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Upcycling mangosteen peels into niosomal-entrapped extract for underarm care products

Narongrit Lahpun[1], *, Yanin Rakmitr[1] and Thitima Wannasiri[1]

[1]Cosmetic Science, Faculty of Science and Technology, Phranakhon Rajabhat University 10220, Thailand
* Corresponding author: narongrit.l@pnru.ac.th

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Abstract

People with underarms body odor often lack self-confidence. Mangosteen peel extract (MPE) has been extensively studied for its ability to reduce body odor by developing niosome product as the delivery system with slow and efficient release to upcycle food waste into cosmeceutical innovations. The MPE antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with total flavonoid content (TFC), and anti-tyrosinase activity assessed using dopachrome method. The MPE obtained by ethanol maceration showed antioxidant activity with an with IC_{50} value of 230.28 mg/L, and the flavonoid content was 4,742.59 ± 2.62 mg quantitative easing (QE) /100 g dry weight. The anti-tyrosinase activity was 67.04 mg/L. The MPE was entrapped by niosomes using the thin-film method, and the particle size was reduced with an ultrasonic bath. The ratio of niosome formula between Span 60 (SP60), cholesterol, and MPE at 1:1:0.1 giving the highest entrapment efficiency of 65.05% ± 0.01, and zeta potential value of -46.1 mV was selected for further study. The underarm care products containing niosomes passed the accelerated stability test using the heating-cooling cycle method with no phase separation. The α-mangostin release comparison between the MPE and the niosomes was carried out using Franz diffusion cells. The release profile of the niosomes was more gradual than for MPE indicating that niosomes can be utilized as an innovative ingredient for underarm care products.

Keywords: Antiperspirant, Delivery system, Mangosteen extract, Niosome, Cosmeceutical

1. Introduction

Mangosteen peels are used as a sustainable active component in cosmeceuticals to improve skin health and fulfill consumer demand for natural products. The peels are utilized in facial creams for their antioxidant and anti-aging properties. Using mangosteen peels in cosmetics reduces waste and taps into the growing market for sustainable products, potentially increasing profitability for manufacturers. However, market competition and consumer awareness impact their economic viability [1].

Some underarm health care products contain harsh chemicals as skin health hazards. Parabens prevent the growth of harmful bacteria and are often used as preservatives in cosmetics. However, they have been linked to estrogenic activity, which may increase the risk of breast cancer due to their ability to bind to DNA and induce cell proliferation in breast tissue [2]. Aluminum compounds are often found in antiperspirants but can cause skin irritation. They have also been related to endocrine disruption [3]. The potential health concerns of using underarm products have led to regulatory measures and increased public awareness resulting in safer formulations with favorable health outcomes.

Mangosteen (*Garcinia mangostana*) peels are high in bioactive components, specifically xanthones, flavonoids, and phenolic compounds with a variety of biological activities. This makes them ideal for cosmetic applications, particularly in formulations focused on skin health. Mangosteen peels contain α-mangostin as the major xanthone with antibacterial, antioxidant, and anti-inflammatory effects [4]. Compounds in the peels such as epicatechin and gartanin contribute to the antioxidant activity and improve skin protection against oxidative stress [5]. Phenolic compounds in the peels including various organic acids exhibit strong antioxidant and antibacterial activities which help to prevent body odor and maintain skin health [5].

Niosome technology provides considerable benefits for underarm products designed to improve skin health and hygiene. Niosomes utilize non-ionic surfactant vesicles to facilitate the absorption and effectiveness of active substances, making them especially useful in cosmetic formulations. Active compounds encapsulated in niosomes have various benefits, including improved epidermal penetration, controlled release, and increased solubility and stability. Niosomes enable the encapsulation of both hydrophilic and lipophilic molecules, hence enhancing the stability and solubility of active components [6]. They are also a potential solution for overcoming the complexities of cosmetic formulations which invariably include both hydrophilic and lipophilic components. Niosomes can control and sustain the release of active ingredients, thereby enhancing their bioavailability and effectiveness [7]. A long-lasting active ingredient is an ideal property of an antiperspirant because the slow release of active components prolongs the prevention of underarm malodor.

