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RESEARCH ARTICLE

Development of Anti Acne Cream (w/o/w Multiple Emulsion) Containing Green Tea Leaf Waste

Naniek Widyaningrum [1,*]
[1]Pharmacy program, Medicine Faculty, Sultan Agung Islamic University
[*] Corresponding author email: naniek@unissula.ac.id

ABSTRAK

Latar belakang: Ampas dari seduhan daun teh hijau hingga saat ini belum dimanfaatkan, ampas tersebut masih dianggap sebagai limbah yang tidak berguna. Penelitian sebelumnya ditemukan ampas daun teh hijau masih memiliki kandungan EGCG yang cukup tinggi. EGCG memiliki manfaat sebagai antibakteri. Pemanfaatan limbah ampas teh hijau dalam formulasi hingga diversifikasi produk multiple emulsion w/o/w hingga saat ini belum pernah dilaporkan. **Tujuan:** mendapatkan dosis dan formula limbah ampas daun teh hijau dalam sediaan krim *multiple emulsion* w/o/w yang efektif pada *Propionibacterium acnes* (P.acnes) bakteri penyebab jerawat.
Metode: Tahap satu pada penelitian ini yaitu penyarian limbah ampas daun teh hijau dilakukan dengan metode infundasi dan fraksinasi menggunakan etil asetat. Tahap kedua yaitu uji aktivitas anti bakteri fraksi etil asetat ekstrak ampas daun teh hijau pada bakteri P.acnes dengan konsentrasi 1% hingga 6%. Tahap ketiga yaitu uji sifat fisik formulasi dengan *multiple emulsion* w/o/w.
Hasil: Konsentrasi fraksi etil asetat ekstrak daun teh hijau 6% merupakan konsentrasi optimum dalam menghambat pertumbuhan bakteri P.acnes dengan zona hambat 32,6 mm ±0,57. Formula sediaan *multiple emulsion* w/o/w dengan zat aktif daun teh hijau 6% memiliki sifat fisik yang baik dengan luas daya sebar 90,4 cm2± 0,03, pH 5,00 ± 0,02, dan analisis mikroskopik menunjukkan adanya *multiple emulsion* w/o/w.
Kesimpulan: Konsentrasi 6% ampas daun teh hijau dalam formula *multiple emulsion* memiliki sifat fisik dan aktivitas antibakteri yang baik sehingga dapat digunakan sebagai acuan formulasi. Perlu dilakukan uji stabilitas formula multiple emulsion.

Kata kunci : Multiple emulsion W/O/W, ampas daun teh hijau, Anti jerawat, P.acnes

ABSTRACT

Background: *To date, green tea leaf waste is not well utilized, the waste is remain considered as a waste. Previous studies found that green tea leaf waste still have a fairly high EGCG content. EGCG has benefits as an antibacterial. Utilization of green tea leaf waste started from formulation until the diversification of multiple emulsion w/o/w products has not been reported.* ***Objective:*** *To develop anti acne cream (W/O/W) multiple emulsion containing green tea leaf waste and evaluate its antibacterial activity against acne-inducing bacteria of Propionibacterium acnes (P.acnes).*
Methods: *Phase one of this research was green tea leaves was extracted by infundation and fractionation method using ethyl acetate and prepared in different concentration (1% to 6%). The second stage was ethyl acetate fraction of green tea leaf extract was tested for its antibacterial activity against P.acnes. Third stage was tested physical properties of formulation with w/o/w multiple emulsion.*
Results: *The optimal antibacterial activity of ethyl acetate fraction of green tea leaf extract against P.acne was at the concentration of 6%, with inhibition zone of 32.6 mm ±0.57. The formula of w/o/w multiple emulsion loaded with green tea leaves waste of 6% active substance demonstrated a good physical properties which can spread 90.4 cm2 ± 0.03, pH 5.00 ± 0.02 and microscopic analysis showed multiple emulsion w/o/w.*
Conclusion: *The concentration of 6% green tea leaf waste formulated in multiple emulsion had a good physical and antibacterial activity for a referred standard. It is necessary to test the stability of multiple emulsion formula.*

