor, School of Pharmacy, Centurion University of Technology and Management, Gopalpur, Balasore, Odisha, India. (*Corresponding Author)

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Abstract

Annona muricata L.'s soursop leaf extract contains alkaloids, flavonoids, steroids, and saponins as its active components. There is antibacterial effect in this extract. This research aims to maximise the utilisation of VCO, Tween 80, and PEG 400 in the nanoemulsion system in order to develop a nanoemulgel and evaluate its antibacterial efficacy. In addition, carbopol 940 will be used as a gelling agent. The combination of VCO, Tween 80, and PEG 400 in the nanoemulsion formula has been successfully optimised via the use of Design Expert 13.0's simplex lattice design technique. This approach led to the creation of 14 unique nanoemulsion formulas. Oils, surfactants, cosurfactants, and extracts should be combined to form a nanoemulsion. The mixture should be homogenised using a vortex before being sonicated. The physical characteristics of the nanoemulsion were determined by assessing the pH, emulsification time, and transmittance percentage. Carbopol 940 was added to the nanoemulgel after the optimal formula for nanoemulsion was assessed in terms of particle size, polydispersity index, and zeta potential. The optimal formula yields a homogeneous, clear nanoemulsion with a transmittance of 91.97±1.11%, an emulsification time of 56.42±0.72 seconds, and a pH of 5.67±0.24. It consists of 10.86% VCO, 67.33% Tween 80, and 21.81% PEG 400. Particle size, polydispersity index, and zeta potential in the optimal formula were 229.47±38.79 nm, 0.41±0.10, and -39.13±0.19 mV, respectively. The physical properties of the nanoemulgel were evaluated, and it was found to be homogenous with the following values: 5.83±0.24 pH, 5.57±0.25 cm spreadability, 3.80±0.25 seconds adhesive force, 11479.33±167.49 cP viscosity, and 9.67±0.47 mm, 7.33±0.47 mm, and 5.67±0.47 mm for P. acnes, Staphylococcus aureus, and Staphylococcus epidermidis, respectively. The nanoemulgel soursop leaf extract's physical characteristics are suitable, and its antibacterial activity is in the medium range.

INTRODUCTION

The skin, which is the outermost layer of the body, is essential to the body because it houses nerve endings, acts as a hydrophobic barrier, and regulates body temperature. The outermost layer of the body is called the skin. Numerous bacteria, such as P. acnes, Staphylococcus aureus, and Staphylococcus epidermidis, call the skin home. Every single one of these microbes is a part of the normal human microbiome. The results of the research conducted by Claire and Lake (2018) indicate that some

bacteria in the environment are harmful and may result in illnesses like acne. The majority of people with this prevalent inflammatory sickness that manifests in the pilosebaceous unit, acne vulgaris, are teens and young adults. Teenagers and young adults account for the bulk of people with acne vulgaris. The skin types on the chest, arms, back, and back are the most susceptible to acne, according to Cong et al.'s 2019 study.

Acne may be caused by a multitude of reasons, including bacteria such as S. aureus, P. acnes, and S. epidermidis. Acne is most likely the outcome of a complex interplay between these variables. Under normal circumstances, it is rare for these bacteria to create skin issues; but, if the skin's state changes, they might become alarming once more. The sebaceous glands and skin perspiration create sebum and other fluids. Different types of fluids are produced by these glands.

There are many key components that make up sebum, but the most crucial ones include fatty acids, water, salt, urea, and amino acids. These materials are taken up by the bacteria that live on the skin's surface and used as a food source. It's possible that some skin disorders or increased sebum production lead to a rise in the invasiveness of bacteria. This is a matter that warrants consideration. The development of acne is significantly influenced by bacteria, which operate via an inflammatory chemotactic mechanism. This results in the development of acne. Bacteria may adapt to changes in the chemical composition of their immediate environment by means of this procedure. Furthermore, bacteria are capable of generating lipolytic enzymes, which modify the quantity of sebum and cause the material to solidify and create a mass. According to Claire and Lake (2018), acne may develop when the sebaceous gland's ducts become partially obstructed. Acne may occur as a result of this.