Public demand for natural extracts as underarm health care products is increasing because these products are used by socially conscious individuals to boost their confidence. Mangosteen peel extract is beneficial for the skin but a delivery mechanism using niosomes increases efficacy. This research focused on the properties and delivery profiles of mangosteen peel extract and the niosomal-entrapped extract. The accelerated stability of underarm health care formulations with niosomes was also tested to ensure the physical stability of the products.

2. Materials and methods

2.1 Chemicals

The chemicals used included: absolute ethanol (QRëC™), absolute methanol (QRëC™), absolute chloroform (VWR®), Span 60® (Namsiang Group), Span 80® (Namsiang Group), Tween 80® (Namsiang Group), cholesterol (Myskinrecipes), 2-2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich), L-ascorbic acid (Ajax Finechem), aluminum chloride hydrated (Ajax Finechem), sodium nitrite (Fluka), sodium hydroxide pellets (Ajax Finechem), quercetin ≥95% HPLC solid (Sigma-Aldrich), kojic acid (Sigma-Aldrich), 3-4-dihydroxy-L-phenylalanine ≥ 98% (Sigma-Aldrich), tyrosinase from mushroom (Sigma-Aldrich), phosphate buffer solution 0.2 M pH 6.8 (Thermo Scientific), phosphate buffer solution 0.2 M pH 7.4 (Thermo Scientific).

2.2 Plant materials

The plant materials (*Garcinia mangostana* Linn. peels) used in this study were purchased in Lan Saka district, Nakhon Si Thammarat Province, Thailand.

2.3 Preparation of mangosteen peel extracts (MPE)

The mangosteen peels were sliced into small pieces and then immersed in ethanol (95%v/v) at a 1:3 ratio for 42 hours. After maceration, the extracts were filtered using Whatman® number 4 filter papers and the solvent was evaporated using a rotary evaporator (Heidolph) at 60.5 °C to produce crude mangosteen peel extract (MPE). The extract was kept in a cool dark place until use [8-9]. The following formula was used to calculate the percentage yield (%yield):

$$\%Yield = \frac{Weight\ of\ mangosteen\ extract\ (g)}{Dry\ weight\ of\ Mangosteen\ peel\ (g)} \times 100\%$$

2.4 Preparation of mangosteen peel extracts loaded niosome (MPEN)

A 1:1 solution of methanol and chloroform was mixed with the constituents of formulations SP60, SP80, and TW80 (surfactant, cholesterol, and MPE), as shown in Table 1. The mixtures were then evaporated using a rotary evaporator at 60 °C until a film formed, followed by 30 minutes of ultrasonic bath shaking at 50 ° after addition of 10 mL of MPE solution [10].

Table 1 Preparation of MPEN formulations

Formulation	Weight Ratio		
	Non-ionic Surfactant	Cholesterol	MPE
SP60	1	1	0.1
SP80	1	1	0.1
TW80	1	1	0.1

SP60: Span 60®, SP80: Span 80® and TW80: Tween 80®

2.5 Characterizations of MPEN

A zetasizer (Malvern Mastersizer 3000) was used to measure the particle size, polydispersity index (PDI), and zeta potential of MPEN, and a transmission electron microscope (Talos F200X) was used to analyze the morphology of MPEN after positive staining with uranyl acetate (0.5% w/w) [11].

2.6 Entrapment efficiency (%EE) of MPEN

The MPEN sample was diluted at a 1:1 ratio with methanol and centrifuged for 30 minutes at 10,000 rpm and 4 °C. The supernatant was measured at λ Max 243 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1900i). The following equation [11-13] was used to calculate the entrapment efficiency.

$$\%EE = \frac{Ct - Cf}{Ct} \times 100\%$$

where C_t is the concentration of total α-mangostin and C_f is the concentration of free α-mangostin. The experiments were conducted in triplicate with the results expressed as the mean ± standard deviation (S.D.).

