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

ORIGINAL ARTICLE

Cytotoxic and Antimigratory Effects on Michigan Cancer Foundation-7 Cells of *Morinda citrifolia* L. Leaf Extract and Formulation of Tablets from Extract

Supavadee Boontha, Benjaporn Buranrat[1], Tasana Pitaksuteepong[2]

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

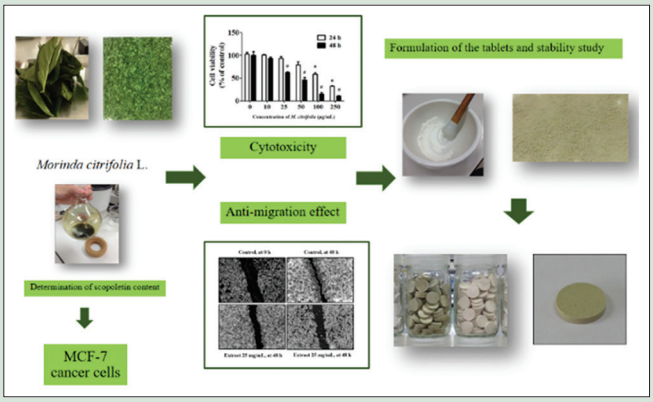
ABSTRACT

Background: Cancer is one of the deadliest diseases known to man. Efforts to combat the disease remain ongoing. *Morinda citrifolia* L. is a medicinal plant which is gaining interest as a natural chemotherapeutic agent for breast cancer treatment. **Objective:** This study aimed to determine the anticancer activity of *M. citrifolia* leaf extract on Michigan cancer foundation 7 (MCF-7) breast cancer cells as well as formulate tablets containing the extract to assess their viability. **Materials and Methods:** *M. citrifolia* leaf extract was prepared using the maceration method. Cytotoxicity and cell migration suppression, representing anticancer activity, were also assessed in MCF-7 cells by the sulforhodamine B assay and wound-healing assay. Tablets containing the extract were formulated using the wet granulation method. The prepared tablets were then evaluated regarding their physical properties and scopoletin content, a marker in the extract, before and after stability testing. **Results:** The extract showed cytotoxicity on MCF-7 cells in a dose- and time-dependent manner with 50% inhibitory concentration values of 39.9 ± 3.5 µg/mL for 48 h. In addition, it showed an antimigratory effect on MCF-7 cells with a significant effect at 25 µg/mL. The prepared tablets had good characteristics and were found to meet the requirements of the United States Pharmacopeia. Moreover, they could maintain their physical properties and scopoletin content over a 2-month period. **Conclusion:** This study showed that *M. citrifolia* leaf extract exhibited potential anti-breast cancer activity. The prepared tablets containing the extract could be a potential formulation for breast cancer treatment. However, the underlying mechanism of the extract as an antibreast cancer agent requires further investigation in subsequent study.

Key words: Antibreast cancer, antimigratory effect, *Morinda citrifolia* L. leaf, scopoletin content, tablets

SUMMARY

- *Morinda citrifolia* leaf extract had scopoletin content and could also inhibit Michigan cancer foundation 7 human breast cancer cell viability and cell migration
- Tablets containing the extract were formulated and consisted of 0.4% w/w *M. citrifolia* extract, polyvinylpyrrolidone K-30, corn starch (F-1) or sodium starch glycolate (F-2), magnesium stearate, talcum and microcrystalline cellulose
- The tablets prepared as formulation F-2 had good physical properties within the acceptable range of the United States Pharmacopeia requirements through a stability period. Thus, it could be a promising formulation to develop for the treatment of breast cancer.



Abbreviations Used: DT: Disintegration time; HPLC: High performance liquid chromatography; IC_{50}: 50% inhibitory concentration; MCF-7: Michigan cancer foundation 7; SRB: Sulforhodamine B assay; USP: The United State pharmacopeia.