The soursop plant, also known as Annona muricata L., possesses naturally occurring antibacterial characteristics, according to scientific research. The antibacterial, anti-inflammatory, and antioxidant properties of it are attributed to the presence of bioactive components such glycosides, steroids, alkaloids, terpenoids, tannins, flavonoids, and eugenol, according to Vijayameena et al. (2013). These characteristics and the existence of these substances may be related. At a dose of 150 mg/mL, the soursop leaf methanol extract demonstrated significant antibacterial activity. This demonstrated the extract's antibacterial efficacy.

This extract's ability to effectively prevent the growth of S. aureus bacteria was what caused the impact. As per Haro et al. (2014), the extract is shown to be an efficient means of inhibiting the growth of harmful bacteria by the establishment of an inhibitory zone with a diameter of 14.1 mm. The area around the location where the extract is administered is referred to as the "inhibitory zone". This is the area where the application of the extract clearly stops or inhibits the growth of germs. The results of the investigation suggest that at certain doses, soursop leaf extract has antibacterial activity against S. aureus.

One approach to improving the efficiency and usability of semi-solid formulations is to include gel matrices with nanoemulsions during the formulation process. The two chemicals may be combined to achieve this. An effort is made to resolve a number of issues that might come from applying nanoemulsions topically in this study. Poor dispersion and viscosity are two of these issues that might make it more difficult for medications to be delivered to the skin (Elmarzugi et al., 2015).

Furthermore, it is best to prevent both of these issues. It is anticipated that this procedure will enhance the topical medication's formulation if the nanoemulsion is distributed into a gel foundation. Nanoemulsions provide many benefits due to their enhanced stability and better carrier characteristics, particularly for hydrophobic drugs (Kute and Saudagar, 2013). These benefits are especially beneficial for hydrophobic medicines. Owing to its increased viscosity, gel foundation may be applied to the skin with more effectiveness.

The objective of this study was to determine whether or not the nanoemulsion's constituent parts—VCO, tween 80, and PEG 400—had the potential to be optimised. The analysis of the response variables for emulsification time, pH, and % transmittance was done using the simplex lattice design approach.

RESEARCH METHODOLOGY

Extraction of Soursop leaf

For the production of soursop leaf extract, the maceration technique is used. This process needs the combination of 300 grammes of dry powder and 3000 millilitres of 70% ethanol at a ratio of 1:10. An opaque container is used to hold the mixture for a period of two days, during which time it is stirred once every twelve hours.

After increasing the concentration of the liquid extract using a rotary evaporator that was set to a regulated temperature of 40 degrees Celsius, the liquid extract was condensed in a water bath at temperatures that were lower than 65 degrees Celsius. The procedure results in the production of a highly concentrated ethanol extract from the leaves of the soursop plant, as stated by Vijayameena et al. (2013).

Ternary Diagramming

For the purpose of plotting the oils, surfactants, and co-surfactants that have been chosen on the basis of the findings of the solubility test, a ternary phase diagram is used. When VCO (VCO), Tween 80, and PEG 400 were mixed together in a number of different ratios, including 1:19, 2:8, 3:7, and 4: (6), a total of sixteen different ternary phase compositions were produced. These compositions were developed by mixing the three chemical substances.

A combination of VCO, tween 80, and PEG 400 is then left to remain undisturbed for a period of twenty-four hours in order to facilitate the separation process. This is done in order to make the separation process simpler. Subsequently, the substance that has been produced is examined using a UV-Vis spectrophotometer after being diluted in 25 millilitres of distilled water.

This is done in order to determine the degree of transparency of the material. A ternary phase diagram was created with the assistance of the Prosim software in order to provide an illustration of the nanoemulsion region (Mardiyanto et al., 2018).

Table 1: Ternal Phase Comparison Virgin coconut oil, Tween 80, and PEG-400

Oil : Smix	Oil (%)	Surfactant (%)	Cosurfactant (%)
01:09	10	80	10
		70	20
		60	30
		50	40
02:08	20	70	10
		60	20
		50	30
		40	40
03:07	30	60	10
		50	20
		40	30
		30	40
04:06	40	50	10
		40	20
		30	30
		20	40

Optimising and Preparing Nanoemulsions Using Simplex Lattice Architecture

It is possible to ascertain the upper and lower threshold values of oil, surfactant, and co-surfactant by making use of the ternary diagram that was developed in the phase that came before this one. Utilising the Simplex Lattice Design technique, the dependent variables (response) of transmittance, emulsification time, and pH were evaluated with the use of the Design Expert 13 software.

