Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Cytotoxicity and Cell Migration Suppression by Noni Fruit Extract on Michigan Cancer Foundation-7 Human Breast Cancer Cells and Development of Topical Microemulsions

Supavadee Boontha[1], Noppamas Kaewjaiboon[1], Phitchayut Rattanatanyapat[1], Wanwalee Nanto[1], Salisa Taolam[1], Benjaporn Buranrat[2], Tasana Pitaksuteepong[3]

[1]Departments of Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of Phayao, Phayao, [2]Faculty of Medicine, Mahasarakham University, Maha Sarakham, [3]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

Submitted: 03-08-2018 Revised: 11-09-2018 Published: 17-01-2019

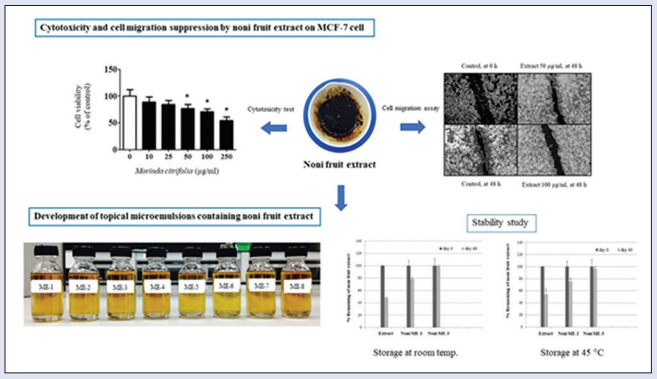
ABSTRACT

Background: There is limited data about the anti-breast cancer activity of noni fruit ethanolic extract. Topical route is gaining interest as an alternative route for breast cancer treatment. **Objectives:** The aims of this study were to investigate the effect of noni fruit extract on cytotoxicity and cell migration suppression in Michigan Cancer Foundation-7 (MCF-7) human breast cancer cells and to develop topical microemulsions (MEs). **Materials and Methods:** The noni fruit ethanolic extract was prepared. Rutin content, a marker, in the extract was analyzed, and the antioxidant activity of the extract was determined. Cytotoxicity and cell migration suppression, indicating anti-breast cancer activity, were also assessed in MCF-7 cells by SRB assay and wound-healing assay. The MEs were developed by titration method. The developed noni MEs were evaluated regarding their physical appearance, viscosity, and pH before and after stability test. **Results:** Results showed that the rutin content in the noni extract was 4.02 ± 0.34 mg/g. The extract possessed antioxidant activity with inhibitory concentration (IC_{50}) of 1.03 ± 0.01 mg/ml. It also showed cytotoxicity with IC_{50} values of 158.4 ± 12.5 µg/ml and cell migration suppression on MCF-7 cells with significant effect at 100 µg/ml. Eight ME systems were selected to incorporate the extract. **Conclusion:** Noni extract had potential anti-breast cancer activity. The ME, named noni ME-3, consisting of 0.7% w/w noni extract, 56% w/w olive oil, 20% w/w polysorbate 80, 20% w/w sorbitan oleate, and 4% w/w water phase, was shown to be the most attractive noni ME for the topical treatment of breast cancer.

Key words: Breast cancer, cytotoxicity, microemulsions, noni fruit, topical

SUMMARY

- The noni fruit extract had antioxidant activity and could also potentially inhibit Michigan Cancer Foundation-7 human breast cancer cell viability and cell migration
- The topical microemulsion containing the noni fruit extract was successfully developed. The best system consisted of 0.7% w/w noni extract, 56% w/w olive oil, 20% w/w polysorbate 80, 20% w/w sorbitan oleate, and 4% w/w water phase.



Abbreviations used: HPLC: High-performance liquid chromatography; SRB: Sulforhodamine B assay; RT: Room temperature.

Correspondence:

Dr. Supavadee Boontha,
Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, University of Phayao, 19 Moo 2, Tambon Maeka, Ampur Mueng, Phayao 56000, Thailand.
E-mail: supavadee.bo@up.ac.th

DOI: 10.4103/pm.pm_403_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Morinda citrifolia L., commonly known as noni, belongs to the Rubiaceae family. Noni is a small plant native to Southeast Asia. It has attained cross-cultural relevance as an alternative herbal treatment for indications such as cancer, inflammation, hypertension, and diabetes.[1-4] Currently, noni fruits have been widely studied for anti-breast cancer activity.[5-7] Wang *et al.*[6] have reported that noni fruit juice, under the commercial name Tahitian fruit juice (TNJ), could prevent breast cancer at the initiation stage on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in female Sprague–Dawley rats. Clafshenkel *et al.*[7] also reported that TNJ could prevent mammary breast cancer at the initiation stage of chemical carcinogenesis in female mammary tumor virus-*neu* mice. The *in vitro* cytotoxicity of the methanolic extracts of noni fruit and the polysaccharide fraction isolated from noni fruit extracts against Michigan Cancer Foundation-7 (MCF-7) breast cancer cell has also been reported.[8,9] Based on these results, noni fruits are considered as an alternative source of chemopreventive agent for breast cancer. Besides noni juice, an ethanolic crude extract of noni fruits has also been reported to inhibit the proliferation of breast cancer cells.[10] Many

Cite this article as: Boontha S, Kaewjaiboon N, Rattanatanyapat P, Nanto W, Taolam S, Buranrat B, *et al.* Cytotoxicity and cell migration suppression by noni fruit extract on michigan cancer Foundation-7 human breast cancer cells and development of topical microemulsions. Phcog Mag 2018;14:S499-506.

research studies have shown that noni fruit contains several phenolic compounds including ursolic acid, kaempferol, quercetin, and rutin.[11-13] Rutin is a glycoside of the flavonoid quercetin which exhibits significant oxygen radical-scavenging properties both *in vitro* and *in vivo* studies.[14] It has been reported to decrease both the risk and progression of cancer with their decrease in the production of reactive oxygen species.[15,16] In addition, rutin has been documented as a marker of antioxidant activity of noni.[17] For anti-breast cancer activity, rutin was reported to display antiproliferative activity against MCF-7 cancer cells.[18,19] Moreover, rutin has been reported as an adjuvant agent of tamoxifen by overcoming the drug efflux from the cancer cells.[18] Because of the previously mentioned reasons, the rutin content can be represented for the antiproliferative activity of the noni extract against breast cancer cells. Currently, breast cancer chemopreventive activity of noni juice has been reported when noni juice was given to animals only via the oral route.[6,7] Topical route is gaining interest in this study as an alternative route for breast cancer treatment because it is safe, easy to administer, and pain free. However, topical application of the extract is difficult to obtain dose effective because the extract is generally low solubility and poor skin permeation. Hence, a drug delivery system is needed.

