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RESEARCH ARTICLE

Pineapple fruit extract (*Ananas comosus* L. Merr) as an antioxidant and anti-acne agent made with the nano-emulsion gel delivery system

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Abstract

Background: Pineapple (*Ananas comosus* L. Merr) contains a lot of fibre, minerals, and vitamins. Consequently, it has anti-inflammatory, antibacterial, antioxidant, and antidiabetic benefits. **Objective:** The purpose of this research was to produce pineapple extracts in the form of nano-emulsion gel for more effective use as an antioxidant and anti-acne. **Method:** The extraction was done by maceration. The resulting extracts were formulated with tween 20 and propylene glycol, while kollisolv PYR served as the oil phase. A nano-emulsion was then formed and formulated into a gel. **Result:** In this study, the nano-emulsion gel produced was transparent yellowish-white, slightly viscous, with a characteristic odour, a particle size of 10.8 nm ± 0.5, a polydispersity index of 0.239 Đ ± 0.1 and a zeta potential of -33.6 mV ± 1.3. The ability of this nano-emulsion gel to spread was good, 10.28 cm - 14.48 cm, with 1.31 seconds of adhesion and the result of the viscosity test of 3667.4 ± 106.03 cP. In the antioxidant test, the %RSA was produced in the range of 17.70 - 70.32% and the IC_{50} value was 0.1 µg/mL so it was included in the category of very strong antioxidant. While the anti-acne test has an average bacterial inhibition zone of 10.5 mm ± 0.1 which is in the category of strong anti-acne. **Conclusion:** It was concluded that pineapple fruit extract with a nano-emulsion gel base can produce good nanoparticle preparations, which have very strong antioxidant and anti-acne activities.

Introduction

The COVID-19 pandemic requires people to work from home so the skin becomes more exposed to blue light from laptops or smartphones which may cause various skin problems, including facial wrinkles, hyperpigmentation, and premature ageing (Arjmandi *et al*., 2018). However, to protect the skin from exposure to sunlight which contains free radicals and blue light, antioxidants are needed to stabilise and prevent inflammation and acne (Rahmatullah *et al*., 2019).

Pineapple (*Ananas Comosus* L. Merr) is a fruit which is widely consumed in various forms. The fruit is free of cholesterol and fat and low in sodium and calories. In addition, pineapple fruit also contains many benefits including anti-inflammatory, antibacterial, antioxidant, and antidiabetic (Das *et al*., 2019; Kargutkar & Brijesh, 2017). It is also widely used in the cosmetic treatment of acne and ageing; and is often used as a sunscreen (Ajagun-Ogunleye & Ebuehi, 2020; Freitas *et al*., 2015).

The dermatological and cosmetic uses of nanoparticles have also been studied extensively in recent years (Contri *et al*., 2015). The preparation was formulated into a stable nano-emulsion in food, drug, cosmetics, and skin care products (Sungpud *et al*., 2020). Some of

the advantages of nano-emulsion-based processes include surface area enlargement, good stability, skin irritation reduction, anti-degradation, and drug delivery enhancement at the intracellular level (Vinardell & Mitjans, 2015). Extracts from pineapple (*Ananas comosus* L. Merr) have been widely studied, for their uses as sunscreens (Freitas *et al*., 2015), and body lotion (Rahmatullah *et al*., 2019).

This research was conducted to develop formulations and dosage forms to increase the effectiveness in use and make resulting products more innovative. This nano-emulsion gel dosage form containing pineapple fruit extract was selected based on the gel's typical role in increasing the bioavailability and optimal absorption of the extracts. Also, the extracts are easy to apply and are expected to be able to optimise their therapeutic effect.

Methods

Materials

The ingredients used are pineapple (*Ananas comosus* L. Merr), kollisolv pyr, tween 20, propylene glycol, carbopol, methyl paraben, propyl paraben, triethanolamine (TEA), aquadest, ethanol 96%, 2.2-diphenyl-1-picrylhydrazyl (DPPH) powder, methanol, *Staphylococcus aureus* ATCC 25923, 0.9% of NaCl solution, chloramphenicol preparations (for positive control), NA media (nutrient agar), and NB media (nutrient broth).