This research was carried out in order to determine the effectiveness of the methodology. A total of fourteen distinct formulations for nanoemulsions were developed as a result of the results of this inquiry. The percentage of transmittance response, the length of time necessary for emulsification, and the pH were all reevaluated in order to determine which formulation of the nanoemulsion would be the most successful. An analysis of significant features, including the percentage of transmittance, the length of time necessary for emulsification, and the pH, was performed in order to determine which formula may be considered the most effective. After that, the differences between these features and the values that were predicted by the Simplex Lattice Design were analysed and compared (Mardiyanto et al., 2018).

The preparation of a nanoemulsion with soursop leaf extract

The nanoemulsion was created using a combination of VCO, tween 80, and PEG 400A solution. This combination included fifty milligrammes of diluted soursop leaf extract (as shown in Table 2) with distilled water. After that, the mixture was vortexed for five minutes and subjected to further sonication for ten minutes until it reached a homogenous state. After adding Tween 80, the liquid was sonicated for ten minutes. A five-minute vortex mixing process ensued next. Fithri et al. (2017) state that PEG 400 was the last addition made. Subsequently, the liquid was blended by simultaneously applying sonication for ten minutes and a vortex for five minutes.

Table 2: SLD experimental design of soursop leaf extract nanoemulsion formula

Formula	Extract (mg)	VCO (%)	Tween 80 (%)	PEG 400 (%)
1	40	30	60	20
2	40	10	60	30
3	40	30	60	10
4	40	10	80	10
5	40	10	70	20
6	40	20	70	10
7	40	10	70	20
8	40	10	80	20
9	40	20	80	10
10	40	13.33	63.33	23.33
11	40	13.33	63.33	13.33
12	40	23.33	63.33	13.33
13	40	17	66.67	16.67
14	40	20	60	30

Assessment of Nanoemulsions

1. **Transmittance %:** A combination of 100 millilitres of distilled water and 10 microliters of Smix—a blend of oils, surfactants, and cosurfactants—was present. The mixture's ratio was 1:10000. The percentage of transmittance at a wavelength of 650 nm was calculated using a UV-Vis Spectrophotometer (Pratiwi, 2021).
2. **Time required for nanoemulsions to emulsify:** For the purpose of dispersing a nanoemulsion that had a volume of 20 microliters into 12.5 millilitres of clean water, a magnetic stirrer was used at a speed of 150 revolutions per minute. In order to achieve a uniform combination or milk that does not include any oil clumps, according to research carried out by Indrati et al. in 2020, it has been shown that a little quantity is need for. The formulation of the nanoemulgel soursop leaf extract is shown in Table 3.

Material	Composition (% w/w)
Nano emulsions	22
Carbopol-940	2
Triethanolamine	1
Nipagin	0.3
Distilled water	Up to 100ml

3. **pH of Nanoemulsions:** Using a pH metre, the pH of the nanoemulsion may be measured by submerging the electrode of the pH metre into the nanoemulsion. Exactly one hundred microliters of Smix is mixed with five millilitres of distilled water. The homogenization of the mixture is accomplished by turning it over for a period of one minute. In order to confirm that the values have achieved a stable condition and are not changing any more, the readings from the pH metre are collected after a five-minute interval (Pratiwi, 2021).
4. **Particle Size, Zeta Potential, and Polydispersity Index (PDI):** In order to conduct the analysis, the nanoemulsion was first diluted with distilled water at a ratio of 1:100, and then a Particle Size Analyzer (PSA) was used. Mardiyanto et al. (2018) performed triangular measurements on three distinct times. One of these occurrences was in 2018.

Preparation of Nanoemulgel Containing Soursop Leaf Extract

Following the steps outlined in Table 3, the nanoemulgel is made by first dispersing 2.5 grammes of Carbopol 940 in distilled water as a gelling agent, and then adding nanoemulsions while continuously stirring the mixture. According to Nikam et al. (2018), first triethanolamine and then nipagin are added to the mixture, and then the mixture is stirred until it becomes homogeneous.