Microemulsions (MEs) can preferably be used as the delivery system for enhancing skin permeation of noni fruit extract because they have been documented to enhance transdermal drug delivery by improving the solubility of an active compound, resulting in increasing concentration gradients, as well as by altering the stratum corneum structure.[20-22] MEs are isotropic clear and thermodynamically stable colloidal dispersions. They consist of oil, water, and surfactant, frequently in combination with a co-surfactant. Recently, MEs have been shown to successfully deliver α-santalol via topical route to breast skin, resulting in the reduction of the percentage incidence of breast tumor and the number of tumors per rat.[23] Therefore, the aims of this study were to investigate the effect of noni fruit extract on cytotoxicity and cell migration suppression in MCF-7 human breast cancer cells and to develop topical MEs for the extract.

MATERIALS AND METHODS

Materials

Acetonitrile, ethanol, and methanol were purchased from RCI Labscan (Bangkok, Thailand). Acetic acid was purchased from Prolabo (Briare, France). Rutin was purchased from Sigma-Aldrich (Buchs, Switzerland). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Steinheim, Germany). Ascorbic acid was obtained from Sigma-Aldrich (Shanghai, China). A human breast cancer cell line (MCF-7) was obtained from the American Type Culture Collection (ATCC #HTB-22; Manassas, Virginia, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and other cell culture reagents were purchased from Gibco-Thermo Fisher Scientific, Inc., Waltham, MA, USA. Trichloroacetic acid (TCA), sulforhodamine B (SRB), and crystal violet were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Olive oil was obtained from Giralda (Madrid, Spain). Isopropyl myristate (IPM) was obtained from TTK science (Bangkok, Thailand). Polyethylene glycol (PEG40) hydrogenated castor oil (Cremophor® RH40) and Sorbitan oleate (Span® 80) were obtained from Namsiang Co., Ltd., Bangkok, Thailand. Polysorbate 80 (Tween® 80) was purchased from Srichand United Dispensary (Bangkok, Thailand).

Preparation of noni fruit extract

The fruits of *M citrifolia* L. were collected from Phayao province, Thailand, during April–November, 2014. Plant identification was performed and deposited by the Applied Thai Traditional Medicine, Faculty of Medicine, Mahasarakham University (specimen no. MSUT_7225), and the Faculty of Sciences, Mahasarakham University, Thailand. The fresh fruits were washed, chopped, and air dried. They were further dried at 50°C using a hot-air oven (Memmert, Schwabach, Germany) for 48 h. After grounding into powder, they were macerated using 95% ethanol. The filtrate was then filtered and concentrated using a rotary evaporator (Heidolph, Germany), with controlled temperature at 50°C. Percentage yield of the crude extract was calculated by the following equation:

% Yield = (weight of dried crude extract [g]/weight of dried noni fruit powder [g]) × 100.

Determination of rutin content in noni fruit extract

The rutin content, a marker, in the extract was analyzed using high-performance liquid chromatography (HPLC) (LC-20AD, Shimadzu, Japan) equipped with a ultraviolet-visible (UV-Vis) detector (SPD-20A, Shimadzu, Japan). Chromatography was performed on phenomenex luna C18 column (5 µm, 150 mm × 4.60 mm) with a mobile phase consisting of 0.01 M acetic acid: methanol: acetonitrile (50:45:5) and a flow rate of 1.0 ml/min. The volume injected was 20 µl with detection at 254 nm. The ruin content was calculated based on the peak area of HPLC chromatogram.

Sample preparation was made by dissolving the extract (125 mg) in 25 ml of methanol. The sample was then sonicated by using a sonication bath (S100H, Elma, Singen, Germany) until completely dissolved. The sample was filtrated through 0.45-µm nylon membrane filters (Vertical Chromatography Co., Ltd., Bangkok, Thailand) and analyzed for rutin content by HPLC analysis.

Determination of antioxidant activity of noni fruit extract

The antioxidant activity of the extract was determined using DPPH radical scavenging assay. Briefly, the stock solution of the extract was prepared in 95% ethanol at the concentration of 2.5 mg/ml. Then, 2 ml of the solutions at various concentrations (0.1–2.5 mg/ml) was mixed with 2 ml of 0.2 mM DPPH in a test tube. After incubation at room temperature (RT) for 60 min, the optical density at 515 nm (OD_{515}) of the samples was measured using UV-Vis spectrophotometer (V-630, Jasco, Tokyo, Japan). Ascorbic acid was used as a positive control. The experiments were carried out in triplicate. The ability to scavenge the DPPH radical was calculated by the following equation:

% Radical scavenging = (1 − [sample OD_{515}/blank OD_{515}]) × 100

where the sample OD_{515} is the absorbance of the test sample and the blank OD_{515} is the absorbance of the blank (without test sample) at 515 nm.

Determination of effects of noni fruit extract on cytotoxicity and cell migration in Michigan Cancer Foundation-7 cancer cells

Cell culture and cell cytotoxicity assay

The MCF-7 cells were cultured according to ATCC's recommendations at 37°C and 5% CO_2 in DMEM medium supplemented with 10% FBS, 100 U/ml penicillin G, and 100 µg/ml streptomycin. The DMEM media was replaced every 3 days, trypsinized with 0.25% trypsin-EDTA, and subcultured in the same medium. To determine the effect of the extract on MCF-7 cell cytotoxicity, the SRB assay was used.[24] In brief, 1×10^4 cells/well of the MCF-7 cell line were cultured for 24 h in a 96-well culture plate. After that, the new medium (200 µl) containing various concentrations of noni extract (0–1000 µg/ml) was added and further incubated for 48 h. The MCF-7 cells were then fixed with 10% TCA for 60 min, stained with 0.4% SRB for 30 min, washed of excess SRB dye, and dissolved with 200 µl of 10 mM Tris base solution (pH 7.4). The absorbance values were measured at a wavelength of 540 nm by

using a microplate reader (Opsys MR™, Dynex Technologies, USA). Cell cytotoxicity was expressed as the percentage of the cell viability when the cells were treated with samples compared with the cell viability of the control group.