2.7 Accelerated stability test of MPEN

A heating-cooling cycle was used to assess the emulsion stability throughout acceleration. The MPEN samples were kept in a refrigerator (SBC-P2DB, Panasonic) at 4 °C for 24 hours, and then placed in an incubator (IN110, Memmert) at 45 °C for another 24 hours. One cycle was assigned to this process. Five experimental cycles were conducted to examine the sample separation.

2.8 DPPH Method

The MPE antioxidant activity was measured by the DPPH method. Briefly, a 0.10 mM DPPH solution in ethanol was mixed with MPE solution (200–500 mg/L) in deionized (DI) water using a L-ascorbic acid solution (4–10 mg/L) in DI water as a positive control. Then, 1.60 ml of the sample solution was pipetted into 2.40 ml of DPPH solution. After shaking vigorously, the mixture was allowed to rest at room temperature for 30 minutes in the dark. Three repetitions of the experiment were conducted using a UV-Vis spectrophotometer at 516 nm [14]. The antioxidant activity was determined and represented as 50% inhibitory concentration using the following formula:

$$Inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

where A_{sample} is the absorbance of the sample (DPPH solution with sample) and $A_{control}$ is the absorbance of the control (DPPH solution without the sample but replaced with ethanol solution).

2.9 Determination of total flavonoid content

The total flavonoid content (TFC) was determined by the aluminum chloride colorimetric assay [15]. First, 3 ml of DI water was placed in a 10 mL volumetric flask followed by 0.3 mL of 5% sodium nitrite. Then, 1 mL of MPE (3,000 µg/mL) was added and the mixture was left for 5 minutes. After that, 0.3 ml of 10% aluminium chloride solution was added, shaken and left for 6 minutes. Then, 2.5 ml of 1 M sodium hydroxide solution was added, and the volume was made up to 10 ml with distilled water. The absorbance was measured immediately at 510 nm by a *UV-Vis* spectrophotometer with results expressed as mg of quercetin equivalent (QE)/100 g dry weight. The TFC was calculated using the following formula:

$$C = \frac{cV}{m}$$

where C = total flavonoid content mgQE/g dry sample, c = concentration of quercetin obtained from the calibration curve in mg/mL, V = volume of solvent used for extraction in ml, and m = weight (g) of the dried sample used. All the tests were performed in triplicate.

2.10 Anti-tyrosinase activity

The dopachrome method was used to determine the anti-tyrosinase enzyme activity [16-17]. A 96-well plate was filled with 40 µL of MPE solution (60, 70, 80, 90, and 100 mg/mL). Then, 120 µL of 3 mM L-dopa solution (dissolved in PBS) and 40 µL of 2,000 Unit/ml tyrosinase solution in a sodium phosphate buffer at pH 6.8 (PBS) were added. The mixed solutions were maintained at 37 °C for 30 minutes to identify the appropriate tyrosinase concentration for MPE tyrosinase inhibitory action. A microplate reader was used to measure the absorbance at 475 nm. Kojic acid (1, 1.5, 2, 2.5, and 3 mg/mL) was chosen as a positive control with DI water utilized as a solvent control and blank. The tyrosine inhibition rate (%) was calculated by the following formula:

$$The\ inhibition\ rate\ (\%) = \left(1 - \frac{\Delta OD_{sample}}{\Delta OD_{control}}\right) \times 100\%$$

where ΔOD_{sample} and $\Delta OD_{control}$ represent the absorbance of the sample and the control measured at 475 nm, respectively. The half inhibitory concentration (IC_{50}) value was determined by the regression of a dose-response curve at which 50% of the target activity was lost.