Assessment of Nanoemulgel

1. **Homogeneity of Nanoemulgel:** The glass object had 0.25 grammes of nanoemulgel uniformly spaced at the top, middle, and bottom. The shortcomings of the preparation are then evaluated by a tactile assessment (Elmarzugi et al., 2015).
2. **pH Analysis:** To conduct this experiment, 5 millilitres of distilled water are combined with 0.5 grammes of the drug under investigation. Afterwards, the probe is submerged in the mixture for a minute in order to measure the mixture's pH. The results of a research carried out in 2020 by Ting and colleagues indicate that the pH of the nanoemulgel may be determined by looking for a change in colour in the pH stick.
3. **Viscosity of Nanoemulgel:** During the procedure, a viscometer is used to accurately measure one hundred millilitres of nanoemulgel preparation. Once the viscosimeter scale has achieved a steady condition and the reading has been taken, the numerical measurement result will be shown on the screen. In the study by Mulia et al. (2018), the experiment was carried out three times.
4. **Dispersion of Nanoemulgel:** A round glass with a diameter of 15 centimetres is used for the test, and the process that is followed for the test is to apply 0.5 grammes of nanoemulgel onto the glass. A second glass is then placed on top of the first glass once it has been allowed to settle for a certain amount of time, which is one minute. The diameter of the dispersion of the preparation is going to be measured in order to fulfil the requirements of this measurement. The specimen is then allowed to remain undisturbed for an additional minute before the diameter that has attained stability is measured (Mulia et al., 2018). This is done in order to ensure reliability. Additionally, an extra load of one hundred grammes is made accessible to the specimen once this has been completed.
5. **Adhesion Test:** The testing technique is carried out by first applying 0.5 grammes of nanoemulgel onto a circular glass with a diameter of 15 centimetres. This is the beginning of the operation. An extra layer of glass is placed on top, and it is provided with the opportunity to remain in place for a duration of one minute. For the purpose of this specific measurement, the diameter of the spread of the preparation is being measured. After this, an additional weight of one hundred grammes is added to the specimen, and it is then allowed to remain undisturbed for one minute until the diameter that has attained stability is measured (Mulia et al., 2018). This process is repeated until the specimen has reached the desired level of stability.
6. **Antibacterial Activity Test:** The methodology known as sumuran diffusion is the way that is used in the process of carrying out research on the quantitative antibacterial activity. At the proper quantities, each test bacterial suspension was injected. Two hundred microliters of P. acnes were placed on blood agar media, and two hundred microliters of S. aureus and S. epidermidis were placed on nutritional agar medium (NA). In order to create a hole in the substrate, a pasteur

pipette with a diameter of six millimetres is used throughout the process. The wells that were allocated for the experiment were filled with precisely twenty microliters of extract solution, twenty microliters of nanoemulgel solution containing soursop leaf extract, twenty microliters of 1% mycin gel solution as a positive control, and twenty microliters of nanoemulgel solution that did not include any extract as a negative control. The use of a laminar air flow (LAF) system was used in order to ensure that each of these solutions was deposited in an aseptic way. In the course of an incubation technique that was carried out in anaerobic conditions at a temperature of 37 degrees Celsius, the bacteria that are responsible for acne were exposed to a duration of forty-eight hours. In contrast, bacteria belonging to the species Staphylococcus aureus and Staphylococcus epidermidis were maintained in an incubator at the same temperature for a period of twenty-four hours before being removed from the incubator. The evaluation is carried out on the basis of the observation of inhibitory zones that are created to be formed. The interpretation of these zones is then accomplished by recognising obvious sections that demonstrate the absence of bacterial growth. During the course of the experiment, there were three separate occurrences that were carried out (Wijayanti et al., 2021).

