CYTOTOXIC ACTIVITY OF ESSENTIAL OILS OF *MENTHA* SPP. ON HUMAN CARCINOMA CELLS

Wanlop Weecharangsan[1, *], Worapan Sithithaworn[1], Pongpan Siripong[2]

[1] Faculty of Pharmacy, Srinakharinwirot University, Nakhonnayok 26120, Thailand
[2] National Cancer Institute of Thailand, Bangkok, Thailand

ABSTRACT: The study aimed to evaluate the cytotoxic activity of essential oils of *Mentha arvensis* and *Mentha spicata* in human carcinoma cells. The essential oils were extracted by hydrodistillation. The chemical compositions of the essential oils were determined using GC-MS analysis. The extracted essential oils were dissolved in DMSO and diluted with growth medium at the essential oil concentrations of 1, 10, 100, 1,000 and 10,000 μg/mL, and evaluated for cytotoxic activity in KB and HeLa human carcinoma cells using MTT assay. The major components of the *Mentha arvensis* and *Mentha spicata* essential oils were menthol, menthone and limonene, and limonene and carvone, respectively. All essential oils showed the cytotoxic activity with IC_{50} value of 142.0 ± 92.2 and 243.3 ± 151.1 μg/mL against KB cells and with IC_{50} value of 178.4 ± 92.1 and 779.4 ± 673.3 μg/mL against HeLa cells with *Mentha arvensis* and *Mentha spicata*, respectively. This study suggests that the essential oils of these *Mentha* spp. have potential to be promising candidates for therapeutic and preventive application against cancers.

Keywords: Cytotoxic activity, essential oil, *Mentha arvensis*, *Mentha spicata*

INTRODUCTION

Currently, cancer is one of the most severe diseases, and the incidence of death rate of cancer patients have been increasing in Thailand and in the world. Considerable attention has been focused on the prevention of cancer. The essential oils of plants including *Eugenia caryophyllata* [1], *Myrica gale* L. [2], *Salvia bracteata* and *Salvia rubifolia* [3]), *Cymbopogon flexuosus* [4], *Amomum tsao-ko* [5] have been reported to be cytotoxic to human cancer cells.

Mentha is spp. the edible plants available in Thailand and other tropical countries. The essential oils of *Mentha* spp. have been shown to exert biological activities including antibacterial [6, 7], fumigating [8], anticandida [9, 10]. The extracts of *Mentha piperita* and *Mentha aquatica* have been reported to cytotoxic to cancer cells [11-13].

In this present study, the essential oils of *Mentha arvensis* and *Mentha spicata* were evaluated for cytotoxic activity in KB human oral carcinoma and HeLa human cervical carcinoma cells. The physical property was characterized, and the components of the essential oils were also analyzed.

* *Correspondence to: Wanlop Weecharangsan*
E-mail: wanlop@swu.ac.th

MATERIALS AND METHODS

Plant material

Mentha arvensis L. and *Mentha spicata* L., family Lamiaceae, were grown in the experimental botanical garden of the Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhonnayok, Thailand. The plants were grown in March 2010, and harvested in June 2010.

Extraction of essential oils

The essential oils were obtained from freshly harvested leaves (1,000 g) by hydrodistillation for 2 h. The extracted essential oils were dried over anhydrous sodium sulfate and stored in the dark at -20°C until analyzed and tested.

Analysis of volatile oil using gas chromatography-mass spectroscopy (GC-MS)

The essential oils were analyzed using an Agilent GC/MS (Model 7685 Series Injector)

Cite this article as: Weecharangsan W, Sithithaworn W, Siripong P. Cytotoxic activity of essential oils of *mentha* spp. on human carcinoma cells. J Health Res. 2014: 28(1); 9-12.

Table 1 Physical characteristics of essential oils of *Mentha* spp.

Mentha spp.	Oil yield (w/w) (%)	Refractive index (at 25°C)	Density (g/mL)	Physical aspect
M. arvensis	0.54	1.45	0.906	clear
M. spicata	0.23	1.48	0.862	viscous clear

equipped with a capillary column HP-INNOWax (30 m × 0.32 mm, 0.25 µm thickness). Helium gas was used as the carrier gas at an inlet pressure of 1.34 psi, with temperature programming initially set at 80°C and increasing to 200°C at 5°C/min. MS was performed by EI positive mode at an ionization voltage of 70 eV. The oil components were identified by comparing their mass spectra and relative retention indices of the peaks with those of standard compounds including menthol, menthone, pulegone (Fluka Chemie, Buchs, Switzerland), and limonene (Sigma-Aldrich, St. Louis, MO, USA) under the same conditions. MS profiles of chemical compositions were matched to Wiley library. The percentage composition was calculated from GC peak area.