2.11 An timicrobial activity

The bacterial strains were obtained from the Faculty of Science at King Mongkut's Institute of Technology in Ladkrabang, Thailand, and the antibacterial activity was determined using the well diffusion method [9]. Nutrient agar was added to *Staphylococcus aureus* ATCC 25923 cultures. The MPE was then added at 500 mg/mL (50 µL/well) concentration. Ten units of the antibiotic penicillin were used as the positive control, with DI water as the negative control.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aureus* MPE were determined via a two-fold serial dilution technique [9]. The *S. aureus* bacteria were tested at MPE concentrations of 0.97, 1.95, 3.90, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 mg/mL and incubated at 37 °C for 24 hours. The transparent samples were injected onto the culture medium and incubated for 24 hours at 37 °C to determine the lowest MPE concentration that inhibited bacterial growth.

2.12 In vitro release study of α-mangostin

Strat-M™ membranes (Sigma-Aldrich, Strat-M™) were soaked in phosphate buffered saline pH 7.4 (PBS) for 16 hours at room temperature. After soaking, the membranes were wiped. Then, 1 ml aliquots of the MPE and MPEN samples were applied to the membrane. The automated transdermal diffusion cell sampling system (SYP-SP-2-10, LOGAN) was utilized for one-hour interval sample collections (12 hours). The instrument was filled with PBS solution, stir speed 500 rpm, and temperature 32 ± 1 °C. The collected samples were investigated by a UV-Vis spectrophotometer at 243 nm [9, 13, 18].

2.13 Formulation of underarm care products

The MPEN were used to formulate two oil in water (O/W) underarm care products. Tables 2 and 3 display the dry serum antiperspirant and underarm mask cream compositions, respectively. The oil and water phases were made independently, and the two phases were heated to 75 to 80 °C. The water phase was then homogenized with the addition of the oil phase until an emulsion formed.

2.14 Creaming index (%CI) of the underarm care product

The stability of an emulsion formulation is measured by the creaming index percentage (%CI) [19]. The products were placed in separate centrifuge tubes and subjected to 1500, 3000, and 6000 rpm for 30 minutes. The %CI of the underarm care product was calculated by the following formula:

$$\%CI = \left(\frac{CC}{CT}\right) \times 100$$

where CC is the total height of the cream layer and CT is the total height of the emulsion layer.

Table 2 Compositions and concentrations of MPEN in dry serum antiperspirant formulation

Composition	%W/W
Water phase	
DI Water	add to 100
Disodium EDTA	0.2
Glycerine	1
Propylene Glycol	2
Polyvinyl Alcohol	1
Carboxymethyl Cellulose	0.65
Niacinamide	3
Aluminum Chlrohydrate	7
MPEN	1
Polysorbate 80	3
Silica	3.5
Phenoxyethanol	0.5
Oil phase	
Steareth-2	6.5
Steareth-21	5
Glyceryl Monostearate	1
Cetyl Alcohol	0.8
Isopropyl Myristate	2
Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate	0.1
Cyclopentaloxane	1
Dimethicone (and) Dimethicone/Vinyl Dimethicone Crosspolymer	1
Sorbitan Oleate	1.5
Fragrance	0.5

Table 3 Compositions and concentrations of MPEN in underarm mask cream formulation

Composition	%W/W
Water phase	
DI Water	add to 100
Disodium EDTA	0.2
Glycerine	2
Propylene Glycol	2
Polyvinyl Alcohol	1
Ammonium Acrloyldimethyltaurate/VP Copolymer	2
Niacinamide	5
Witch Hazel	1
Allantoin	1
MPEN	1
Phenoxyethanol	0.5
Oil phase	
Steareth-21	4
Glyceryl Monostearate	1
Cetyl Alcohol	2
Shea Butter	3
Petrolatum	2
Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate	0.1
Isopropyl Myristate	2
Cyclopentaloxane	1.5
Dimethicone (and) Dimethicone/Vinyl Dimethicone Crosspolymer	1
Polysorbate 80	1
Sorbitan Oleate	1
Fragrance	0.1

2.15 Accelerated stability test of underarm care products

A heating-cooling cycle was utilized to assess the stability of the underarm care products via acceleration. After storing at 4 °C for 24 hours in a refrigerator (SBC-P2DB, Panasonic), the MPEN samples were placed in an incubator (IN110, Memmert) at 45 °C for a further 24 hours. This procedure signified a single cycle. Measurements of the emulsion separation, pH value using a pH meter (ST300, OHAUS), viscosity by a viscometer

(DV2T, Brookfield), and color change using a skin colorimeter (CL400 Courage + Khazaka electrical GmbH) were taken during five experimental cycles.