Cell migration suppression

To determine the effects of the extract on cell migration suppression, wound-healing assay was used to determine the metastasis of cancer cells.[25,26] In brief, 2.5 × 10^5 MCF-7 cells were seeded into 24-well plates and cultured for 24 h. Then, MCF-7 cells were scratched to make wound with a sterile 0.2-ml pipette tip, and the cells were washed with phosphate-buffered saline buffer (pH 7.4). The new complete medium (1 ml) containing various concentrations of noni extract (0–100 μg/ml) was added, and images were taken from 0 to 48 h by an inverted microscope (×10) (TS100, Nikon, Japan). Cell migration suppression was expressed as the percentage of the relative closure of the scratched wound to the distant of uncovered region.

Construction of pseudoternary phase diagrams

The systems consisted of olive oil or IPM as an oil phase. PEG40 hydrogenated castor oil or polysorbate 80 served as a surfactant. Sorbitan oleate and water were used as a co-surfactant and an aqueous phase, respectively. The weight ratios of surfactant and co-surfactant (K_m) were varied at 1:1, 2:1, and 3:1 for the development of pseudoternary phase diagrams. The pseudoternary phase diagrams were constructed using water titration method at ambient temperature. For each phase diagram, the weight ratio of oil and surfactant mixed with co-surfactant (S_{mix}) were varied from 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These mixtures were tittered drop by drop with de-ionized water under magnetic stirring. After each mixing, the sample was allowed to equilibrate for 24 h. The samples were visually observed and classified as MEs when clear liquids were obtained. The formation of MEs was confirmed using a polarized light microscope (BA300, Motic, Hong Kong). The percentage area of the phase diagram that covered ME regions was determined by a cut-and-weight method.[27]

Preparation of blank microemulsions and noni microemulsions

After the identification of ME region in the pseudoternary phase diagrams, the MEs were selected from the diagrams. The extract was incorporated into the selected MEs by dissolving the extract in water phase before mixing with other ingredients.

Characterization of blank microemulsions and noni microemulsions

The characteristics of the blank and noni MEs were studied as follows:

Physical appearance

Physical appearance of MEs was determined by visual examination in terms of phase separation, clarity, and transparency.

Viscosity

The viscosity of MEs was measured at 25°C by using a Brookfield DV-II programmable rheometer equipped with a CP52 cone and plate spindle (Brookfield Engineering laboratories Inc., Massachusetts, USA).

pH

The pH value for MEs was determined at 25°C by using a pH meter (SevenEasy, Mettler Toledo, USA).

Stability study

The stability of the noni MEs was performed at RT and 45°C for 2 months. The physical appearance, viscosity, and pH of the noni MEs were evaluated as described above. In addition, the rutin content, indicating the chemical stability of noni MEs, was analyzed before and after the stability test. Briefly, the MEs were thoroughly mixed with methanol at a ratio of 1:1. The samples were then centrifuged at 5000 rpm for 30 min. The supernatant was further filtered through 0.45-μm nylon membrane filter, and the filtrate was analyzed for rutin content by using HPLC as previously described. The percentage of remaining rutin content was calculated.

Statistical methods

All experiments were repeated three times, and the data were expressed as the mean value ± standard deviation. Statistical data were analyzed using one-way analysis of variance, followed by Turkey's *post hoc* test. The analyses were conducted using Sigma Stat software version 3.5 (Systat Software Inc., San Jose, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Rutin content and antioxidant activity of the noni fruit extract

Appearance of the extract was a viscous yellowish-brown resin. The percentage yield of the extract obtained was approximately 9.83% (w/w) [Table 1]. With HPLC technique, the rutin peaks were well resolved under isocratic elution with retention times of 4.3 min, and the rutin content in the extract was 4.02 ± 0.34 mg/g.

According to the decreasing risk and progression of cancer associated with antioxidant activity of rutin, antioxidant activity of the noni fruit extract was determined. The noni fruit extract has antioxidant activity with IC_{50} of 1.03 ± 0.01 mg/ml.

Table 1: Percentage yield, rutin content, and antioxidant activity of the extract

Sample	Yield (% w/w)	Rutin content (mg/g)	IC_{50} of DPPH radical scavenging activity
Noni fruit extract	9.83	4.02±0.34	1.03±0.01 mg/ml
Ascorbic acid		-	8.66±0.26 μg/ml

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; IC_{50}: Half maximal inhibitory concentration

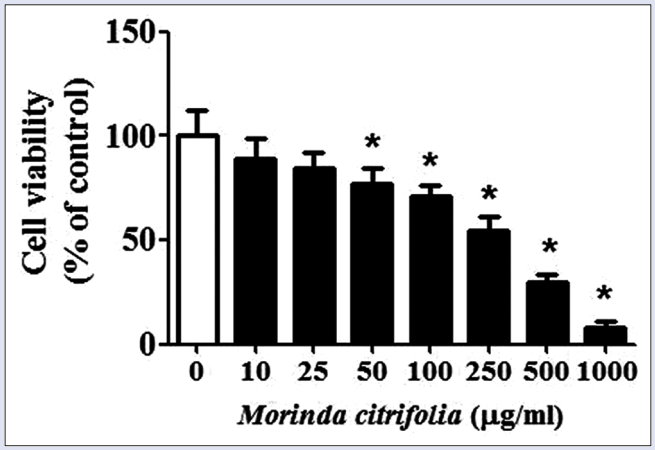


Figure 1: Effect of noni fruit extract on Michigan Cancer Foundation-7 cell viability. The cells were treated with various concentrations of 0–250 μg/ml extract for 48 h, and the cell viability was determined by sulforhodamine B assay. The results are presented as percentage of control and represented as mean ± standard error of the mean values from three independent experiments. *$P < 0.05$ as compared with the control group

Effects of noni fruit extract on cytotoxicity in Michigan cancer foundation-7 cancer cells

The results showed that the extract had cytotoxicity on MCF-7 cells. Compared with the control group, the extract reduced the number of MCF-7 cells in a dose-dependent manner with IC_{50} value of 158.4 ± 12.5 μg/ml [Figure 1]. The results are in agreement with the work of Moongkarndi *et al.*,[10] in which the ethanolic noni fruit extract showed cytotoxic activity on human breast cancer cells.