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/pr.pr_66_19

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

INTRODUCTION

Medicinal plants in the fight against cancer are currently a topic of focus because treatment with anticancer drugs have shown high toxicity and high drug resistance rates,[1] frequently leading to cancer treatment failure. Among cancers, breast cancer causes the greatest proportion of morbidity and mortality among women worldwide.[2,3] Thus, novel chemotherapeutic agents with greater effects and less toxicity are needed for breast cancer treatment.

Morinda citrifolia L., commonly called Noni or Yor in Thai, is an edible and medicinal tropical plant in the *Rubiaceae* family. The plant grows in many tropical regions of the world, including Thailand and other countries in Southeast Asia.[4,5] The leaves and fruits of *M. citrifolia* are used around the world as internal and external treatments in traditional medicine. Additionally, they have been used extensively as a dietary supplement for many diseases including hypertension, diabetes, arthritis, headaches, cancer, and many more.[5-9] In Thailand, the leaves of *M. citrifolia* are usually used for cooking in a popular Thai dish called

Cite this article as: Boontha S, Buranrat B, Pitaksuteepong T. Cytotoxic and antimigratory effects on Michigan cancer foundation-7 cells of *Morinda citrifolia* L. leaf extract and formulation of tablets from extract. Phcog Res 2020;12:24-8.

"Hor Mhok Bai Yor." Moreover, it has been used in traditional herbal remedies for both single remedies and combination remedies with other ingredients, since the last century.[10] Thus, the leaves of *M. citrifolia* can be used in either food or herbal medicine.

Thani *et al.*[10] reported that the methanolic *M. citrifolia* leaf extract exhibited cytotoxic effects on many cancer cell lines. It also inhibited Michigan cancer foundation 7 (MCF-7) cell viability with an 50% inhibitory concentration (IC_{50}) value of 245.00 ± 8.37 μg/mL. This study revealed that the ethanolic *M. citrifolia* leaf extract had low cytotoxic effect on MCF-7 cells with an IC_{50} value higher than 600 μg/mL. Meli *et al.*[11] reported that the ethanolic *M. citrifolia* shoot extract exhibited highly cytotoxic effects on breast cancer (MDA-MB-231) and colorectal cancer (HT-29) cell lines with an IC_{50} value of 49.72 μg/mL and 65.43 μg/mL, respectively. Therefore, *M. citrifolia* leaf extract will be investigated as an alternative natural source and safer chemotherapeutic agent for breast cancer treatment. Besides cytotoxic effect, the antimigratory effect on breast cancer cell, indicating cancer cell metastasis, is necessary to study the antibreast cancer effect of the medicinal plant extract.[12] Wound scratch-healing assay is generally used to determine the ability of cancer cells to migrate.[13] There are only a few studies concerning the anti-migratory effect of the ethanolic *M. citrifolia* leaf extract on MCF-7 cells. Therefore, the cytotoxic and anti-migratory effects of the extract on MCF-7 breast cancer cells were evaluated in this study.

It has been reported that *M. citrifolia* contains a number of major components including scopoletin, octanoic acid, potassium, Vitamin C, terpenoids, alkaloids, anthraquinones, β-sitosterol, carotene, flavone glycosides, rutin, and many more.[14] Scopoletin, a coumarin compound, can be found in *M. citrifolia* and various medicinal plants, including *Artemisia*, *Brunfelsia, Mallotius* and *Solanum* species.[15] Scopoletin has been reported to have antioxidant and anti-inflammatory activities as well as exhibiting a cytotoxic effect on cancer cells.[16,17] Moreover, it has been used as a reference compound for the cytotoxic activity of *M. citrifolia* leaf extract.[10] Hence, scopoletin content can represent the cytotoxic activity of the extract on the MCF-7 cancer cells in this study. Herbal dietary supplement products are currently available in many different dosage forms, including powders, capsules, tablets or liquids.[18,19] When comparing all the formulations, tablets are more stable and preferable, while giving an accurate amount of the drug and being in a convenient dosage form.[20,21]