7. **Optimum Formula Approach:** Test findings on nanoemulsions containing soursop leaf extract were recorded, and they included the percentage of transmittance, the amount of time it took to emulsify, and the pH of the microemulsion. The use of the Simplex Lattice Design approach is utilised at the stage of data analysis, which is where the Design Expert programme is utilised. Based on the findings of the study that has been carried out, the objective of this stage is to determine the formula that achieves the best possible outcomes. Through the use of this method, it is possible to identify formulae that, when applied to certain variable settings, provide the best possible outcomes.
8. **Inhibition Zone Approach:** The antibacterial activity of the inhibitory zone was evaluated three times at different locations using a calliper, and the results of the measurements were averaged. Finally, the findings of the tests were compared to one another. This allows for the acquisition of a value of the inhibitory zone size that is more precise and representative, and which indicates the antibacterial activity of the sample. This may be accomplished by performing what is described above.
9. **Statistical Approach:** In order to do statistical analysis on the data that pertains to the characteristics of nanoemulsions, the Simplex Lattice Design approach was used in combination with the Design Expert programme. A one sample t-test was carried out with the aid of SPSS software in order to provide verification and analysis of the results of the prediction that was accomplished. Ninety-five percent was the level of confidence that was used. During this phase, it is feasible to conduct a more in-depth study of the accuracy of the predictions that were produced by the statistical model that was used.

RESULT AND DISCUSSION

1. **Soursop Leaf Extraction:** In the course of the extraction procedure, the maceration method was used to extract 400 grammes of simplisia powder, which ultimately led to the production of a dry extract that weighed 33.34 grammes. Using a comparison between the weight of the simplisia that was used and the weight of the extract that was generated, it was established that the extraction

yield was 8.34%. Maceration was picked as the method of choice because of its straightforwardness, its uniqueness, and the ease with which it may be applied. Ethanol at a concentration of seventy percent was chosen as the universal solvent because of its ability to extract both polar and nonpolar molecules with the maximum possible degree of activity (Tambun et al., 2021).

2. **Solubility of Extracts:** Dried extracts are placed through a solubility test to confirm their potential before the formulation procedure. Consequently, this is because choosing the best suitable carrier requires careful consideration of a number of factors, including solubility. Extracts are more advantageous for therapeutic purposes due to their relatively easy dissolution, and they also aid in the creation of high-quality nanoemulsions. According to Mardiyanto et al. (2018), the results of their research showed that the soursop leaf extract was the most soluble in VCO (6 mg/mL), Tween 80 (18 mg/mL), and PEG 400 (9 mg/mL). Consequently, the three components that would be employed were decided upon: oil, surfactant, and cosurfactant phases. Lowering the quantity of solvent needed to dissolve the extract often improves its solubility in that specific solvent. This occurs as a result of the extract being dissolved in the solvent.
3. **Ternary phase diagram:** Phase diagrams are used to ascertain the concentration ranges of various components. Pseudo ternary diagrams, in particular, are employed for this purpose. These constituents include VCO (oil), Tween 80 (surfactant), and PEG 400 (cosurfactant), among others. This endeavour aims to find the highest and lowest thresholds for the phases of oil, surfactant, and cosurfactant as well as locations where nanoemulsion is generated throughout the process. As per the results of Elmarzugi et al. (2015), this procedure is carried out without using soursop leaf extract.

   Examining the ternary phase diagram, one finds that the nanoemulsion located within the area enclosed by the blue line in Figure 1 comes into being spontaneously. As mentioned by Mardiyanto et al. (2018), using a greater concentration of Tween 80 and PEG 400 may enable the creation of clear or transparent nanoemulsions. This might be attributed to the fact that surfactants and cosurfactants can decrease the interfacial tension of the oil surface, increasing the stability of the nanoemulsions.

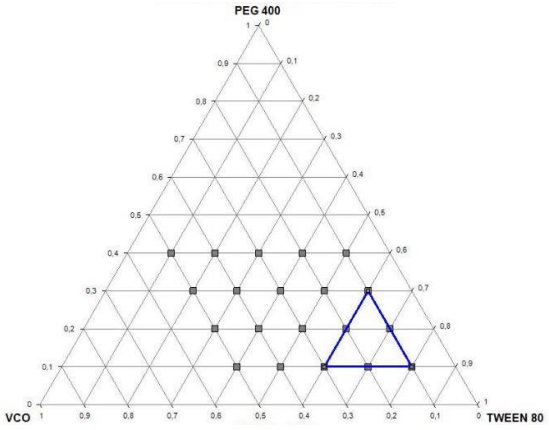

Figure 1: Ternary diagram of *virgin coconut oil*, Tween 80, PEG 400. The area in the blue line indicated a clear solution when the mixture was diluted in distilled water