Cell culture

KB human oral carcinoma cells were obtained from Natural Products Research Section, Reasearch Division, National Cancer Institute, Bangkok, Thailand. HeLa human cervical carcinoma cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were maintained as a monolayer culture in MEM (Invitrogen, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum, 100 µg/mL streptomycin 100 U/mL penicillin and 1% amphotericin B. The cells were maintained at 37°C in a humidified incubator with 5% CO_2.

Cytotoxic activity assay

KB or HeLa cells were seeded into 96-well plates at a density of 1.5×10^4 and 3×10^4 cells/cm^2, respectively, in 100 µl of completed medium. After 16-18 h of incubation, the medium was removed and the cells were rinsed with phosphate-buffered saline (PBS, pH 7.4), and then added with the medium containing increasing concentrations of the essential oils (1-10,000 µg/mL). The cells were incubated with the essential oils for 72 h at 37°C in a humidified incubator with 5% CO_2. The final concentration of DMSO in the culture medium was maintained less than 0.7% (v/v) to avoid toxicity of the solvent. Untreated cells and cells incubated with 1 µM doxorubicin were used as controls. After treatment, the cells were incubated with 100 µl of MTT solution (1 mg/mL in medium without FBS) for 4 h at 37 °C. The medium was removed, and 100 µl of DMSO was added to each well to dissolve the formazan crystals. The absorbance of formazan solution was detected at 570 nm by a microplate spectrophotometer (Zenyth 200 rt; Anthos Labtech Instruments GmbH, Salzburg, Austria). The viability of untreated control cells was arbitrarily defined as 100%.

Table 2 Chemical compositions of essential oils of *Mentha* spp.

Component (%)	*Rt*[a]	*M. arvensis*	*M. spicata*
Limonene	2.65	0.13	44.12
Menthone	5.75	1.04	0.72
Neo-Menthol	8.06	1.92	-
Menthol	8.88	92.38	0.80
Pulegone	8.96	-	0.61
Dihydrocarvyl acetate	9.47	-	1.92
Carvone	10.68	-	41.31

[a]Retention time (min)

Statistical analysis

The results are represented quadruplicately as the mean±standard error (S.E.). Statistical significance of differences in growth inhibition were examined using one-way analysis of variance (ANOVA) followed by an LSD post hoc test. The treated cells were compared to the untreated cells. The significance level was set at $p < 0.05$.

RESULTS

Physical characteristics and chemical compositions of essential oils of Mentha spp.

The yields of the essential oils based on the weight of fresh samples of *Mentha arvensis* and *Mentha spicata* were 0.54 and 0.23%, respectively. Table 1 presents the characteristics of the essential oils. The physical characteristics of *Mentha* spp. essential oils were different. *Mentha arvensis* essential oil was a clear liquid, and that of *Mentha spicata* was viscous clear liquid.

The chemical compositions of the essential oils of *Mentha* spp. are listed in Table 2. The components of the essential oil of *Mentha arvensis* were menthol (92.38%), neo-menthol (1.92%), menthone (1.04%) and limonene (0.13%). The essential oil of *Mentha spicata* contained limonene (44.12%), carvone (41.31%), dihydrocarvyl acetate (1.92%), menthol (0.80%),