Effects of noni fruit extract on cell migration in Michigan cancer foundation-7 cancer cells

In general, metastasis is the process of tumor cell migration from the primary site to distant organs via the circulatory system and

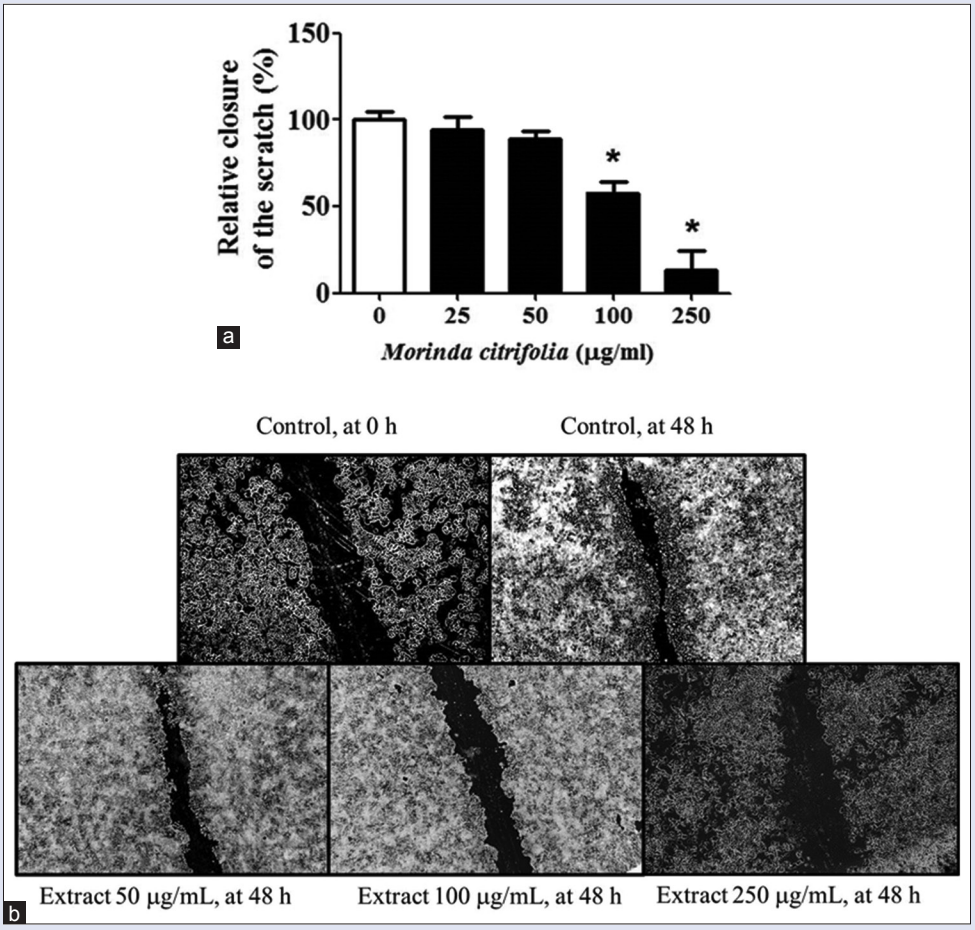


Figure 2: Effect of noni fruit extract on Michigan Cancer Foundation-7 cell migration. The cell monolayers were scratched by a 0.2-ml pipette tip, and then the cells were treated with the extract at 50, 100, and 250 μg/ml for 48 h. (a) The results are presented as percentage of control and represented as mean ± standard error of the mean values from three independent experiments. *$P < 0.05$ as compared with the control group. (b) Pictures of Michigan Cancer Foundation-7 cell migration which were monitored and captured by an inverted microscope (×10)

Table 2: Composition of the surfactant blends and their final HLB values

Type of oil	Surfactant	Co-surfactant	Weight ratio	Final HLB	Microemulsions area (%)
Olive oil	PEG40 hydrogenated castor oil	Sorbitan oleate	1:1	9.15	9.65
HLB=7.0			2:1	10.80	9.08
			3:1	11.58	7.88
	Polysorbate 80		1:1	9.65	9.99
			2:1	11.47	7.70
			3:1	12.33	5.11
Isopropyl myristate	PEG40 hydrogenated castor oil	Sorbitan oleate	1:1	9.15	0.39
HLB=11.1			2:1	10.80	3.69
			3:1	11.58	9.48
	Polysorbate 80		1:1	9.65	7.86
			2:1	11.47	3.40
			3:1	12.33	1.97

HLB: Hydrophilic-lipophilic balance

Table 3: Compositions of the selected blank microemulsions

Formulation	Oil	S_{mix}	km	Oil (%)	S_{mix} (%)	Water (%)
Blank ME-1	Olive oil	PEG40 hydrogenated castor oil: sorbitan oleate	1:1	52.0	40.0	8.0
Blank ME-2	Olive oil		1:1	44.0	48.0	8.0
Blank ME-3	Olive oil	Polysorbate 80:sorbitan oleate	1:1	56.0	40.0	4.0
Blank ME-4	Olive oil		1:1	36.0	56.0	8.0
Blank ME-5	IPM	PEG40 hydrogenated castor oil: sorbitan oleate	3:1	56.0	36.0	8.0
Blank ME-6	IPM		3:1	48.0	44.0	8.0
Blank ME-7	IPM	Polysorbate 80:sorbitan oleate	1:1	36.0	52.0	12.0
Blank ME-8	IPM		1:1	32.0	56.0	12.0