Thus, this study aimed to determine the anticancer effect of *M. citrifolia* L. leaf extract on MCF-7 cancer cells as well as formulate tablets containing the extract.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol was purchased from RCI Labscan (Bangkok, Thailand). Acetic acid, acetonitrile, and methanol (high-performance liquid chromatography [HPLC] grade) were purchased from Prolabo (Briare, France). Scopoletin were purchased from Sigma-Aldrich (Steinheim, Germany). MCF-7, a human breast cancer cell line, was purchased from the American Type Culture Collection (ATCC) (ATCC #HTB-22; Manassas, Virginia, USA). Dulbecco's modified Eagle's medium, fetal bovine serum, and other reagents for cell culture were purchased from Gibco-Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Trichloroacetic acid, sulforhodamine B (SRB) and crystal violet were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Corn starch, sodium starch glycolate, magnesium stearate, and talcum were obtained from Union Science Co. Ltd. (Chiang Mai, Thailand). Polyvinylpyrrolidone K-30 (PVP K-30) and microcrystalline cellulose were obtained from TTK science (Bangkok, Thailand).

Preparation of *Morinda citrifolia* extract

The *M. citrifolia* sample was identified and deposited in the Applied Thai Traditional Medicine Department, Faculty of Medicine, Mahasarakham University (specimen voucher no. MSUT_7225) and the Faculty of Science, Mahasarakham University, Thailand. The fresh leaves of *M. citrifolia* were washed, cut into small pieces and then dried in the air. They were also dried at 50°C using a hot-air oven (Memmert, Schwabach, Germany) for 48 h. Subsequently, they were macerated using 95% ethanol, followed by the leaf pieces being ground into a powder. After filtration, the filtrate was further concentrated at a controlled temperature of 50°C by a rotary evaporator (Heidolph, Germany). The percentage yield of the obtained extract was determined by comparing the crude extract dried weight (g) to the *M. citrifolia* leaf powder dried weight (g).

Determination of scopoletin content in the extract

Scopoletin content was determined to be a marker for the obtained extract using HPLC (CBM-20Alite®, Shimadzu, Japan). The assay was performed on a phenomenex luna C_{18} column (150 mm × 4.60 mm, 5 μm). The mobile phase consisted of 0.01M acetic acid, acetonitrile and methanol at a ratio of 60:20:20. The flow rate was run at 1.0 mL/min. The sample (20 μl) was added for ultraviolet detection at 254 nm. Scopoletin content was determined according to the peak area of the chromatogram.

Cell culture and cell viability method

The effects of the extract on cancer cell viability were examined using the SRB method, as previously described.[22,23] The MCF-7 cells were exposed to various concentrations of the extract (0–250 μg/mL) for 24–48 h. After that, the MCF-7 cells had their viability determined in terms of the percentage of cell viability by comparing with the untreated control groups. The IC_{50} was then calculated from the dose-response curve.

Cell migration method

The effects of the extract on cancer cell migration was examined using the wound healing method, as previously described.[22,23] Briefly, MCF-7 cells were scratched and then the complete medium containing various concentrations (0–50 μg/mL) of the extract was added. The wound distance (%) was determined by taking a photo of the uncovered area of the wound and then comparing with the control group.

Preparation and characterization of tablets containing extract

The tablets containing *M. citrifolia* extract were prepared by the wet granulation method. The ingredients of the tablets consisted of PVP K-30 as a binder, corn starch or sodium starch glycolate as a disintegrant with magnesium stearate, and talcum as glidants, while microcrystalline cellulose served as a filler. The tablet formulations are shown in Table 1. The composition of tablet formulations are shown in Table 1.