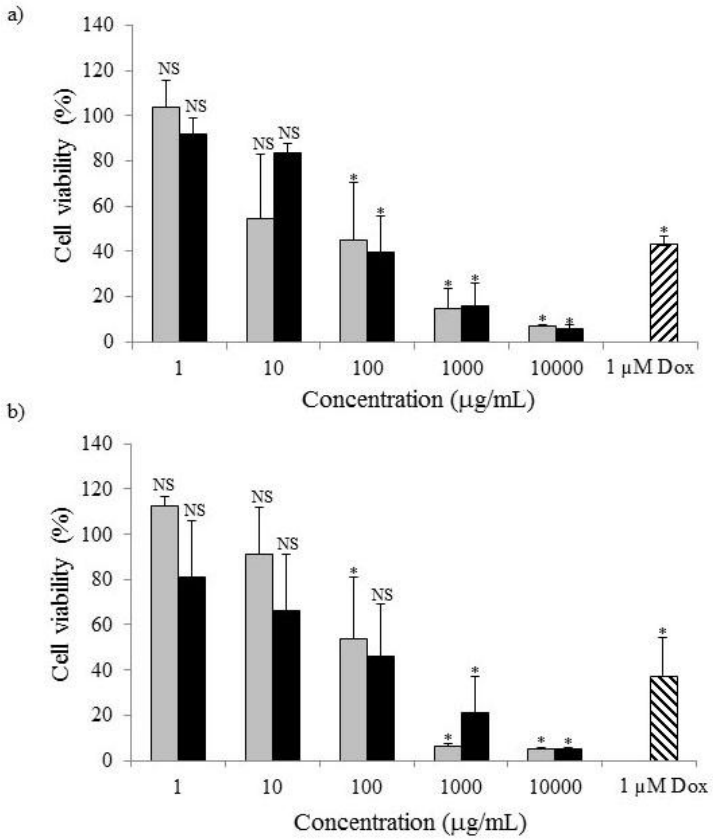

Figure 1 Antiproliferative activity of the essential oils of *Mentha arvensis* and *Mentha spicata* with increasing concentrations of the essential oils (1-10,000 μg/mL) in KB human oral carcinoma (a) and HeLa human cervical carcinoma cells (b). Cells were incubated with the essential oils in growth medium for 72 h. Grey bar: *Mentha arvensis*; dark bar: *Mentha spicata*; dash bar: 1 μM doxorubicin. NS, not significant; *, $p < 0.05$ when compared with untreated cells.

Table 3 IC_{50} of *Mentha* essential oils on KB human oral carcinoma and HeLa human cervical carcinoma cells

***Mentha* spp.**	**IC_{50}[a] ± S.E. (μg/mL)**	
	KB	**HeLa**
M. arvensis	142.0 ± 92.2	178.4 ± 92.1
M. spicata	243.3 ± 151.1	779.4 ± 673.3

[a]IC_{50} values are the concentrations which inhibit cell growth 50% of the control. Each value represents quadruplicately the mean ± SE.

menthone (0.72%) and pulegone (0.61%).

Cytotoxic activity

KB human oral carcinoma and HeLa human cervical carcinoma cells were used as a representative of human carcinoma cells. The cytotoxic activity of the essential oils of *Mentha arvensis* and *Mentha spicata* was evaluated in KB human oral carcinoma and HeLa human cervical carcinoma cells. Figure 1 shows the cytotoxic activity of the essential oils of *Mentha arvensis* and *Mentha spicata* increased with an increasing concentration of the essential oils in KB human oral carcinoma and HeLa human cervical carcinoma cells. Doxorubicin, a chemotherapeutic drug used as a positive control at a concentration of 1.0 μM, caused cell growth inhibition of 56.1±2.8 ($p < 0.05$) and 62.4±16.8% ($p < 0.05$), in KB and HeLa cells, respectively. The essential oils of *Mentha arvensis* and *Mentha spicata* showed the cytotoxic activity with IC_{50} values of 142.0 and 243.3 μg/mL, and 178.4 and 779.4 μg/mL in KB and HeLa cells, respectively (Table 3).

DISCUSSION

The essential oils of plants have been reported to be cytotoxic to human cancer cells [1-5]. Our results showed that the essential oils of *Mentha arvensis* and *Mentha spicata* had potent cytotoxic in KB human oral carcinoma and HeLa human cervical carcinoma cells. The cytotoxic activity of *Mentha* essential oils could be due to the major compositions including menthol, limonene, carvone. *Mentha arvensis* had major component of

menthol (92.38%), and *Mentha spicata* of limonene (44.12%) and carvone (41.31%).

Menthol inhibited growth of DU145 human prostate cancer cell with increasing concentration [14]. Menthol induced cell death in T24 human bladder cancer cells [15]. Limonene, one of the components in the essential oil of *Tanacetum gracile* inhibited HL-60 human leukemia cells, and induced cell apoptosis [16]. L-limonene, one of the components in the extract of *Foeniculum vulgare* inhibited MCF7 breast cancer cells and HepG2 liver cancer cells [17]. Hydroisobenzofuran analogs of sclerophytin prepared from (*S*)-(+)-carvone exhibited growth inhibition in KB3 carcinoma, RPMI-8226 leukemia, and HOP-92 non-small cell lung cancer cells [18].