IPM: Isopropyl myristate; S_{mix}: Surfactant mixed with co-surfactant

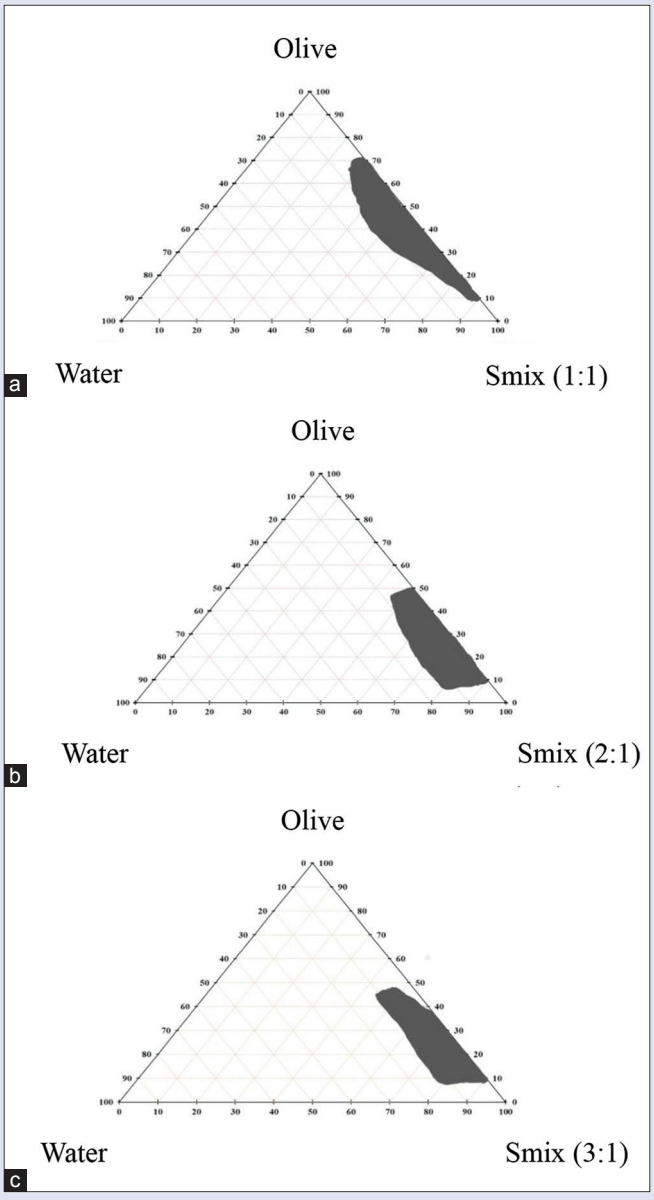


Figure 3: Pseudoternary phase diagrams of olive oil, water, S_{mix} (polyethylene glycol hydrogenated castor oil: sorbitan oleate) at K_m ratio of (a) 1:1, (b) 2:1 and (c) 3:1

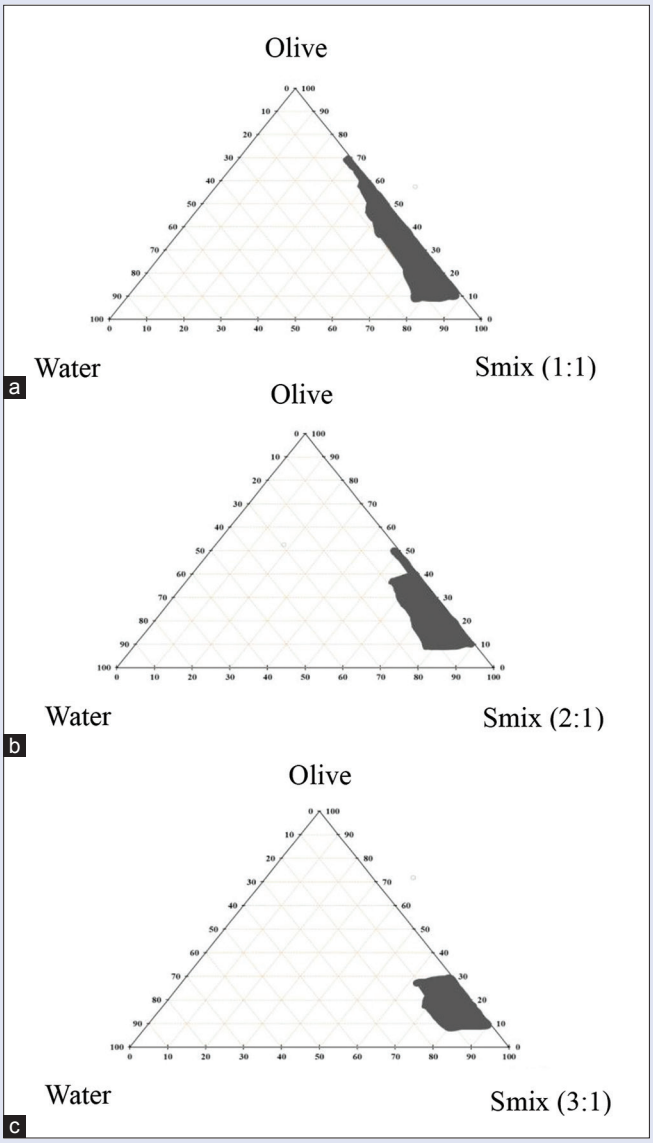


Figure 4: Pseudoternary phase diagrams of olive oil, water, S_{mix} (Polysorbate 80: sorbitan oleate) at K_m ratio of (a) 1:1, (b) 2:1 and (c) 3:1

establishes a secondary tumor. Such metastases are the majority cause of cancer-associated mortalities.[26] In this study, wound-healing assay was used to determine cell migration suppression of the extract on MCF-7 cancer cells. If the extract could suppress migration of the MCF-7 cells, the scratched wound closer was inhibited. The results demonstrated that the extract could inhibit the migration of MCF-7 breast cancer cells in a dose-dependent manner and showed significant effect at 100 μg/ml [Figure 2a]. At this concentration, the extract inhibited MCF-7 cancer cell migration by approximately 40% when compared with the control group [Figure 2b].

From the results, ethanolic noni fruit extract has a major effect on cell migration suppression compared with cytotoxicity and antioxidant effects. This study demonstrated that noni fruit extract can be potentially used as an anticancer agent for breast cancer treatment with its cytotoxic activity and suppression of breast cancer cell metastasis.

Construction of pseudoternary phase diagrams

In this study, olive oil and IPM were selected as an oil phase. PEG40 hydrogenated castor oil and polysorbate 80 were used as a surfactant, and sorbitan oleate was used as a co-surfactant because all components are particularly accepted as safe and nontoxic agents for use in a number of pharmaceutical and cosmetic product.[28,29] The pseudoternary phase diagrams using olive oil or IPM as an oil phase are shown in Figures 3-6. The results showed that the percentage area of ME regions in the pseudoternary phase diagrams decreased following increasing in the Km value [Figures 3, 4 and 6]. However, the pseudoternary phase diagrams of IPM with S_{mix} of PEG40 hydrogenated castor oil and sorbitan oleate showed different results [Figure 5]. These results could be explained by the hydrophilic–lipophilic balance (HLB) value of the used surfactant blends that matches the HLB value of the used oil which provided the lowest interfacial tension between the oil and water phase.[27] However, the pseudoternary phase diagrams of IPM with S_{mix} of polysorbate 80 and sorbitan oleate showed unexpected results. This might be due to not only the HLB value, but also the component of formulation which plays a major role in the formation of ME. The percentage area of ME regions from each pseudoternary phase diagram and the final HLB value of each surfactant blends are shown in Table 2.

Two representative MEs of each pseudoternary phase diagram were selected. The compositions of the eight MEs, named blank ME-1–blank ME-8, are given in Table 3. The extract was added to MEs at 0.7% w/w.