4. **Simplex Lattice Design for Nanoemulsion Formula Optimisation:** The results of the assessment conducted for the percent transmittance response, emulsification time, and pH are presented in Table 4 and Figure 2, correspondingly. Concerning the proportion of light that was permitted to traverse, an observed range of 83.2% to 96.4% was established. By employing the percentage transmittance, one can approximate the dimensions of the particles that are contained within nanoemulsions. It is plausible to deduce that the oil particles carrying the active chemical are in a state undispersed within the nanoemulsion formulation, given the optical clarity and high transmittance (low absorbance) of the nanoemulsions. This is due to the fact that the nanoemulsion formula has an exceptionally high transmittance. To determine the formula that comprises the optimal solution, techniques such as simplex lattice design analysis are implemented. The letter A represents the reaction, while the letters B and C represent Tween 80 and PEG 400, respectively. The model equation, denoted as Y in Table 5, is presented as the response. The reader was provided with this equation.

The correlation between the percentage of transmittance response to Tween 80, PEG 400, and VCO, as well as the interaction between VCO and PEG 400, demonstrate a positive impact that ultimately results in an augmentation of the transmission rate percentage. However, contrary to the observed negative reaction in the interaction between Tween 80 and VCO, which suggested a reduction in transmittance at the specified percentage of transmittance, this interaction did not demonstrate such a reaction. As demonstrated by the results, the percentage of transmittance was said to have decreased due to the interaction between Tween 80 and PEG 400.

A total of fourteen separate investigations were undertaken to ascertain the emulsification duration, which was ultimately determined to be sixty-seven seconds to the fifty-first. The primary aim of this experiment was to ascertain the duration required for a mixture consisting of identical components to be formed through the dissolution process in a conducting medium. This aim was successfully achieved through the implementation of a methodology that entailed the observation of the dissipation of nanoemulsion particles. As illustrated in Figure 2, the investigation of the simplex lattice design yields a plot representing the percent transmittant response. In order to delineate the relationship between the response and the variables, one may employ a linear model, as illustrated by Equation 2. This model is applicable to the description of this relationship. The variables An (Tween 80), B (An (VCO)), and C (PEG 400)) are denoted by the equation, which also represents the emulsification time response signified by Y. Additionally, the equation incorporates the emulsification time response. Due to the interaction between each component, a positive coefficient is generated, which affects the emulsification time response to PEG 400, Tween 80, and VCO. The generation of the positive coefficient can be attributed to this interaction. An imperative consequence of this advancement is the reduction in the duration required for emulsification.

The nanoemulsions obtained from fourteen distinct experiments, which exhibited pH values ranging from 5 to 6, were determined to be compatible with the acceptable pH range of 4.5 to 6.5 for topical administration, as indicated by the pH analysis. This was ascertained by adjusting the pH value of the nanoemulsions to a range of 5 to 6. To ascertain the suitability of nanoemulsion compositions for topical application, the execution of this test is imperative. By utilising the findings of the study conducted by Wijayanti et al. (2021) employing a simplex lattice configuration, it becomes possible

to ascertain the most effective formula. The pH response to VCO, Tween 80, and PEG 400 are all positively correlated with the pH produced during the reaction due to the interaction between the two substances. This holds true for the relationship between Tween 80 and PEG 400 as well. A negative reaction is initiated due to the interaction between PEG 400 and VCO; this ultimately causes the pH value that is regarded as the equilibrium to decrease. The obtained response values are evaluated in the following phase to determine which formula produced the most accurate results. The formulation with 10.86% VCO, 67.33% Tween 80, and 21.81% PEG 400 was anticipated to produce the most effective nanoemulsion, according to the projections. It was expected that the response values would be influenced by the following variables: an emulsification time of 54.71 seconds, a pH of 5.3, and a transmittance of 96.40%. The formula's desirability value of 0.703, which is in close proximity to 1, provides clear evidence that this strategy is the most effective.