Pineapple extracts

The pineapples were cleaned, cut, mashed and then dried using a cabinet dryer at a temperature of 50℃. A total of 200 g of dried and mashed pineapple was produced. Simplicia was extracted with two litres of 96% ethanol for 120 hours. The product was then filtered, after which the ethanol was removed with a rotary evaporator and a water bath for three days.

Gel nano-emulsion formulation

Nano-emulsion was made by mixing pineapple extract, tween 20 and kollisolv pyr with an ultrasonic homogeniser. Thereafter, propylene glycol was mixed and then homogenised again. The gel base was made by mixing carbopol into distilled water until it expanded. Then, methyl and propyl paraben were dissolved with warmed glycerine, then the mixture was homogenised. Following this stage, nano-emulsion of pineapple extract and triethanolamine (TEA) were gradually added, until the desired gel base was formed and desired gel thickness achieved.

Organoleptic and homogeneity test

The organoleptic test was done by examining the physical characteristics of the nano-emulsion gel which includes the colour, shape, and smell. Then the homogeneity test was carried out using two slides and the sample was placed on one of them evenly.

Determination of particle size and zeta potential

The clear solution samples were analysed using a particle size analyser. Then the zeta potential was analysed using a zeta sizer.

pH test

The pH levels of the nano-emulsion gel were determined using a pH meter.

Spreadability test

It was carried out using 0.5 g of the preparation which was placed on a glass base with a scale and then covered with a cover slip whose weight was known and left for one minute. Followed by the addition of a load of 50 g, 100 g, 200 g, 500 g, 1 kg, and 2 kg and let to stand for another one minute, after which the constant diameter was measured.

Adhesion test

It was carried out using 0.05 g of the sample which was placed between two glasses of objects on the adhesion test equipment, then pressed a load of one kg for five minutes then lifted and given a load of 100 g on the tool, recording the release time of the nano-emulsion gel.

Viscosity test

It was carried out using a Brookfield viscometer by immersing the spindle on the viscometer in 100 g of the preparation that had been put in a glass beaker and at the appropriate speed.

Antioxidant test

A solution of 0.068 mM 2.2-diphenyl-1-picrylhydrazyl (DPPH) was made and dissolved in a volumetric flask with methanol to 100 mL. Pineapple extracts with concentrations of 300 ppm, 600 ppm, 900 ppm, 1200 ppm, and 1500 ppm (3mg/10mL, 6mg/10mL, 9mg/10mL, 12mg/10mL, and 15mg/10mL) were dissolved using methanol. A total of 2 mL of each concentration was taken and put into a test tube. Then,

2 mL of 0.068 mM DPPH was added to each test tube. The mixture was incubated for one hour in a dark room, homogenised and the absorbance was measured using a spectrophotometer at a wavelength of 514.7 nm. Then the %RSA (Radical Scavenging Activity) and IC_{50} (Inhibition Concentration 50%) were calculated.

Anti-acne test

Anti-acne activity test was carried out by the diffusion method. Diffusion began by transferring the bacterial suspension to a petri dish medium (nutrient agar and nutrient broth) levelled on the surface with a sterile spreader. Blank discs, antibiotic discs, and discs containing pineapple extract were used. The disc was placed on the surface of the media. The paper discs were arranged and spaced from one another. The petri plates were incubated at 36°C for 24 hours. After this, the results were observed using a Scan-500 tool to automatically measure the inhibition zones formed in the petri dishes.

Results

Sample preparation

The preparation of pineapple fruit extract using the maceration technique obtained a thick extract weighing 95.8425 g. The yield of the extract was calculated by finding the percentage by weight (w/w) of the final extract and dried pineapple fruit powder for maceration. The extract yield calculation data is as follows:

$$\text{Yields} = \frac{\textit{Thick extract weight}}{\textit{Initial weight of simplicia}} \times 100\%$$

$$\text{Yields} = \frac{95{,}8425\ grams}{200\ grams} \times 100\%$$

Yields = 47.8 %

Preparation and formulation of nano-emulsion gel from pineapple fruit extract

Organoleptic test results and homogeneity

Formula 1 has a thick consistency, transparent yellowish-white colour, with a distinctive odour and was homogeneous. Formulas 2 and 3 had slightly thick consistencies, transparent yellowish-white colour, with a distinctive odour and were homogeneous.