3. Results and discussion

3.1 Yield of crude extract

Results showed that the 95% v/v MPE ethanolic extract had a yield of 45.10% w/w and a yellowish-brown color. More phytochemicals from the plant or herb can be extracted by macerating the sample in ethanol for over 24 hours, with a longer extraction period giving a higher extract concentration [8 - 9]. A yellow solid remained when the solvent was evaporated [20].

3.2 Transmission electron microscopy (TEM) of MPEN

The morphology of MPEN with Span 60®, Span 80® and Tween 80® is shown in Figure 1. The samples were spherical indicating that the niosomes were unilamellar vesicles.

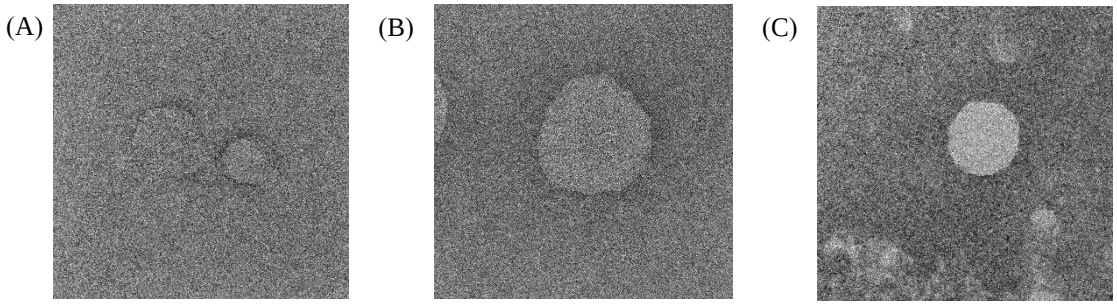

Figure 1 MPEN under TEM: **(**A**)** Niosomes with Span 60®, **(**B**)** Niosomes with Span 80®, **(**C**)** Niosomes with Tween 80®.

The niosomes used in the thin-film hydration approach were a multilamellar vesicles (MLVs) with irregular spherical shapes. The sonicator-induced size reduction mechanism resulted in unilamellar niosome vesicles in this experiment [21].

3.3 Size, PDI, Zeta potential, %EE and layer separation of MPEN

The MPEN formulations with Span 60® (SP60), Span 80® (SP80), and Tween 80® (TW80) were compared. The SP60 formula had the smallest particle size, while the TW80 had the lowest polydispersity index (PDI) values, and zeta potential of -20.2 mV. The zeta potentials obtained using SP60 and SP80 were comparable. The %EE of SP60 was highest at 65.05 ± 0.01 with TW 80 and SP80 showing comparable results. TW80 exhibited layer separation. The size, PDI, zeta potential, %EE, and layer separation of the formulations are shown in Table 4.

SP60 was chosen for this experiment because it had the highest entrapment efficiency, the narrowest particle size distribution and the highest zeta potential. With no separation, the SP60 formulation was suitable for developing underarm care product formulas. The layer separation impacted the zeta potential value, which represents the electrical potential difference between the surface of a charged molecular particle and the surrounding liquid. A value close to zero may cause neighboring particles to collide with sedimentation or separation of layers. This result was consistent with the MPEN stability test. The highest %EE value of Span 60® was due to the HLB value of the surfactant used, which influences the encapsulation ability of the substance in the niosomes [21].

Table 4 The results of MPEN evaluation.