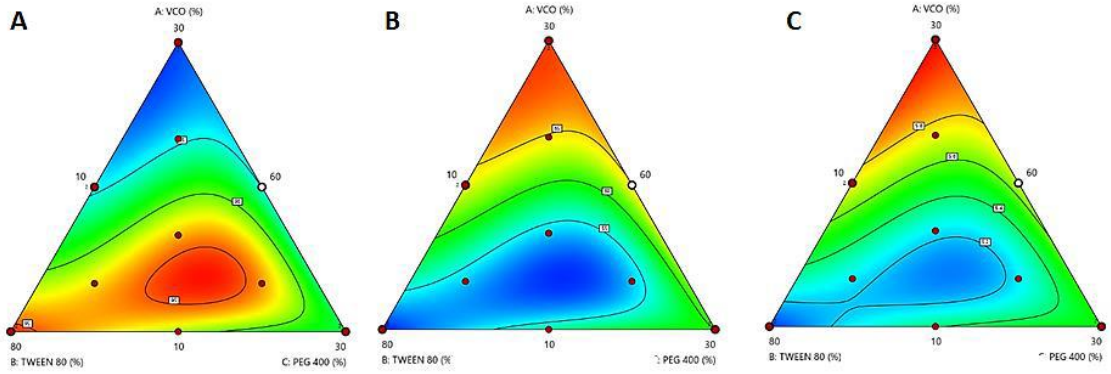

Figure 2: Contour plot responses of transmittance (A), emulsification time (B), and pH (C)

Table 4. Experimental Formula Response Data Using *Simplex Lattice Design*

Run	Factor			Response		
	VCO (%)	Tween 80 (%)	PEG 400 (%)	% Transmittant	Emulsification Time	pH
1	20	70	10	85.7	62	5.7
2	13.33	63.33	23.33	94.5	53	5.2
3	10	60	30	86.3	61	5.5
4	30	60	10	82.6	67	6
5	13.33	73.33	13.33	93.4	53	5.2
6	10	80	10	96.4	50	5
7	17	66.67	16.67	92.7	55	5.3
8	30	60	10	80.6	69	6
9	23.33	63.33	13.33	85.1	64	5.7
10	10	80	10	95.2	51	5.1
11	10	70	20	90.8	57	5.4
12	10	60	30	88.6	59	5.5
13	20	70	10	83.2	66	5.8
14	20	70	10	85.7	62	5.7

5. **Optimum Formula Verification:** The best formula is then verified, test results are analysed using a single sample T-test, and the results are contrasted with the value that was first predicted at the start of the procedure. The process that was used to determine the P-value was based on the study's findings. Table 6 shows that the results of the software recommendations and the verification's findings did not vary statistically significantly ($p > 0.05$). If the polydispersity value is 0.5, the whole

sample's particle size distribution is considered to be uniform. Danaei et al. (2018) state that a closer value to zero means a more uniform particle size distribution, which makes it possible to characterise the nanoemulsion formula more precisely. This is as a result of the particles' more uniform density. Potential zeta values greater than +30 mV or less than -30 mV will prevent the formation of particle aggregates, which might result in coalescence. This is due to the potential for coalescence to happen.

6. **Nanoemulgel Evaluation:** An evaluation was conducted on the nanoemulsion preparations, encompassing parameters such as pH, visual uniformity, dispersion, adhesion, and viscosity. An evaluation has been conducted. The nanoemulgel texture flatness test ensures that the formulation is applied to the skin in a consistent manner throughout, whereas the homogeneity test ensures that all of the formulation's components are adequately combined. Based on the findings pertaining to homogeneity, it is feasible to deduce that the application of the preparation to the glass object does not induce the segregation of any phases or particles. The Nanoemulgel's pH, as determined through pH testing, is 5.83, which falls within the acceptable range of normal epidermis pH, which is approximately 4.5 to 6.5. The data presented herein is derived from a study conducted by Wijayanti et al. in 2021. Furthermore, this provides further evidence that the product is safe to consume.