Particle size test results, polydispersity index and zeta potential

These tests were carried out in triplicates. The resulting particle size of the sample was 10.8 nm ± 0.5 with a polydispersity index of 0.239 Đ ± 0.1, and a zeta potential of -33.6 mV ± 1.3 (see Table I). From the results obtained, the nano-emulsion gel had good particle size values, which ranged between 10-200 nm (Mathialagan *et al*., 2020), while the good polydispersity value is less than 0.5. Values closer to 0 indicate more homogeneous particle size distributions and a stable nano-emulsion formula (Suciati *et al*., 2014). For a good zeta potential, nanoparticle preparations with negative values higher than -30 mV or positive values higher than +30 mV tend to have higher stability (Laxmi *et al*., 2015; Silva *et al*., 2012).

Table I: Particle size test results, polydispersity index and zeta potential

Parameters	Results	Standard
Particle size	10.8 ± 0.5 nm	10-200 nm
Polydispersity index	0.239 ± 0.1	<0.5
Zeta potential	-33.6 ± 1.3 mV	< -30 mV or > +30 mV

The results of the test of the dispersive power of the pineapple fruit extract nano-emulsion gel.

pH test results

Tests were carried out in triplicate. The pH test results from formula 1 were 4.5 ± 0.005, formula 2 was 5.1 ± 0.071 and formula 3 was 5.1 ± 0.013. The results showed all the formulas met the criteria for normal skin pH (4.5-6.5) (Mulia *et al*., 2018).

Spreadability test results

The results of this test indicate that the nano-emulsion gel has a high spreadability (Table II). The higher the dispersion value, the better the consistency of the gel, and the good release of the active substance (Forestryana *et al*., 2020).

Table II: Spreadability test results

Formula	Load weight						
	Cover glass	50 g	100 g	200 g	500 g	1 kg	2 kg
1	9.15 cm	11.28 cm	12.80 cm	14.15 cm	14.85 cm	15.73 cm	16.08 cm
2	11.00 cm	12.95 cm	14.50 cm	14.65 cm	14.98 cm	15.35 cm	16.75 cm
3	10.28 cm	12.13 cm	13.35 cm	13.48 cm	13.85 cm	14.38 cm	14.48 cm

Adhesion test results

The longer the gel is attached to the skin, the more active the substances absorbed and therefore it provides an optimal therapeutic effect (Ariyani & Wulandari, 2020). As shown in Table III, it can be seen the results of the adhesion test show that the preparation has good adhesion.

Table III: Adhesion test results

Formula	1	2	3
Result (seconds)	1.41	1.38	1.31

Viscosity test results

Prepared gels that appear too soft or too thick are characterised as "*a bad gel*". The expected viscosity value of the gel base is 500-10000 cP (Nurahmanto *et al*., 2017). During this test, the Brookfield Viscometer with spindle number 64 and a speed of 100 rpm was used. The results are shown in Table IV.

Table IV: Viscosity test results

Formula	1	2	3
Results (cP)	4143.8 ± 137.17	3792.2 ± 56.67	3667.4 ± 106.03

Antioxidant test results

The antioxidant activity of the pineapple extracts was determined. The concentration values; 300 ppm, 600 ppm, 900 ppm, 1200 ppm, and 1500 ppm had %RSA values which were 17.7% ± 0.11; 32.9% ± 0.00; 48.3% ± 0.02; 58.4% ± 0.02; and 70.3% ± 0.06 respectively (Table V), with a linear regression equation of y = 435.64x + 6.3197 and obtained R^2 of 0.9927 (Figure 1).

The IC_{50} value produced from pineapple fruit extract was 0.1 µg/mL, hence, it is categorised as a very strong antioxidant at a value of <50 µg/mL (Putri & Hidajati, 2015) (Figure 2).