Keywords: *W/O/W Multiple emulsion, green tea leaf waste, Anti-acne, P.acnes*

INTRODUCTION

Epigallocatechin gallate (EGCG) in green tea leaf has been shown to have antibacterial activity. EGCG can also kill antibiotic resistant Acinetobacter baumani. A study by Widyaningrum et al. (2012) showed that green tea leaf ethanolic extract in a standard formula of 7% cream preparation has the same efficacy as Ristra acne cream® in inhibiting the activity of Staphylococcus aureus (S. aureus) bacteria. Widyaningrum et al. (2015a) also stated that the ethyl acetate fraction of green tea leaves extract of 1% to 6% concentration has antibacterial activity against P. acnes and Staphylococcus epidermidis (S. epidermidis), and 6% concentration is the recommended concentration

as anti-acne dose.

Green tea leaf waste has not been utilized. Based on preliminary studies, green tea leaf waste still contain EGCG although not as much as the first processed green leaf tea (Widyaningrum, 2017). Waste based product are expected to reduce the cost of production to benefit the middle society without reducing its quality and effectiveness.

EGCG has been shown to have unstable properties, easily decomposable by heat. Therefore, development of multiple emulsion formulation is needed to maintain EGCG stability in cream products. EGCG properties are polar, so the multiple emulsion used is water in oil in water (W/O/W) so that EGCG can be soluble in the active water phase and can be protected by the oil phase. The preparations were made comfortable and not sticky because the water phase is in the outer form. To develop an effective emulsion cream formulation as an antibacterial treatment and physical properties of green tea waste multiple emulsion were evaluated against P. acnes to meet the standard requirements.

METHODS

a. Extraction

Green tea leaf waste obtained from PT. Sari Kemuning, the highest EGCG producing source in tea plantation in Central Java (Widyaningrum, et al., 2015c) with 30 minutes stew processing, the waste is then extraction and fractionation. The green tea leaf was extracted by infundation method using distilled water at the temperature of 90oC for 30 minutes, then extreme cold and add buffer solution to pH 4 (Widyaningrum, et al., 2015a).

b. Fractionation

Performed by adding 1: 1 ethyl acetate in separating funnel, then the water phase was discarded and its ethyl acetate phase was evaporated with rotary evaporator until it became dry powder (Widyaningrum, et al., 2015a).

c. Antibacterial activity

P.acnes was incubated in a blood agar medium for 72 hours in order to make the blood under anaerobic conditions, the bacterial suspension using 0.9% sterile NaCl. The cell adherence was synchronized with Mc.Farland 0.5 standard. The sterile lid foam was inserted into a tube containing bacteria, and then pressed. Press on the wall of the tube to keep it from getting too wet. The cotton was applied to a blood agar medium that had been incubated for about 2 hours until flattened, then made wells on the medium using sterile iron. The medium was filled with ethyl acetate fraction of green tea leaf extract of 1% to 6% concentration, then incubated at 37°C for 72 hours under anaerobic condition (Niyomkam, et al., 2010).

d. Formulation

The cream preparation formula used in this study was based on the formula proposed by Mahmood, et al. (2014) which is modified as follows:

Primary emulsion (W/O)	
Paraffin oil	24
Cetyl dimethicone copolyol	4.25
Ethyl acetate fraction of green tea leaf extracts	6
Magnesium sulfate	0.7
Distilled water	100
Multiple emulsion (W/O/W)	
Primary emulsion	80
Polyoxyethylene (20) cetyl ether	3.75
Cetomacrogol 1000®	2.5
Hydroxypropyl methylcellulose (HPMC)	1.25
Distilled water	100

