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DEVELOPMENT AND EVALUATION OF ANTI-ACNE MICROEMULSION GEL CONTAINING EXTRACTS OF MANGOSTEEN FRUIT RIND

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INTRODUCTION

Acne vulgaris is one of the most common skin disorders which can result in comedos or severe inflammatory lesions in the face, back and chest with a large number of sebaceous follicles[1]. The pathogenesis of acne is complex but dependent on four key factors including androgen-mediated stimulation of sebaceous gland activity, follicular hyperkeratinization, colonization of the bacterium *Propionibacterium* acnes, and inflammation[2-3]. *Staphylococcus epidermidis (S. epidermidis)* and *Propionibacterium acnes (P. acnes)* have been recognized as major skin bacteria that cause the formation of acne comedos[1]. Over the last decade, antibiotics have been used to treat acne vulgaris, however, there are problems in increasing resistance to existing anti-microbial agents, side effects and sometimes high cost of treatment[4-5]. To overcome the problem of antibiotic resistance, medicinal plants have been progressively studied as alternative treatments for diseases[6].

Mangosteen (*Garcinia mangostana* L.) is a medicinal plant and its ethanolic fruit peel extracts have potential for inhibiting acne - causing bacteria[7]. The major xanthone in mangosteen fruit rind is mangostin which exhibits medicinal benefits. α-mangostin was report to possess a strong inhibitory effect against *P. acnes* and *S. epidermidis* which is common pus-forming bacteria which present in acne lesions[8].

Microemulsions offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, improved drug solubilization of lipophilic drugs and bioavailability. Microemulsions were reviewed for several applications, such as topical, oral, parenteral use and cosmetics. Concerning dermal application, microemulsions increase the transdermal permeation of drugs by acting as a penetration enhancer[9]. Microemulsion of azelaic acid used in many skin disorders, prepared using the monosodium salts have been evaluated. The results suggested that azelaic sodium microemulsions could be used to optimize drug targeting in acne treatment[10]. Thus, the aim of this study was to develop anti-acne microemulsion gel containing extracts of mangosteen fruit rind and the physicochemical properties and chemical stability were evaluated.

MATERIALS AND METHODS

Crude extract preparation

Mangosteen fruits (*Garcinia mangostana* L.) were purchased from a local grocery store (Bangkok, Thailand). The fresh fruit peels were chopped into small pieces and extracted with 95% ethanol for three days, three times, at room temperature. The filtrates were pooled and concentrated by a rotary evaporator at 40°C. The obtained semisolid extracts were lyophilized and then kept in a desiccator at 4°C until further used.

Phase diagram studies

The ingredients used in formulations were either isopropyl myristate (IPM) or soybean oil as oil phase, tween 80, span 80, and brij 35 as the surfactant, propylene glycol, polyethylene glycol 400 (PEG 400), glycerin, ethanol 95% v/v, and propan-2-diol as the cosurfactant and distilled water as an aqueous phase. To determine the composition of microemulsions, pseudoternary phase diagrams were constructed keeping the ratios of surfactant/cosurfactant i.e., 1:0.5 and 1:0.25 w/w and varying the remaining 2 components. After each mixing the sample was allowed to settle and its physical condition i.e., color, clarity and flowability was reviewed. Clear and transparent formulations were indicative of a stable microemulsion, and their compositions were recorded.

Preparation of microemulsion gels

Based on the phase diagram, four different formulas were selected from the microemulsion region. Exactly 1% w/w of crude extract of mangosteen fruit rind was dissolved in the oil, surfactant, and cosurfactant mixtures, and water was added drop by drop with gentle mixing at room temperature.

Characterization and evaluation of microemulsion gel formulations:

Physicochemical characterization

All microemulsion gel formulations were evaluated for appearance (transparency, clarity). pH was determined using a pH meter (Model 210A$^+$, Thermo Orion, Germany). Viscosity measurement was

carried out using a Brookfiled Digital Rheometer (Model DV-II, Brookfield Engineering Laboratories, USA).