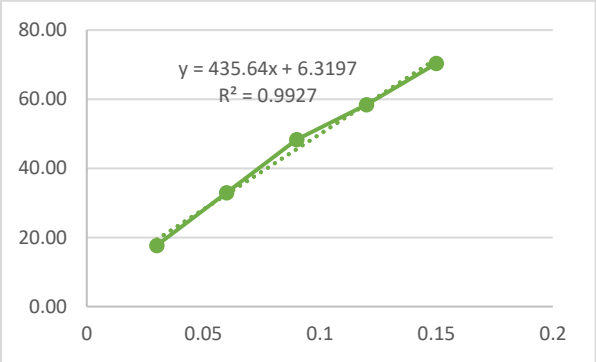
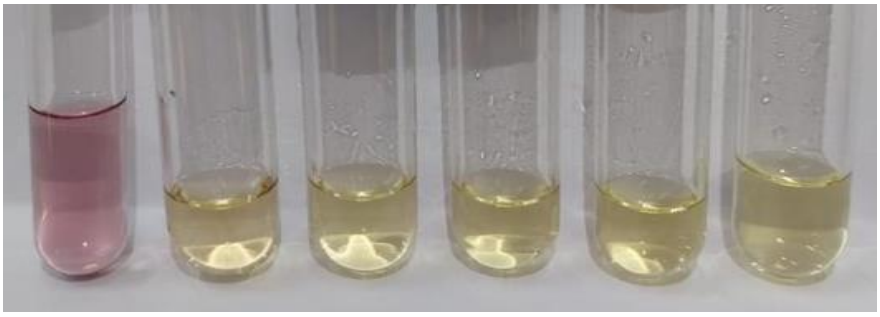


Figure 1: Graph of antioxidant activity of pineapple extract

Table V: Percentage of radical scavenging activity (RSA)

Concentration (µg/mL)	Replication			% Radical scavenging activity (RSA)			Average % RSA	SD
	I	II	III	I	II	III		
Blank	-	0.4102	-	-	-	-	-	-
0.03	0.338	0.3377	0.3371	17.60	17.67	17.82	17.70	0.11
0.06	0.2751	0.2751	0.2751	32.94	48.29	32.94	32.94	0.00
0.09	0.2121	0.2121	0.212	48.29	48.29	48.32	48.30	0.02
0.12	0.1707	0.1707	0.1708	58.39	58.39	58.36	58.38	0.02
0.15	0.1216	0.122	0.1216	70.36	70.26	70.36	70.32	0.06



(blank, extract 300 ppm, extract 600 ppm, extract 900 ppm, extract 1200 ppm, and extract 1500 ppm)

Figure 2: Antioxidant test results with DPPH extract of pineapple (*Ananas comosus* L. Merr)

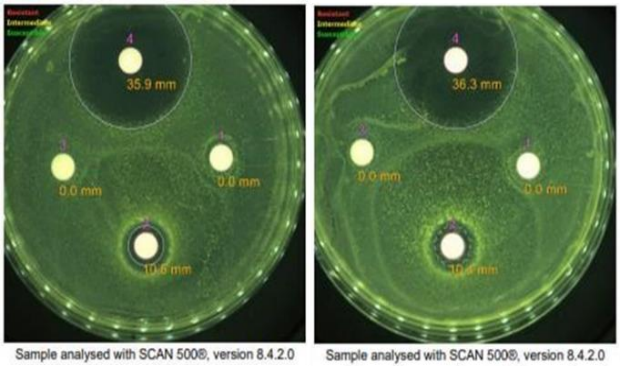
Anti-acne test results

The anti-acne activity was seen from the average results of the inhibition zone against *Staphylococcus aureus* formed in pineapple fruit extract in one-fold dilution, (10.5 mm ± 0.1) while at the ten-fold dilution, there was no inhibition zone as seen in Table VI.

Table VI: Anti-acne test results

Parameters	Inhibition zone (mm)		Average	SD
Replication	1	2		
Positive control	35.9	36.3	36.1	0.3
Negative control	0.0	0.0	0.0	0.0
P1x	10.5	10.4	10.5	0.1
P10x	0.0	0.0	0.0	0.0

The average results of the inhibition zone found in the positive control (36.1 mm ± 0.3), negative control (0.0 mm ± 0.0), P1x (10.5 mm ± 0.1) and P10x (0.0 mm ± 0.0). These results indicate that pineapple fruit extract has anti-acne effectiveness which is categorised as "***strong***" because it has an inhibitory power in the range of 10-20 mm (Trisia *et al*., 2018) (Figure 3).



a b

(a) replication of 1 inhibitory zone positive control (chloramphenicol) and extracts P1x, P10x, and negative control (sterile distilled water); (b) replication of 2 inhibitory zones positive control (chloramphenicol) and extracts P1x, P10x, and negative control (sterile distilled water).