CONCLUSION

The essential oils of *Mentha arvensis* and *Mentha spicata* have potent growth inhibition in KB human oral carcinoma and HeLa human cervical carcinoma cells in dose-dependent manners. This study suggests that the essential oils of these *Mentha* spp. have potential to be promising candidates for therapeutic or preventive applications against cancers.

ACKNOWLEDGEMENT

This study was financially supported by Srinakharinwirot University (No. 199/2553).

DECLARATION OF INTEREST

The authors have no conflict of interest.

REFERENCES

1. Yoo CB, Han KT, Cho KS, Ha J, Park HJ, Nam JH, et al. Eugenol isolated from the essential oil of *Eugenia caryophyllata* induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells. Cancer Lett. 2005; 255(1): 41-52.
2. Sylvestre M, Legault J, Dufour D, Pichette A. Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. Phytomedicine. 2005; 12(4): 299-304.
3. Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA, et al. Essential oil of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J Ethnopharmacol. 2009; 126(2): 265-72.
4. Sharma PR, Monhe DM, Muthiah S, Pal HC, Shahi AK, Saxena AK, et al. Anticancer activity of an essential oil from *Cymbopogon flexuosus*. Chem Biol Interact. 2009; 179(2-3): 160-8.
5. Yang Y, Yang Y, Runwei Y, Guolin Z.Cytotoxic, apoptotic and antioxidant activity of the essential oil of *Amomum tsao-ko*. Bioresource Technol. 2010; 101: 4205-11.
6. Imai H, Osawa K, Yasuda H, Hamashima H, Arai T, Sasatsu M. Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. Microbios. 2001; 106(suppl.1): 31-9.
7. Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S. Antibacterial properties of the essential oils from Thai medical plants. Fitoterapia 2005; 76(2): 233-6.
8. Lee SE, Lee BH, Choi WS, Park BS, Kim JG, Campbell BC. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L). Pest Manag Sci. 2001; 57(6): 548-53.
9. Tampieri MP, Galuppi R, Macchioni F, Park BS, Kim JG, Campbell BC. The inhibition of *Candida albicans* by selected essential oils and their major components. Mycopathologia. 2005; 159(3): 339-45.
10. Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C. Anti-*Candida* activity of Brazilian medicinal plants. J Ethnopharmacol. 2005; 97(2): 305-11.
11. Kumar A, Samarth RM, Yasmeen S, Sharma A, Sugahara T, Terado T, et al. Anticancer and radioprotective potentials of *Mentha piperita*. Biofactors 2004; 22(1-4): 87-91.
12. Jain D, Pathak N, Khan S, Raghuram GV, Bhargava A, Samarth R, et al. Evaluation of cytotoxicity and anticarcinogenic potential of Mentha leaf extracts. Int J Toxicol. 2011; 30(2): 225-36.
13. Conforti F, Ioele G, Statti GA, Marrelli M, Ragno G, Menichini F. Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. Food Chem Toxicol. 2008; 46(10): 3325-32.
14. Wang Y, Wang X, Yang Z, Zhu G, Chen D, Meng Z. Menthol inhibits the proliferation and motility of prostate cancer DU145 cells. Pathol Oncol Res. 2012; 18(4): 903-10.
15. Li Q, Wang X, Yang Z, Wang B, Li S. Menthol induces cell death via the TRPM8 channel in the human bladder cancer cell line T24. Oncology 2009; 77(6): 335-41.
16. Verma M, Singh SK, Bhushan S, Pal HC, Kitchlu S, Koul MK, et al. Induction of mitochondrial-dependent apoptosis by an essential oil from *Tanacetum gracile*. Planta Med. 2008; 74(5): 515-20.
17. Mohamad RH, El-Bastawesy AM, Abdel-Monem MG, Noor AM, Al-Mehdar HA, Sharawy SM, et al. Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). J Med Food. 2011; 14(9): 986-1001.
18. Bateman TD, Joshi AL, Moon K, Galitovskaya EN, Upreti M, Chambers TC, et al. Synthesis and anticancer activity of sclerophytin-inspired hydroisobenzofurans. Bioorg Med Chem Lett. 2012; 19(24): 6898-901.