Formulation Code	Size (nm.)	PDI	Zeta potential (mV)	%EE	Layer separation
SP60	247	0.542	-46.1	65.05 ± 0.01	-
SP80	249.2	0.612	-45.9	37.79 ± 0.02	-
TW80	312	0.481	-20.2	37.66 ± 0.03	+

SP60: Span 60®, SP80: Span 80® and TW80: Tween 80®
Symbol +: Layer separation, Symbol -: No layer separation.

3.4 DPPH, TFC and tyrosinase inhibitions of MPE

The MFE antioxidant activity measured by the DPPH assay gave an IC_{50} value of 230.28 mg/L. Vitamin C at 7.30 mg/L had higher antioxidant activity than MPE.

The antioxidant activity of MPE extracted with an ethanol solvent by the DPPH method gave higher antioxidant values for fresh peels than the dried form. The fresh sample was not heat dried before extraction. The phytochemicals in the fresh sample degraded when exposed to high temperatures during the drying process [14].

The flavonoid content of MPE was 4,742.59 ± 2.69 mg QE/100 g dry weight using the aluminum chloride colorimetric method. The calibration curve of quercetin is shown in Figure 2.

MPE contained high flavonoids including α- mangostin, and the ethanolic extraction showed high potential to extract flavonoids [8, 12, 15, 20].

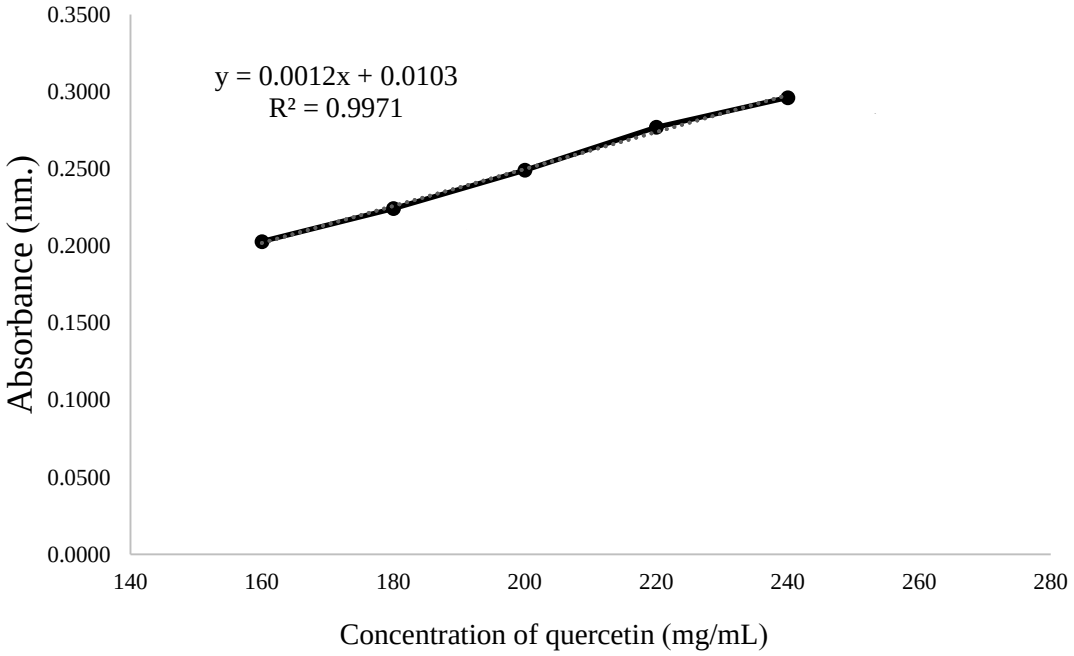

Figure 2 Calibration curve of standard quercetin for the determination of TFC.

The dopachrome method determined that MPE inhibited the enzyme tyrosinase with an IC_{50} value of 67.04 mg/mL compared to kojic acid at 1.10 mg/mL suggesting that kojic acid had better anti-tyrosinase activity than MPE.

The anti-tyrosinase enzyme activity in mangosteen peel is attributed to its xanthone contents, particularly α-mangostin which reducing melanin production and make the skin brighter [16, 22].