Characterization of blank and noni microemulsions

The physical appearance of blank ME prepared from olive oil was light yellow in color, while that prepared from IPM was clear colorless. Using polysorbate 80 and sorbitan oleate as a surfactant blend, the color the

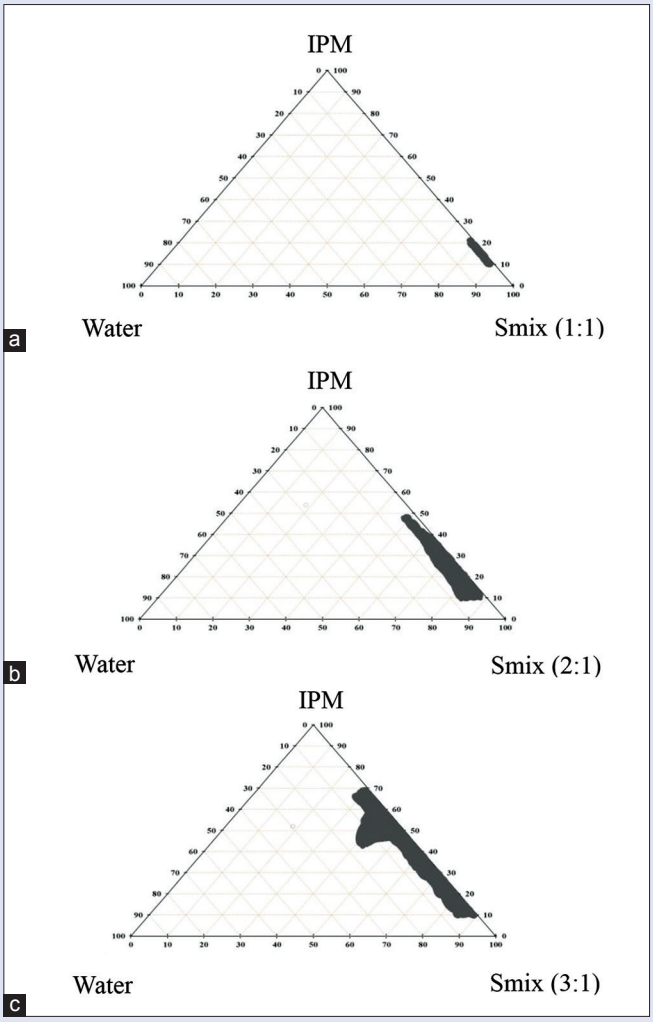


Figure 5: Pseudoternary phase diagrams isopropyl myristate (IPM), water, S_{mix} (polyethylene glycol hydrogenated castor oil: sorbitan oleate) at K_m ratio of (a) 1:1, (b) 2:1 and (c) 3:1

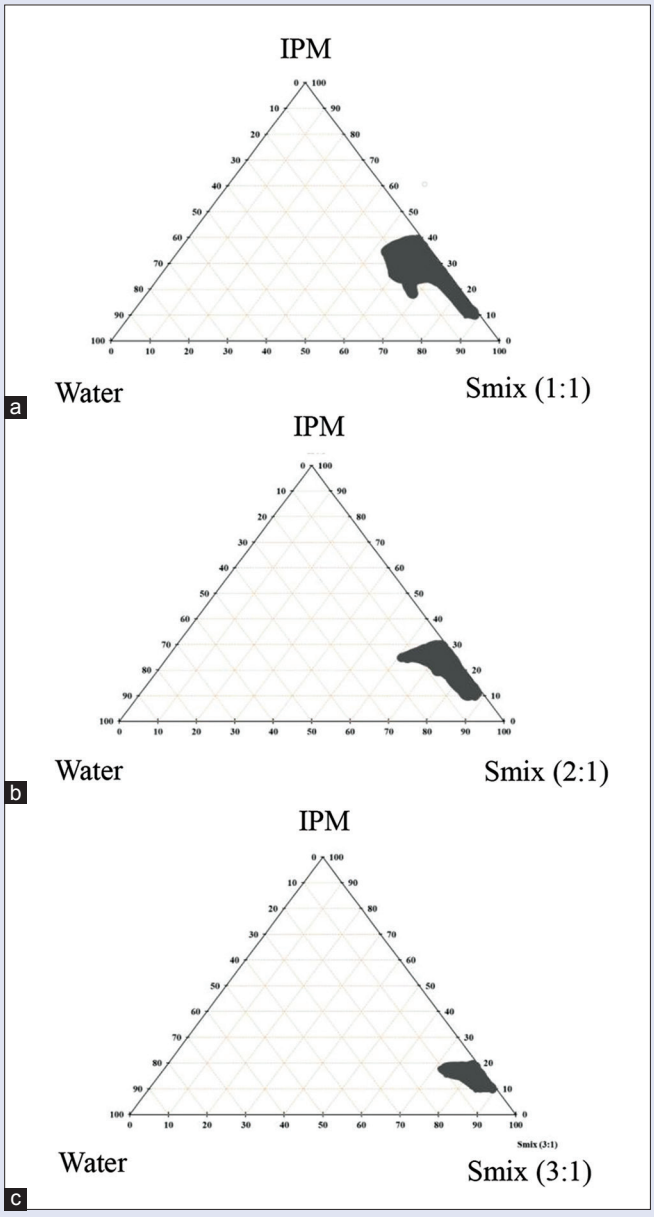


Figure 6: Pseudoternary phase diagrams isopropyl myristate (IPM), water, S_{mix} (Polysorbate 80: sorbitan oleate) at K_m ratio of (a) 1:1, (b) 2:1 and (c) 3:1

MEs obtained was more yellow than that of MEs prepared with PEG40 hydrogenated castor oil and sorbitan oleate. According to the color of the extract, the noni MEs prepared were dark yellow in color than that the blank ME prepared. Eight MEs containing the extract were given product code as noni ME-1–noni ME-8 [Tables 4 and 5]. The mean viscosity and pH of the blank and noni ME are shown in Tables 4 and 5. The viscosities of the blank ME and the noni ME prepared were in the range of 140–700 cps in all formulations. Incorporation of the extract slightly decreased the viscosity of MEs. However, the extract did not change the general characteristics of the prepared MEs. The pH of all blank ME prepared was in the range of 7.0–7.9, whereas that of noni ME prepared was in the range of 6.2–6.8, which was an appropriate pH range for topical formulation. The lower pH of the prepared noni ME was due to the acidic properties of the extract.