Two emulsification steps are used. The first step is making of primary emulsion by mixing paraffin oil with cetyl dimethicone copolyol at 75oC, then added ethyl acetate fraction of green tea leaf extract and magnesium sulfate using stirrer with 2000 rpm stirring speed for 15 minutes, followed by 1000 rpm for 10 minutes, continued 500 rpm for 10 minutes. The second step was mixing Polyoxyethylene (20) cetyl ether, Cetomacrogol 1000®, Hydroxypropyl methylcellulose (HPMC) and distilled water at 700 rpm stirring speed.

a. Physical properties test

Spreading ability

Creams weighing 0.5 grams were placed in the middle of a round glass, covered with another glass and left for 1 minute after it added a load of 1 kilogram and left 1 minute later measured the diameter of the spread then measured the area of cream spread by using the formula of the area of the circle (Widyaningrum, 2015b). The test was done 3x replication.

PH measurement test

The pH measurements were performed using a calibrated pH meter with equimolar buffer pH 7 and a potassium hydroxy phthalate buffer pH 4. The electrodes were dipped into a dilute extract mixture with aquades (one part of the mixture was diluted with 9 parts of aquades) and then pH was observed. (Widyaningrum, 2015b).

Microscopic analysis

Microscopic analysis was conducted by using

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Table 1. Inhibition zone of P.acnes bacteria growth

Replication	Inhibition power zone (mm)							
	1%	2%	3%	4%	5%	6%	D water control	Tetracycline control
1	19.2	26	31	31	332	33	0	45
2	19.3	26	31	32	32	32	0	45
3	19.1	25.5	31	31,5	33	33	0	45
Mean ±SD	19.2 ± 0.1	25.83±0.28	31±0	31.5±0.5	32.33±0.57	32.6± 0.57	0±0	45±0

optical microscope with camera (DCM-35 USB 2.0 and minisse image software). Observation was done using 100X magnification.

RESULTS

a. Inhibitory activity of green tea leaf waste against the growth of P.acnes

The antibacterial activity test measured the effectiveness was effective on the material used, basically the higher the concentration the greater the potential of the inhibition. However, the pure natural material does not allow to make a product, it needs the supporting materials/formula so that the effective concentration of the main ingredient or active substance required.

Based on Table 1, the concentration of 6% has a diameter of inhibition (32.6 mm ± 0.57) greater than the 1-5% concentration, but lower than tetracycline positive control.

b. Spreading ability

Spreading ability measured whether a product is easy to be applied to the surface of the skin or not. The bigger the spreading ability the better, but there is a certain limit. The resultant power result is presented in table 2.

Table 2. Spreading ability of multiple emulsion w/o/w

Replication	Area of spreading (cm^2)
1	88.0 ± 0,07
2	90,0 ± 0,04
3	89,0 ± 0,05
mean ± SD	89,0 ± 1,00
positive control (Hazeline®)	90,4 ± 0,03

Based on the spreading result, the w/o/w multiple emulsion showed no significant different in area of dispersion compared to the positive control (P=0.136).

c. pH

Table 3. pH of w/o/w multiple emulsion

Replication	pH
1	5.02 ± 0.01
2	4.98 ± 0.05
3	5.00 ± 0.02
Mean ± SD	5.00 ± 0.02
positive control (Mahmood *et al.*, 2014)	5.04 ± 0.05

Based on the pH, the emulsion cream of green tea leaf emulsion yielded pH 5.00±0.02 according to the positive control of Mahmood, et al (2014) study of 5.04±0.05. Asian skin pH is 4.5-6.5, so the preparation with pH 5 meets the requirements of a topical preparation for the face.