Quantitative analysis

The samples of about 100 mg of each microemulsion gel formulations were taken into 5-ml volumetric flasks. The samples were mixed with a mixture of methanol:distilled water (1:1, v/v) and then was made up to 5 ml with the same solvent. Five hundred microliters aliquots were taken in 5-ml volumetric flasks and then diluted and filled up volume with methanol. The crude extract content was determined by UV-spectrophotometer at 318 nm and calculated from a calibration curve.

Physical and chemical stability

The samples were evaluated for both physical (i.e., appearance, pH, viscosity) and chemical stability studies under a room temperature (30°C) storage condition. The content was determined by a UV-VIS spectroscopic method described earlier at 0, 14, 21, 28 and 42 days. The crude extract content was determined against a calibration curve. Log (crude extract remaining content, %) was plotted against time and the slopes (m) were calculated by a linear regression. The slopes (m) were then substituted into the following equation for the determination of k values:

$$k = m \times 2.303 \quad (1)$$

Shelf life values (the time for 10% loss, t_{90}) were then calculated by the following equation:

$$t_{90} = 0.105/k \quad (2)$$

RESULTS

Phase diagram studies

Isopropyl myristate is predominantly forming microemulsion with surfactant/cosurfactant in larger area of isotropic region than that of soybean oil. Microemulsion gel was performed in the system containing tween 80 greater than that of the system comprised of mixture of tween80 and span80 due to its high HLB of tween80 while brij 35 could not form microemulsion gel with soybean oil. The effect of cosurfactant shown that the extent of microemulsion formation increases in the sequence ethanol> propan-2-diol>PEG 400>propylene glycol = glycerin (data not shown). The systems which were good appearance, spreadability and flowability were selected for further study.

Preparation of microemulsion gels

The microemulsion gel system comprised of 1% w/w mangosteen crude extract, either IPM or soybean oil and appropriate type and amount of surfactant and cosurfactant with or without hydroxypropylmethylcellulose (HPMC), viscosity inducing agent are shown in Table 1. F1 and F2 were chosen in pseudoternary phase diagram regarding to high oil content of IPM while F3 and F4 containing soybean oil, long chain triglycerides. F1 and F3 were selected which represent to form microemulsion gel whereas F2 and F4 were microemulsion formed as fluid state; thus HPMC, the external viscosity inducing agent was applied to form microemulsion gel.

Table 1 The compositions of mangosteen crude extract microemulsion gel formulations (%w/w)

Ingredients	F1	F2	F3	F4
Mangosteen crude extracts	1	1	1	1
Isopropyl myristate	49	49	-	-
Soybean oil	-	-	39	29
Tween 80	16	16	30	24
Span 80	16	16	-	24
Propylene glycol	-	8	-	-
Glycerin	-	-	10	-
PEG 400	-	-	-	12
Propan-2-diol	8	-	-	-
HPMC	-	18	-	18
Distilled water q.s. to	100	100	100	100

Characterization and evaluation of microemulsion gel formulations

All formulations were optically clear, transparent and elegant in appearance. Prepared microemulsion gels were free flowing and possess better spreadability. The physicochemical properties of microemulsion gel formulations are shown in Table 2. The pH of all the formulations was found to be 5.71-6.74. F3 had almost two times higher in viscosity (1794 cps) than that of the other formulations (1030-1085 cps). A UV spectrum of α-mangostin in mangosteen crude extracts solution in methanol

showed the maximum absorption peaks at 318 nm which corresponding to the maximum wavelength obtained from standard α-mangostin. Thus, the wavelength at 318 nm was used for all measurements due to no interference from the solvent. Quantitative analysis of the mangosteen crude extratct exhibited the content of the active compound in the range of 95-110% LA (Table 2).

Table 2 The pH, viscosity and the content of microemulsion gel containing mangosteen crude extract (mean±SD, *n*=3)

Formulation	pH	Viscosity (cps)	Content (%)
F1	6.71 ± 0.32	1085 ± 67	106.96 ± 2.21
F2	6.12 ± 0.60	1030 ± 52	100.67 ± 0.56
F3	5.71 ± 0.11	1794 ± 102	96.53 ± 0.55
F4	6.74 ± 0.34	1068 ± 85	97.85 ± 0.78