Stability study

Following the stability test, the viscosity and pH of all formulations were slightly decreased. Using olive oil as an oil phase, only the noni ME-2 and noni ME-3 were still clear and transparent without any phase separation after storing at RT and 45°C [Tables 4 and 5]. However, using IPM as an oil phase, phase separation was found in all formulations (noni ME-5–noni ME-8) after storing at both conditions. Therefore, the noni ME-2 and noni ME-3 were further determined for the rutin content. As shown in Figure 7, it was found that the unformulated noni extract significantly degraded much faster than the noni extract formulated in ME-2 and ME-3. High temperature, i.e., at 45°C, has a slight effect on the degradation of noni extract compared to RT [Figure 7]. The noni ME-3 was found to be the most stable formulation.

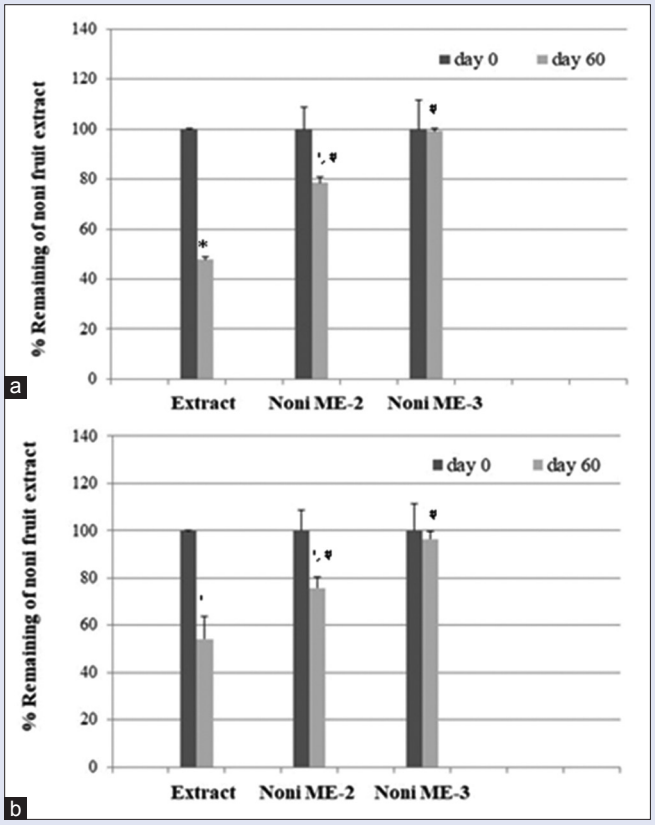


Figure 7: Percentage remaining of unformulated noni fruit extract and the extract formulated in ME2 (noni ME-2) and ME3 (noni ME-3) formulations after storing at different temperatures: (a) RT and (b) 45°C for 2 months. The results are presented as mean ± standard deviation values ($n = 3$). *$P < 0.05$ as compared with day 0 and #$P < 0.05$ as compared with unformulated extract

CONCLUSION

This study demonstrated that noni fruit extract possesses antioxidant activity and could also inhibit MCF-7 cell viability and cell migration. The topical ME containing the noni fruit extract was successfully developed. The system consisted of 0.7% w/w noni extract, 56% w/w olive oil, 20% w/w polysorbate 80, 20% w/w sorbitan oleate, and 4% w/w water phase. The developed noni ME shows potential to be used for the topical treatment of breast cancer.

Acknowledgements

This work was financially supported by a research grant from the School of Pharmaceutical Sciences, University of Phayao, Thailand, and Thailand Research Fund (Grant No. MRG6080071 for Dr. Benjaporn Buranrat). We would also like to express our thanks to Assistant Prof. Dr. Saengrawee Sutthiparinyanont for HPLC technical

Table 4: The physical appearance, viscosity, and pH of blank and noni microemulsions prepared (ME1–ME4) before and after the stability test

Formulation code	Conditions	Phase separation	Viscosity (cps)	pH
Blank ME-1	-	No	543.7±0.7	7.06±0.18
Noni ME-1	Before	No	529.9±14.7	6.30±0.14
	RT	Yes	612.5±16.3	6.05±0.09
	45°C	No	534.8±5.2	5.88±0.10
Blank ME-2	-	No	703.5±0.5	7.13±0.04
Noni ME-2	Before	No	716.7±14.0	6.24±0.13
	RT	No	760.7±17.6	6.19±0.10
	45°C	No	709.1±2.3	6.01±0.04
Blank ME-3	-	No	278.8±0.2	7.29±0.03
Noni ME-3	Before	No	284.5±0.6	6.56±0.07
	RT	No	245.1±1.0	6.32±0.11
	45°C	No	245.1±2.6	5.95±0.08
Blank ME-4	-	No	466.3±0.6	7.14±0.09
Noni ME-4	Before	No	463.7±15.2	6.66±0.05
	RT	Yes	427.0±12.2	6.46±0.06
	45°C	No	422.0±15.8	6.10±0.08

RT: Room temperature

Table 5: The physical appearance, viscosity, and pH of blank and noni microemulsions prepared (ME5-ME8) before and after the stability test

Formulation code	Conditions	Phase separation	Viscosity (cps)	pH
Blank ME-5	-	No	148.8±0.7	7.98±0.05
Noni ME-5	Before	No	176.6±8.4	6.29±0.07
	RT	Yes	120.1±2.0	5.68±0.29
	45°C	Yes	166.7±0.0	5.56±0.08
Blank ME-6	-	No	498.1±2.4	7.58±0.07
Noni ME-6	Before	No	455.4±13.9	6.62±0.09
	RT	Yes	479.2±17.2	5.78±0.02
	45°C	Yes	461.4±12.0	5.65±0.08
Blank ME-7	-	No	140.9±0.2	7.39±0.15
Noni ME-7	Before	No	141.2±2.1	6.78±0.10
	RT	Yes	126.0±1.0	6.12±0.20
	45°C	Yes	126.3±1.2	5.76±0.21
Blank ME-8	-	No	187.5±0.0	7.03±0.03
Noni ME-8	Before	No	193.1±7.5	6.71±0.18
	RT	Yes	168.0±4.6	6.44±0.06
	45°C	Yes	161.1±5.5	6.00±0.03