Table 1: Compositions of the tablet prepared

Compositions	Functions	Amount (mg/tablet)	
		F-1	F-2
Morinda citrifolia extract	Active ingredient	8.0	8.0
PVP K-30 paste (10% w/w)	Binder	38.0	38.0
Corn starch	Disintegrant	83.0	-
Sodium starch glycolate	Disintegrant	-	83.0
Magnesium stearate	Glidant	4.5	4.5
Talcum	Glidant	13.5	13.5
MCC add to	Filler	500	500

PVP K-3: Polyvinylpyrrolidone K-30; MCC: Microcrystalline cellulose

The ingredients were weighed and then mixed together in a mortar using the geometric dilution technique. The extract was dissolved in the binder solution before being mixed with other ingredients. Using a hydraulic press (PerkinElmer, IL, USA), the tablets were compressed with a compaction force of 2 kN and a round flat-faced punch of diameter 10 mm was used. The obtained tablets were then evaluated for their physical properties. The nonofficial evaluation (i.e., thickness and hardness) and official evaluation (i.e., % weight variation, friability test and disintegration test [DT]) were evaluated based on the standard United States Pharmacopeia (USP) guidelines.[24]

Thickness and hardness

Ten tablets were measured using a hardness and thickness tester (Erweka, Germany). Data was reported in terms of mm units for thickness and kilopond (kP) units for hardness.

Weight variation

Twenty tablets were weighed individually and the weight of each tablet was recorded. It was considered acceptable if the average percentage weight variation was in the range of ± 5%.

Friability test

Ten tablets were accurately weighed together and friability was tested using a friability tester (Erweka, Germany) with rotation at 25 rpm for 4 min. The friability of the prepared tablets was determined by comparing the weight of the tablets before rotation (g) to the weight of the tablets after rotation (g). In this test, any free dust was removed from the tablets before weighing, with reweighing carried out following rotation. It was considered acceptable if the friability was not more than 1%.

Disintegration time

Six tablets were tested using a disintegration tester (Erweka, Germany). The DT of the tablet was determined in distilled water at a controlled temperature of 37°C. It was considered acceptable if the DT was not more than 15 min.

Stability test

Physicochemical stability was evaluated at 45°C and relative humidity of 75% for a 2-month period. The prepared tablets and their physical properties were evaluated as previously described, while scopoletin content was determined as follows.

Determination of scopoletin content in tablets

To determine the scopoletin content in the tablets, five obtained tablets were crushed using a mortar and pestle, after which 25 mL of methanol was added for extraction. After extract filtration, filtrate with a volume of 20 µL was analyzed by the HPLC technique.

Statistical analysis

The data values were expressed in terms of mean ± standard deviation and assessed by one-way ANOVA with *post hoc* least significant difference test. The data values were analyzed using Sigma Stat software version 3.5 (Systat Software Inc., San Jose, CA, USA). The data were considered statistically different when $P < 0.05$.

RESULTS

Physical appearance, percentage yield and scopoletin content of *Morinda citrifolia* extract

The extract was a viscous greenish-brown paste with pH of 6.7. The percentage yield of the extract was approximately 30% (w/w). The peaks of the scopoletin had retention times of 4.3 min and it was well-resolved under isocratic elution using the HPLC technique. The scopoletin amount in the extract was 0.58% (w/w).

Effect of extract on cell viability and migration

The effect of *M. citrifolia* leaf extract on breast cancer MCF-7 cell viability was explored by the SRB method. This study indicated that the extract could decrease the cell viability of cancer cells in a dose and time-dependent manner when compared to the control group [Figure 1], with IC_{50} values of 156.9 ± 10.4 µg/mL for 24 h and 39.9 ± 3.5 µg/mL for 48 h. It was found that the extract had a significant role in inhibiting MCF-7 cell viability at a concentration of 25 µg/mL for 24 h and concentration of 100 µg/ml for 48 h. These results revealed that the extract showed four times higher inhibition effect on MCF-7 cell viability with increased incubation time.

The effect of the extract on MCF-7 cell migration was determined by the wound healing assay. The results revealed that the extract inhibited MCF-7 cancer cell migration and was significant at 25–50 µg/mL [Figure 2a and b] with an IC_{50} value of 39.8 ± 16.8 µg/mL. As shown in Figure 2a, cell death seemed to be observed when the MCF-7 cells were treated with 50 µg/mL of the extract for 48 h. This was due to the extract exhibiting a cytotoxic effect on MCF-7 cells with IC_{50} values of 39.9 ± 3.5 µg/mL at 48 h. From the experiments, it was found that the extract had a significant effect on inhibiting MCF-7 cell viability and cell migration.