Physical and chemical stability

The physical stability displayed that there was no significantly changes in appearance, pH and viscosity of all formulations after storage for 6 weeks. The chemical stability results showed that the content of the active compound in F1 and F3 was gradually decreased and was found to be 99.55 % and 94.14 %, respectively but markedly decreased in content was appeared in F2 and F4 (88.43% and 86.41%, respectively) after 42-days storage at room temperature (30°C) (Table 3). Table 4 showed the predicted shelf-life of microemulsion gel formulations containing mangesteen crude extracts storage at room temperature (30°C). With regarding to the data of shelf-life and the drug content after storage for 42 days of microemulsion gel formulation containing mangosteen crude extract, the ranking order of stability was F3> F1>F2=F4

Table 3 The chemical stability of microemulsion gel formulation containing mangosteen crude extract storage at room temperature (30°C) (mean±SD, *n*=3)

Time (days)	F1	F2	F3	F4
0	106.96 ± 2.21	100.67 ± 0.56	96.53 ± 0.55	97.85 ± 0.78
14	100.38 ± 2.09	100.17 ± 1.33	95.09 ± 0.35	96.03 ± 1.51
21	99.91 ± 2.20	97.75 ± 1.03	94.98 ± 0.68	95.23 ± 0.82
28	99.82 ± 1.35	90.30 ± 3.01	94.32 ± 0.29	90.36 ± 0.64
42	99.55 ± 0.39	88.43 ± 0.98	94.14 ± 0.32	86.41 ± 1.80

Table 4 Predicted shelf lives at 30°C of microemulsion gel formulations containing mangosteen crude extract

Formulation	K	t_{90} (days)
F1	1.34×10^{-3}	83.87 ± 28.62
F2	3.56×10^{-3}	30.08 ± 5.19
F3	6.10×10^{-4}	182.25 ± 51.15
F4	3.19×10^{-3}	33.25 ± 3.98

DISCUSSION

In fact, it is generally observed that oils having a high molar mass forms microemulsions far less readily than their low molar mass homologues. Thus, the formulation of microemulsions with long chain triglycerides is expected to be non-trivial such as this case only 40% of soybean oil forms spontaneous microemulsion gel. And also, soybean oil having long chain triglycerides characteristic, amount of surfactant and cosurfactant were needed to be adsorbed toward the oil/water rather than that of IPM. Thus, the formulation consisted of IPM might exhibit more a tendency to form globular structure than that of soybean oil which showed more microemulsion region. In addition, the small molecules of hydrophilic co-surfactant i.e., ethanol, propan-2-diol could possibly intercalate between the molecules of more hydrophilic tween 80 at the o/w interface better than those of cosurfactants. One of the conditions

necessary for microemulsion formation is the high fluidity of the interface. The interfacial fluidity and low interfacial tension can be achieved by using a proper co-surfactant or an optimum temperature. However, the amount of co-surfactant should be quantitatively considered in order to stabilize the formulations.

The pH of all formulations was suitable for topical administration which is within the range of normal healthy skin (4–6.8). The viscosity and the spreadability of the formulation were suitable for topical administration. Comparison among formulations, due to its long chain characteristics, the formulation containing soybean oil showed more stable formulation than those of IPM. The addition of viscosity inducing agent (i.e., F2 and F4) may interrupt the microemulsion system as the drug reservoir. This was possibly due to the distribution of the active substance in polymer which was closely contacted to the environment leading to drug decomposition. The optimum formulation consists of 1% crude extract of mangosteen fruit rind, 39% soybean oil, 30% tween 80, 10% glycerin, and 20% distilled water (all %w/w). The formulation was optically clear, transparent and elegant in appearance. The pH of the formulation was found to be between 5.71. It was clear from the physicochemical data of the system that the developed formulation had high viscosity (1794 cps). The content of crude extract of mangosteen fruit rind in the formulation was found to be 94.14 ± 0.32% after storage 42 days at room temperature (30°C) and the predicted shelf-life was 182.25 ± 51.15 days.

CONCLUSION

From the above results it is clear that, the physical properties of the optimum microemulsion gel formulation containing crude extracts of mangosteen fruit rind not varied to the greater extent and exhibit 6-months shelf life when stored in room temperature. However, further study should be done with in vitro permeation through membrane and in vivo study.

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