3.5 Antibacterial of MPE

The MPE antibacterial activity was determined using the well diffusion method (Figure 3). The clear zone of MPE was 13.88 ± 0.17 mm compared to penicillin at 34.87 mm. The DI water did not inhibit bacteria. MPE was effective against *S. aureus* because it contains xanthones and tannins. These inhibit Gram-positive bacteria, which can penetrate bacterial cells. Gram-positive bacteria have an antibiotic impact because they lack an outer membrane and periplasmic space [8, 9, 20, 23].

The MIC and MBC values of MPE were 1.95 mg/mL and MBC 1.95 mg/mL, respectively indicating that MPE had good inhibitory activity against *S. aureus*. Extraction of the substances was performed using an ethanol solvent. Increasing the MPE extraction time promoted the extraction of xanthones and tannins. A longer extraction time resulted in high concentrations of xanthones and tannins in MPE. The MIC and MBC were tested against *S. aureus* using a small amount of MPE [8, 20, 23]. Uzeh et al. (2012) found that *S. aureus* was responsible for body odor in male and female university students in Nigeria. MPE inhibited *S. aureus*, indicating potential use in developing underarm care products [24].

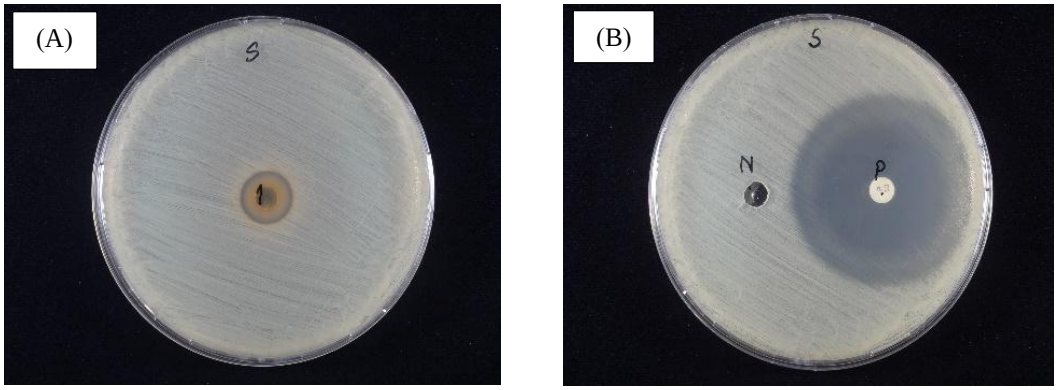

Figure 3 Zone diameter of *S. aureus*: **(**A**)** MPE in an agar dish, **(**B**)** DI water and penicillin in an agar dish

3.6 In vitro release study of α-mangostin

A release profile study of α-mangostin in MPE and MPEN samples was carried out using Franz diffusion cells (Figure 4). The release profile of the MPEN samples was more gradual than MPE. Research revealed that MPE released α-mangostin more readily than MPEN. Thus, encapsulating MPE helped to maintain control over α-mangostin release. The quantity of cholesterol in MPEN enhanced chemical release for a longer duration than MPE leading to larger particle size and increased solubility of the niosomes in various substances, while a slower release rate allowed modification of the strength of the particle barriers [21].