Properties of prepared tablets

The prepared tablets from both formulations had a diameter of 10 ± 0.43 mm with a round shape and flat surface. As shown in Figure 3, the prepared tablets were light brown in color when compared with the tablets without the extract (data not shown). The two prepared tablet formulations had good characteristics. Their properties are shown in Table 2. The prepared tablets showed a narrow weight variation range with friability of <1%. In addition, the prepared tablets were completely disintegrated within 5 min. Therefore, they were found to be acceptable under the USP standards. The thickness of the prepared tablets was in the range of 3.03–3.15 mm, depending on the size of the die. Tablet hardness

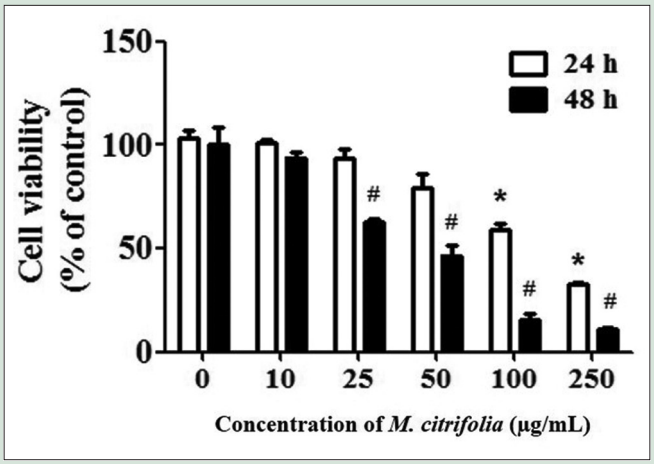


Figure 1: Effect of *Morinda citrifolia* leaf extract on cancer cell, Michigan cancer foundation-7, viability. Cells were exposed to extract (0–250 µg/mL) for 24 and 48 h. All results are percentages of control groups with three independent experiments and represent mean ± standard error of mean values. *$P < 0.05$ as compared with day 0 (cell treated with the extract for 24 h) and #$P < 0.05$ as compared with day 0 (cell treated with the extract for 48 h)

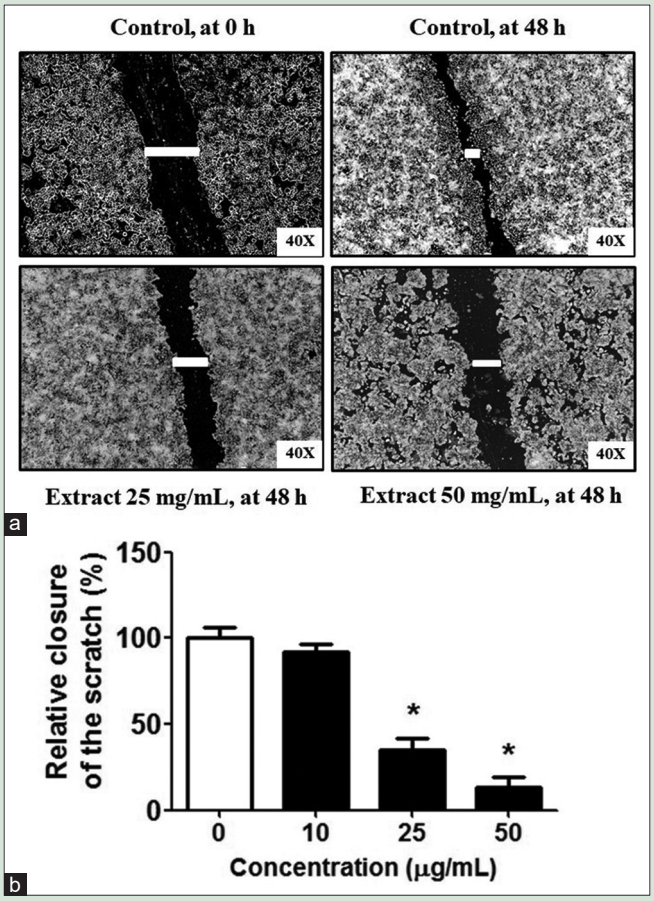


Figure 2: Effect of *Morinda citrifolia* leaf extract on cancer cell, Michigan cancer foundation-7, migration. Cells were exposed to extract (0–50 µg/mL) for 48 h and migration was captured using inverted microscopy (Magnification ×40) (a). All results are percentage of control groups with three independent experiments and represent mean ± standard error of mean values. *P < 0.05 when compared with control (b)