RT: Room temperature

assistance. The authors would like to thank the Faculty of Pharmaceutical Sciences, Naresuan University, for supporting instrument and Mr. Kevin Mark Roebl of the Naresuan University Language Centre for his editing assistance and advice on English expression in this manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Furusawa E, Hirazumi A, Story S, Jensen J. Antitumour potential of a polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (Noni) on sarcoma 180 ascites tumour in mice. Phytother Res 2003;17:1158-64.
2. Yang J, Gadi R, Paulino R, Thomson T. Total phenolics, ascorbic acid, and antioxidant capacity of noni (*Morinda citrifolia* L.) juice and powder as affected by illumination during storage. Food Chem 2010;122:627-32.
3. Gupta RK, Patel AK. Do the health claims made for *Morinda citrifolia* (Noni) harmonize with current scientific knowledge and evaluation of its biological effects. Asian Pac J Cancer Prev 2013;14:4495-9.
4. Basar S, Uhlenhut K, Högger P, Schöne F, Westendorf J. Analgesic and antiinflammatory activity of *Morinda citrifolia* L. (Noni) fruit. Phytother Res 2010;24:38-42.
5. Wanchai A, Armer JM, Stewart BR. Complementary and alternative medicine use among women with breast cancer: A systematic review. Clin J Oncol Nurs 2010;14:E45-55.
6. Wang M, Peng L, Anderson G, Nowicki D. Breast cancer prevention with *Morinda citrifolia* (noni) at the initiation stage. Funct Foods Health Dis 2013;3:203-22.
7. Clafshenkel WP, King TL, Kotlarczyk MP, Cline JM, Foster WG, Davis VL, *et al. Morinda citrifolia* (Noni) juice augments mammary gland differentiation and reduces mammary tumor growth in mice expressing the unactivated c-erbB2 transgene. Evid Based Complement Alternat Med 2012;2012:487423.
8. Arpornsuwan T, Punjanon T. Tumor cell-selective antiproliferative effect of the extract from *Morinda citrifolia* fruits. Phytother Res 2006;20:515-7.
9. Vennila S, Brindha D. *In vitro* cytotoxic and apoptotic activity of polysaccharide rich *Morinda citrofolia* fruit on mcf-7 cells. Asian J Pharm Clin Res 2015;8:1-4.
10. Moongkarndi P, Kosem N, Luanratana O, Jongsomboonkusol S, Pongpan N. Antiproliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line. Fitoterapia 2004;75:375-7.
11. Pak-Dek MS, Abdul-Hamid A, Osman A, Soh CS. Inhibitory effect of *Morinda citrifolia* L. On lipoprotein lipase activity. J Food Sci 2008;73:C595-8.
12. Harada S, Fujita-Hamabe W, Kamiya K, Mizushina Y, Satake T, Tokuyama S, *et al. Morinda citrifolia* fruit juice prevents ischemic neuronal damage through suppression of the development of post-ischemic glucose intolerance. J Nat Med 2010; 64:468-73.
13. Sahib N, Abdul Hamid A, Kitts D, Purnama M, Saari N, Abas F. The effects of *Morinda citrifolia, Momordica charantia and Centella asiatica* extracts on lipoprotein lipase and 3T3-L1 preadipocytes. J Food Biochem 2011;35:1186-205.
14. Ben Sghaier M, Pagano A, Mousslim M, Ammari Y, Kovacic H, Luis J, *et al.* Rutin inhibits proliferation, attenuates superoxide production and decreases adhesion and migration of human cancerous cells. Biomed Pharmacother 2016;84:1972-8.
15. Ekström AM, Serafini M, Nyrén O, Wolk A, Bosetti C, Bellocco R, *et al.* Dietary quercetin intake and risk of gastric cancer: Results from a population-based study in Sweden. Ann Oncol 2011;22:438-43.
16. Lam TK, Rotunno M, Lubin JH, Wacholder S, Consonni D, Pesatori AC, *et al.* Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue and lung cancer risk. Carcinogenesis 2010;31:634-42.
17. Yang J, Gadi R, Thomson T. Antioxidant capacity, total phenols, and ascorbic acid content of noni (*Morinda citrifolia*) fruits and leaves at various stages of maturity. Micronesica. 2011; 41:167-76.
18. Iriti M, Kubina R, Cochis A, Sorrentino R, Varoni EM, Kabała-Dzik A, *et al.* Rutin, a quercetin glycoside, restores chemosensitivity in human breast cancer cells. Phytother Res 2017;31:1529-38.
19. Hansa N, Amin I, Kamaludin R. p53 and cyclin B1 mediated apoptotic effects of apigenin and rutin in ER+-breast cancer MCF-7 cells. J Teknol 2018;80:133-40.
20. Lopes LB. Overcoming the cutaneous barrier with microemulsions. Pharmaceutics 2014;6:52-77.
21. Nastiti CM, Ponto T, Abd E, Grice JE, Benson HA, Roberts MS, *et al.* Topical nano and microemulsions for skin delivery. Pharmaceutics 2017;9. pii: E37.
22. Lu Y, Wu K, Li L, He Y, Cui L, Liang N, *et al.* Characterization and evaluation of an oral microemulsion containing the antitumor diterpenoid compound ent-11alpha-hydroxy-15-oxo-kaur-16-en-19-oic-acid. Int J Nanomedicine 2013;8:1879-86.
23. Dave K, Alsharif FM, Islam S, Dwivedi C, Perumal O. Chemoprevention of breast cancer by transdermal delivery of α-santalol through breast skin and mammary papilla (Nipple). Pharm Res 2017;34:1897-907.
24. Buranrat B, Suwannaloet W, Naowaboot J. Simvastatin potentiates doxorubicin activity against MCF-7 breast cancer cells. Oncol Lett 2017;14:6243-50.
25. Buranrat B, Mairuae N, Kanchanarach W. Cytotoxic and antimigratory effects of *Cratoxy formosum* extract against hepG2 liver cancer cells. Biomed Rep 2017;6:441-8.
26. Amith SR, Wilkinson JM, Fliegel L. Assessing Na+/H exchange and cell effector functionality in metastatic breast cancer. Biochim Open 2016;2:16-23.
27. Junyaprasert VB, Boonsaner P, Leatwimonlak S, Boonme P. Enhancement of the skin permeation of clindamycin phosphate by aerosol OT/1-butanol microemulsions. Drug Dev Ind Pharm 2007;33:874-80.
28. Mahdi ES, Sakeena MH, Abdulkarim MF, Abdullah GZ, Sattar MA, Noor AM, *et al.* Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. Drug Des Devel Ther 2011;5:311-23.
29. Syed HK, Peh KK. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. Acta Pol Pharm 2014;71:301-9.