d. Microscope Analysis

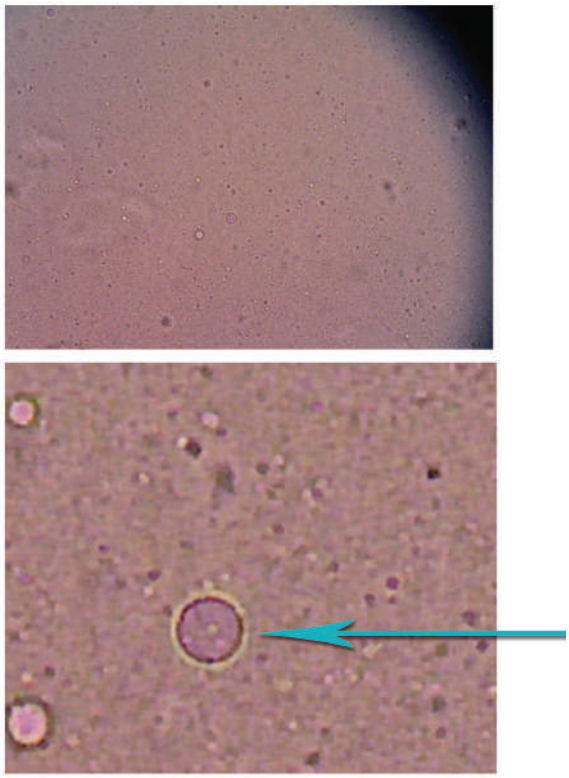

Figure 1. Particle of multiple emulsion of green tea leaf formula

Based on Figure 1, the particle size forms a multiple emulsion consisting of a water-in-oil emulsion layer (W/O/W).

DISCUSSION

P. acnes could not live on aerobic conditions while the dilution medium contains more oxygen in its medium. Its base and cream preparations are also known not to be performed on the dilution method. Therefore, the diffusion method was used. The study of Stratton et al. (2000) showed that epigallocatechin gallate (EGCG) concentration of 10% can cause erythema in the test animals after 5 days and 7% concentration may cause erythema by day 15, while the lower concentrations caused no toxicity within 30 days. Moreover, the antibacterial activity of ethyl acetate fraction of green tea leaf waste extract at a 6 different concentrations (1%, 2%, 3%, 4%, 5%, 6%) were tested against P. acnes and S. epidermidis. The result showed that the higher concentration of ethyl acetate fraction of green tea leaf extract, the higher inhibitory effect against to P. acnes bacteria. Ethyl acetate fraction of green tea leaf extract at the concentration of 6% had the greatest inhibitory activity compared to that of 1% to 5% (32.6 ± 0.57 mm versus 19.2 ± 0.1 to 32.33 ± 0.57 respectively). However, the concentration of 6% as the dosage was selected, although the inhibitory activity was not as positive as control. This could happen because the EGCG in the green tea leaf pulp was lower than that of pure green tea (not the pulp), so the EGCG lower results in lower bacterial activity

Research by Widyaningrum et al. (2017) showed that EGCG levels in green tea leaf pulp are 21.325% w/w while EGCG levels in green tea leaf are 60.98% w/w (Widyaningrum et al., 2015a). This indicates that the concentration of EGCG in green tea leaf is greater than that of in its waste.

Compared with the study of Bashir et al. (2014), green tea leaf originating from Indonesia, Camellia sinensis species have antibacterial activity against bacteria K. pneumoniae, S. pyogenes, S. aureus, S.marcesscens, P.aeruginosa and E. coli, with a large resistance of 3.33 ± 1.52; 5.0 ± 0.0; 0.0 ± 0.0; 9 ± 4.58; 1.33 ± 1.15 and 1.0 ± 0.0 on 100% methanolic extract of green tea leaf. This showed that ethyl acetate fraction of green tea leaf extract in this study has better inhibitory activity against P. acnes.

The tested multiple-cellular formula has a corresponding positive scalability, has a pH corresponding to the skin (4.5-6.5) and microscopic analysis indicating the presence of multiple emulsion. This indicates that the formula used is compatible with the green tea leaf active ingredient. The development of multiple emulsion formulation is expected to maintain the stability of EGCG in the formula. It is necessary to stabilize the multiple emulsion cream formula W/O/W ethyl acetate fraction of green tea leaf extract.

CONCLUSIONS

The fraction of ethyl acetate extract of green tea leaf waste at concentration of 6% can be used as dose of anti-acne cream, in the development of multiple emulsion formulation, the active ingredient of green tea leaf concentration of 6% is compatible with the formula used in this study.

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