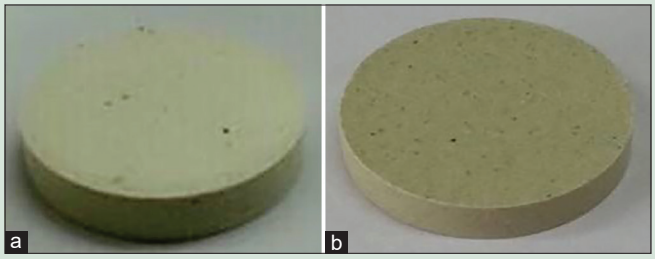


Figure 3: The physical appearance of the tablets prepared (F-1) before (a) and after stability test (b)

Table 2: Physical properties of the tablet prepared before and after stability test

Tablet properties	F-1		F-2	
	Day 0	Day 60	Day 0	Day 60
Thickness (mm)	3.15±0.03	3.09±0.03	3.03±0.03	3.01±0.03
Hardness (kP)	8.76±0.32	5.76±0.55*	11.02±0.81	9.15±0.49
Weight variation (mg)	501.80±2.30	502.30±5.60	508.80±6.10	500.60±3.30
Weight variation (%)	0.97	3.20	3.28	3.47
Friability (%)	0.16	0.76	0.40	0.44
DT (min)	4.54±0.31	0.57±0.21*	3.33±0.15	4.21±1.06

*P<0.05 as compared with day 0. DT: Disintegration time

was in the range of 8.76–11.02 kP, indicating that the tablets should be strong enough to resist the packing and shipping process for the finished product, as revealed by the data from tablet tests.

Stability study

After the stability test, the tablets prepared from the two formulations were a dark brown color. The thickness and hardness of the prepared tablets were decreased, whereas weight variation and % friability were increased [Table 2]. However, the % weight variations and % friability were within the acceptable working range of the USP requirements. The hardness of the tablets prepared using corn starch (F-1) was reduced, resulting in faster DT, while the results of the tablets prepared using sodium starch glycolate (F-2) were different, though not significantly. The tablet formulation F-2 showed better physical properties than tablet formulation F-1 after the stability test. The scopoletin amount in both prepared tablet formulations were reduced after the stability test, as shown in Figure 4. However, they were not significantly different in terms of the decrease in the scopoletin content of the prepared tablets.

DISCUSSION

Presently, medicinal plants are considered to be effective sources of anticancer agents based on their lower toxicity. Many anticancer agents comprise plant-derived compounds including vinblastine, vincristine, paclitaxel, topotecan, and irinotecan.[25] Moreover, herbal dietary supplements are used in traditional cancer treatment. This investigation revealed that the extract from *M. citrifolia* leaves had scopoletin content of 0.58% (w/w) and inhibited viability and migration in MCF-7 cells. The SRB method and wound scratch healing method are usually used to evaluate cell viability and cell metastasis, respectively. Consequently, this study indicated that the extract had anticancer activity on MCF-7 breast cancer cells, as based on its cytotoxic activity on the MCF-7 cells in a dose-and time-dependent manner. This study confirmed that *M. citrifolia* leaf extract exhibited a cytotoxic effect on breast cancer cell lines, as reported by previous studies.[10,11] In this study, the ethanolic *M. citrifolia* leaf extract showed higher potential cytotoxic effect on human MCF-7 cancer cells with lower IC_{50} value when compared with the results from previous research.[10] This might be due to a different in the percentage of extraction solvent. Also, *M. citrifolia* leaf extract showed higher potential cytotoxic effect on breast cancer (MDA-MB-231) cell lines with lower IC_{50} value and shorter incubation time, as previously reported by Meli *et al*.[11] The *M. citrifolia* leaf extract seemed to be selective and safer because previous studies reported that *M. citrifolia* extract did not show any cytotoxic effect on normal cells, including mice fibroblast (L929) and mouse fibroblast (BALB/c 3T3) cell lines after incubation with the extract.[11,26] The suppression effect on MCF-7 cell migration of the extract was observed, with the results showing that the extract had an anti-migratory effect, indicating inhibited metastasis on MCF-7 cells. The mechanism of *M. citrifolia* extract for growth inhibition on MCF-7 cancer cells will be identified by using cell cycle analysis, colony formation, caspase-three activity, and reactive oxygen species formation.