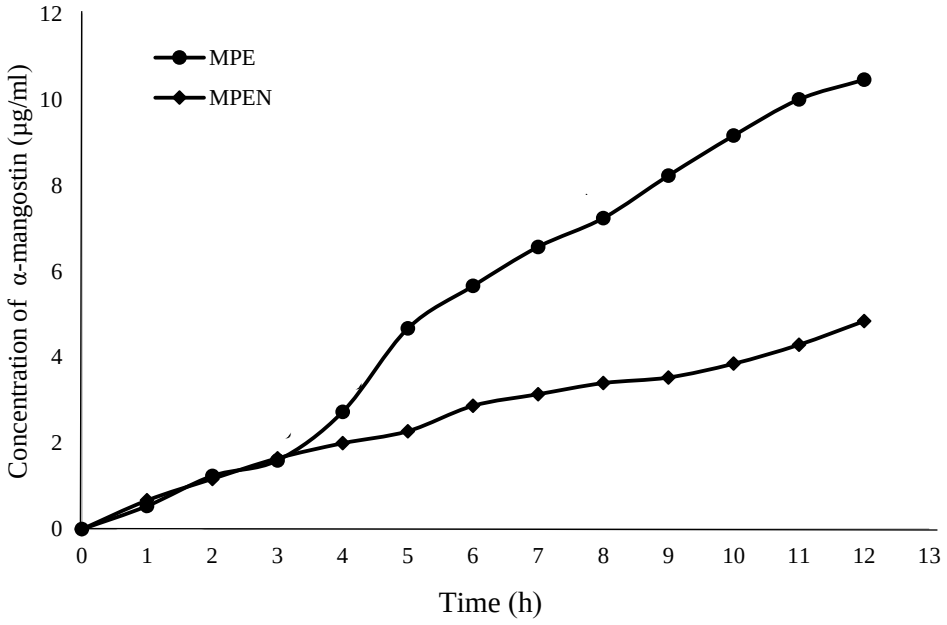

Figure 4 Cumulative release profiles of α-mangostin in the MPE and MPEN samples.

3.7 Evaluation of underarm care products containing MPEN

Results in Table 5 indicate that the dry serum antiperspirant passed the accelerated centrifuge test at 1,500 rpm. After five heating-cooling cycles, the viscosity doubled in value with little change in hue and no phase separation or pH shift (Table 6).

The underarm mask cream passed the accelerated centrifuge test at 1,500, 3,000, and 6,000 rpm (Table 5). After five cycles of heating and cooling, the viscosity increased from 504,200 ± 39,346 to 585,300 ± 7,135 cP (Table 6). No phase separation was recorded, with only a slight shift in hue that was difficult for humans to discern.

Table 5 Creaming index of underarm care products.

Underarm care product	Speed (rpm)	Creaming index (%)
Dry serum antiperspirant	1,500	0
	3,000	3.08 ± 0.75
	6,000	4.16 ± 2.86
Underarm mask cream	1,500	0
	3,000	0
	6,000	0

Table 6 Stability assessment of underarm care products.

Characteristics	Dry serum antiperspirant formulation under storage condition		Underarm mask cream formulation under storage condition	
	Heating-cooling cycle		Heating-cooling cycle	
	Cycle 0	Cycle 5	Cycle 0	Cycle 5
Viscosity (cP)	41,750 ± 0	85,600 ± 3,011	504,200 ± 39346	585,300 ± 7,135
Color (L*)	74.02 ± 0.05	71.84 ± 0.20	75.07 ± 0.03	74.35 ± 0.06
Color (a*)	-2.54 ± 0.18	-2.66 ± 0.34	-1.98 ± 0.38	-2.32 ± 0.01
Color (b*)	2.48 ± 0.05	3.07 ± 0.16	6.16 ± 0.16	7.67 ± 0.05
pH	4.04 ± 0.01	4.04 ± 0.01	5.77 ± 0.01	5.82 ± 0.01
Separation	-	-	-	-

Symbol +: Layer separation, Symbol -: No layer separation.

4. Conclusions

Mangosteen peel extract entrapped in niosomes showed potential to formulate underarm care products. SP60, with a zeta potential value of -46.1 mV and the highest entrapment effectiveness of 65.05% ± 0.01 was chosen for further investigation. The underarm care cream containing niosomes passed the technical acceleration stability test using the heating-cooling cycle method with no phase separation. Franz diffusion cells were used to compare the release of α-mangostin between MPE and MPEN. The release profile of MPEN was more gradual than MPE suggesting that the niosome formulation with Span60® showed promise for use as a novel underarm care product.

5. Acknowledgements

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