Figure 3: Results of the inhibition zone

Discussion

Pureed pineapple Simplicia has the form of fine powder with a brownish colour. A thick black-green extract weighing 95.8425 grams with a distinctive pineapple aroma is obtained from 200 grams of pineapple Simplicia. The extract yields 47.8%. The extract was free from 96% ethanol, as indicated by the absence of the characteristic ester odour of alcohol in the esterification test. The pineapple fruit extract is an active ingredient used in the manufacture of gel-nanoemulsion. Tween 20 acts as a surfactant, kollisolv pyr as a co-surfactant, and carbopol as a gelling agent. Carbopol can also be used as a gelling agent in pineapple extract gel-nanoemulsion preparations because it is a potent gelling agent and produces a more attractive appearance than the gelling agent.

Particle size testing is crucial for nanoemulsion preparations; it ensures the preparation size has entered the appropriate range. The nanoparticle size will also increase the penetration of the preparation into the skin (Jivani, Patel & Prajapati, 2018). In addition, the value of the polydispersity index is also significant for describing the distribution of particles (Taurina *et al.,* 2017). Another critical parameter is the potential zeta value, which depicts the electrical properties of the particle. Potential zeta is very influential on the stability of the preparation because it affects the attraction interaction between particles. So, if the potential zeta value is higher, it can prevent flocculation (Juliantoni, Hajrin & Subaidah, 2020). The pH test results showed that all formulas met the standard skin pH criteria with good absorption of active substances and stability; thus, they could be applied to the skin safely (Mulia, Ramadhan & Krisanti, 2018). The gel consistency is related to viscosity, adhesion, and spreadability. All formulas show that the response of gel viscosity is directly proportional to the spreadability. The lower the viscosity value, the higher the spreadability value. The spreadability of the gel is related to the comfort of use. The soft gel consistency causes the gel to be more evenly distributed; it is easily absorbed into the skin and has a softer impression on the skin compared to stiff gels.

DPPH is a fast and sensitive method for assessing the antioxidant capacity of plant extracts due to the colour change of DPPH in the presence of antioxidants in the test sample. The colour changes from purple to yellow indicates the strong antioxidant capacity of the sample (Vora, Srivastava & Modi, 2018). The IC_{50} value of each extract is determined by applying an extract dose that can reduce the intensity of free radical absorption by 50% (Hidayati *et al.,* 2017). The IC_{50} value produced from the pineapple fruit extract of 0.1 µg/mL is considered a potent antioxidant because it is <50 µg/mL (Putri, 2015). The results of the anti-acne test showed that pineapple fruit extract has anti-acne effectiveness, which is categorised as vital because it has an inhibitory power in the range of 10-20 mm (Trisia *et al.,* 2018). Bromelain, which is contained in pineapple fruit extract, has many pharmacological activities, including anticoagulant, antimicrobial, anti-cancer, antioxidant, anti-inflammatory, and more. It

also exhibits antimicrobial activity against P. acnes bacteria by interfering with the peptidoglycan and polysaccharide components of the bacterial cell membrane, which causes instability in the electrostatic membrane force. In addition, bromelain denatures membrane proteins by forming complex compounds, disrupting their chemical structure and causing protein coagulation and lysis of cell membranes.

Conclusion

The obtained pineapple fruit extract nano-emulsion gel has the characteristics of a good nano-emulsion gel. In addition, the pineapple fruit extract has a very high antioxidant content with an IC_{50} value of 0.1 µg/mL and has a very strong anti-acne activity with an average bacterial inhibition zone of 10.5 mm ± 0.1. this is because the pineapple fruit contains a lot of bromelain compounds. Thus, the nano-emulsion gel of pineapple extract can be used as an antioxidant and anti-acne.

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Conflict of interest

There is no conflict of interest associated with the present study.

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