When preparing the tablet formulation containing the extract, the finished product is light brown, flat and round with satisfactory characteristics according to USP guidelines. The tablet hardness and % friability represent the tablet strength and resistance to friability for the packing and shipping process of the finished product, as described

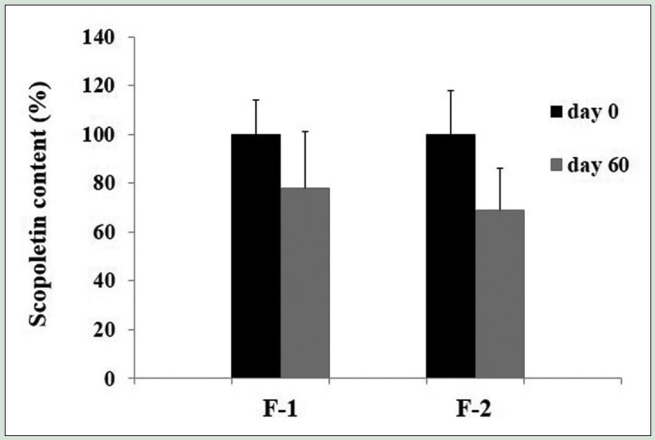


Figure 4: Percentage remaining of scopoletin content of the extract formulated in F-1 and F-2 formulations following stability study. The results are presented as mean ± standard deviation values ($n = 3$)

above, whereas the DT represented the initial prominent step of the tablets to disintegrate into smaller granules in solution, which are then soluted in the medium. Generally, the faster the DT of the tablets, the faster the onset of action will be.[27] In this study, corn starch or sodium starch glycolate was used as a disintegrant and added to give a faster DT. After the stability study, the physical properties of the *M. citrifolia* tablets exhibited no significant differences. This result indicated that the tablets could remain stable throughout the duration of the stability test, with no significant decrease in scopoletin content. However, stability over a longer time period and the dissolution test of the prepared tablets will be determined in future studies.

CONCLUSION

This study revealed that *M. citrifolia* leaf extract had a scopoletin content of 0.58% (w/w) and could inhibit viability and migration in the MCF-7 cell. Therefore, the extract has the potential for development as an anticancer agent for breast cancer. Further, the tablet formulation consisting of 0.4% w/w *M. citrifolia* extract, PVP K-30, sodium starch glycolate, magnesium stearate, talcum, and microcrystalline cellulose could potentially be developed for the treatment of breast cancer. However, further study is needed to understand the underlying mechanism of the extract as a chemotherapeutic agent for breast cancer and the possibility of development as a dietary supplement formulation.

Acknowledgements

The authors would like to express our thanks to Assistant Prof. Dr. Saengrawee Sutthiparinyanont for HPLC technical assistance and Dr. Jolyon Dodgson for his editing assistance and advice on English expression in the manuscript.