As adhesion testing directly influences the manner in which drugs are assimilated, conducting this type of testing is critical. Obat possesses the capacity to undergo absorption upon contact with the epidermis. The results of a research investigation carried out in 2022 by Sultan et al. indicate that nanoemulgel formulations containing soursop leaf extract exhibit an adhering time exceeding one second, thereby confirming satisfactory adhesion. The degree to which the substance disperses across the epidermis is a critical determinant in the dispersion test. It is critical that this procedure be followed so that the rate of drug release and the efficacy of the treatment can be assessed. The testing results indicated that the diameter of the dispersion was 5.57 centimetres, which is consistent with the range of 3–7 centimetres recommended in the scholarly literature. This determination was reached in consideration of the test results. Consequently, it is apparent that the formulation utilised in the preparation process was efficacious in generating an adequate dispersion (Okpalaku et al., 2023).

The viscosity test is employed to determine the extent to which nanoemulgel preparations are user-friendly and to assess the ease of extracting nanoemulgel preparations from their packaging. The determination of the nanoemulgel's viscosity (11479.33 centipoise (cP)) derived from the extract of soursop leaf was the outcome of the measurement. According to the findings of Sultan et al. (2022), gel formulations with a viscosity between 900 and 14,000 cP are considered optimal. This was ascertained through investigation conducted by the scholars. To ensure that nanoemulgel formulations remain within the physiological pH range of the epidermis, which is approximately 4.5 to 6.5, conducting pH testing on these formulations is of the utmost importance (Lukić et al., 2021). This practice ensures that the gel is applied to the skin in a manner that is secure, preventing any skin irritation or distress, irrespective of the method of application. It is crucial to utilise these nanoemulgel formulations with a pH level commensurate with the skin's condition. Consequently, the preservation of the skin's integrity and overall well-being will be a more straightforward task.

Table 6. Verification of optimization results using one sample t-test

Parameters	Predictions	Verification	Significance	Information
% Transmittance	92.73	91.97 ± 1.16	0.401	Not significantly different
Emulsification Time	54.71	52.76 ± 1.18	0.105	Not significantly different
pH	5.3	5.67 ± 0.24	0.163	Not significantly different

7. **Antibacterial Properties:** The microbes P. acnes, Staphylococcus aureus, and Staphylococcus epidermidis were tested for the soursop leaf extract's antibacterial activity. The results showed inhibitory zones of 13 mm, 11 mm, and 10 mm, respectively (Table 7). It was discovered that the soursop leaf extract was efficient against all three types of bacteria. Table 7 shows that the inhibitory zones in the nanoemulgel containing the soursop leaf extract had average dimensions of 9.67 mm, 7.33 mm, and 5.67 mm. The extract was used to get these measurements. The soursop leaf extract nanoemulgel formulation was efficient against P. acnes, S. aureus, and S. epidermidis bacteria, even those in the medium category, as shown by the Davis and Stout inhibitory zones (Sari et al., 2018). This effectiveness was calculated based on the diameter category. One likely reason for this behaviour is the very modest quantity of soursop leaf extract included in the nanoemulgel formulation.

Table 7. Antibacterial Test Results of Soursop Leaf Extract dan Nanoemulgel

Bacteria	Inhibition zone (mm)			Inhibition zone (mm)		
	Extract	Control (+)	Control (-)	Nanoemulgel	Control (+)	Control (-)
P. acnes	13	15	0	9.67 ± 0.47	16.33 ± 0.94	0
S. aureus	11	17	0	7.33 ± 0.47	12.33 ± 0.94	0
S. epidermidis	10	17	0	5.67 ± 0.82	10.33 ± 0.47	0

CONCLUSIONS

It was determined that the best composition of the nanoemulsion was 10.86% VCO, 67.33% Tween 80, and 21.81% PEG 400. This was accomplished via the use of the simplex lattice design approach. This has been shown to be the most effective composition. During the testing of the nanoemulsion, the following results were obtained: a transmittance value of 91.97%, an emulsification duration of 52.76 seconds, a pH of 5.67, a zeta potential of -39.13 mV, a particle size of 229.47 nm, and a PDI of 0.42. These are the findings that were achieved. Following the completion of the assessment of the nanoemulgel's physical characteristics, a nanoemulgel that was homogenous was created. One of the characteristics of this nanoemulgel was that it had a pH value of 5.83±0.24, a dispersion power of 5.57±0.25 cm, adhesion of 3.80±0.25 seconds, viscosity of 11479.33±167.49 cP, and medium category antibacterial activity against P. acnes, S. aureus, and S. epidermidis.

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