Financial support and sponsorship

This study was financially supported by the School of Pharmaceutical Sciences, University of Phayao, Thailand and Thailand Research Fund (MRG6080071).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mitra S, Dash R. Natural products for the management and prevention of breast cancer. Evid Based Complement Alternat Med 2018;2018:1-24.
2. Carter D. New global survey shows an increasing cancer burden. Am J Nurs 2014;114:17.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2016;66:7-30.
4. Pawlus AD, Kinghorn DA. Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). J Pharm Pharmacol 2007;59:1587-609.
5. Wang MY, Peng L, Weidenbacher-Hoper V, Deng S, Anderson G, West BJ. Noni juice improves serum lipid profiles and other risk markers in cigarette smokers. Sci World J 2012;2012:1-8.
6. Nayak BS, Marshall JR, Isitor GA, Adogwa A. Hypoglycemic an hepatoprotective activity of fermented fruit juice of *Morinda citrifolia* (Noni) in diabetic rats. Evid Based Complement Alternat Med 2011;6:1-5.
7. 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.
8. Wang MY, Su C. Cancer preventive effect of *Morinda citrifolia* (Noni). Ann N Y Acad Sci 2001;952:161-8.
9. 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.
10. Thani W, Vallisuta O, Siripong P, Ruangwises N. Anti-proliferative and antioxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. Southeast Asian J Trop Med Public Health 2010;41:482-9.
11. Meli MA, Shafie NH, Loh SP, Rahmat A. Anti-proliferative and apoptosis-inducing effects of *Morinda citrifolia* L. shoot on breast, liver, and colorectal cancer cell lines. Mal J Med Health Sci 2019;15 Suppl 1:129-35.
12. Hulkower KI, Herber RL. Cell migration and invasion assays as tools for drug discovery. Pharmaceutics 2011;3:107-24.
13. Amith SR, Wilkinson JM, Fliegel L. Assessing Na/H+exchange and cell effector functionality in metastatic breast cancer. Biochim Open 2016;2:16-23.
14. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, *et al. Morinda citrifolia* (Noni): A literature review and recent advances in Noni research. Acta Pharmacol Sin 2002;23:1127-41.
15. Seo EJ, Saeed M, Law BY, Wu AG, Kadioglu O, Greten HJ, *et al.* Pharmacogenomics of scopoletin in tumor cells. Molecules 2016;21:496.
16. Witaicenis A, Seito LN, da Silveira Chagas A, de Almeida LD Jr., Luchini AC, Rodrigues-Orsi P, *et al.* Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives. Phytomedicine 2014;21:240-6.
17. Asgar MA, Senawong G, Sripa B, Senawong T. Scopoletin potentiates the anti-cancer effects of cisplatin against cholangiocarcinoma cell lines. Bangladesh J Pharmacol 2015;10:69-77.
18. Gawron-Gzella A, Marek P, Chanaj J, Matławska I. Comparative analysis of pharmaceuticals and dietary supplements containing extracts from the leaves of Ginkgo biloba L. Acta Pol Pharm 2010;67:335-43.
19. West BJ, Deng S, Isami F, Uwaya A, Jensen CJ. The potential health benefits of noni juice: A review of human intervention studies. Foods 2018;7. pii: E58.
20. Nguyen TH, Morton DA, Hapgood KP. Application of the unified compaction curve to link wet granulation and tablet compaction behavior. Powder Technol 2013;240:103-15.
21. Kucinskaite A, Sawicki W, Briedis V, Sznitowska M. Fast disintegrating tablets containing *Rhodiola rosea* L. Extract. Acta Pol Pharm 2007;64:63-7.
22. Buranrat B, Suwannaloet W, Naowaboot J. Simvastatin potentiates doxorubicin activity against MCF-7 breast cancer cells. Oncol Lett 2017;14:6243-50.
23. 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.
24. The United State Pharmacopeial Convention. The United State Pharmacopeia 33-the National Formulary 28. Maryland: United Book Press; 2010.
25. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol 2005;100:72-9.
26. Serafini MR, de Lima CM, Santos RC, Dória GA, de Jesus JY, de Melo MG, *et al.* Pre-clinical toxicity of *Morinda citrifolia* Linn. Leaf extract. Afr J Biotechnol 2011;10:14566-72.
27. Markl D, Zeitler JA. A review of disintegration mechanisms and measurement techniques. Pharm Res 2017;34:890-917.