Original Article

Pharm Sci Asia 2018; 45 (3), 154-160
DOI : 10.29090/psa.2018.03.154

Differentiation-inducing effect in human colon cancer cells of essential oils

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ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form
4 June 2017
Accepted 15 June 2017

KEYWORDS:
Essential oils;
Chemopreventive; Colon cancer; Cell differentiation

ABSTRACT

Essential oils are naturally occurring, volatile aromatic compounds. Many essential oils are suitable for use as flavoring agents and are safe for human consumption. In this study, the effects of essential oils on colon cancer cell differentiation were evaluated. Essential oils regularly used as food ingredients were selected for investigation. These included betel vine oil, citronella oil, clove oil, clove leaf oil, galangal oil, guava leaf oil, hairy basil oil, holy basil oil, kaffir lime oil, lemongrass oil, lesser galangal oil, lime oil, sweet basil oil, and turmeric oil. Human rectum adenocarcinoma cells (RCM-1) was a study model for cell differentiating induction. The result showed that most essential oils were able to induce call differentiation except lemongrass oil and lime oil which showed lower fractions of duct formation index (FFI) than 3-methylthiopropionic acid ethyl ester (MTPE), the positive control from Japanese pickling melon. Hairy basil oil, holy basil oil, and guava leaf oil were highly effective in inducing cell differentiation effect with FFIQ 4.71 ± 0.20, 4.03 ± 0.26, and 3.81 ± 1.05, respectively. The effect of inducing differentiation was interpreted by compared with the FFI of MTPE. Our finding indicated no dose dependent manner in this study. Interestingly high concentration of some essential oils exhibited cytotoxicity on colon cancer cells. The results of this study can be used as preliminary data for further study on novel chemoprevetative agents, with less adverse effects.

1. INTRODUCTION

Colorectal cancer is a global health problem that affects all parts of the world. It is considered as one of the most common cancer in the world[1]. At present, the death from patients with colorectal cancer have been over 600,000 people per annual worldwide [2]. Colorectal cancer, also known as colon cancer or rectal cancer starts from abnormal growth of colon or rectum inner wall that lead to the forming of malignant tumors or adenocarcinoma[3,4]. To date, treatment for colon cancer is by surgery combined with anti-cancer drug therapy to eradicate any remaining of tumorigenic cells[5-7]. However, drug therapy for patients with cancers can cause serious side effects and consumes high economic cost[8]. Therefore, prevention is always better than treatment. Medicinal plants are of interest as alternative medicine for cancer treatment and prevention. Previous studies have reported that natural compounds could be potentially used for treatment and prevention of colon cancers[9,11].

Essential oils are natural compounds of aromatic plants. They can be found in leaves,

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flowers, fruit, seeds, barks, and roots of plants. Essential oils are quite popular worldwide and have been used for consumption and aromatherapy. There is little evidence on essential oils and colon cancer although many researchers have reported on their anti-inflammatory activities[12,14]. Some studies have been reported on anti-proliferative activity of Thai essential oil[15]. Interestingly, Thai edible plants that can also produce essential oils such as citronella (*Cymbopogon nadus* L.), holy basil (*Ocimum tenuiflorum* L.), sweet basil (*Ocimum basilicum* L.), clove (*Syzygium aromaticum* L.), lime (*Citrus aurantifolia* (Christm. et Panz.) Swing), kaffir lime (*Citrus hystrix* L.), hairy basil (*Ocimum americanum* L.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), Galangal (*Alpinia galanga* L.), guava (*Psidium guajava*), betel vine (*Piper betle* L.), lesser galangal (*Boesenbergia rotunda* (L.) Mansf.), and turmeric (*Curcuma longa* L.).

Thai edible plants used in folk medicines have been reported on many biological activities related to cancers. Citronella oil has been reported on cytotoxicity in human breast cancer (MCF-7) and non-tumorigenic (Vero) cell line. Holy basil oil has been reported an ability to prevent fibrosarcoma tumors induced by 20-methylcholanthrene which acted by increasing the level of endogenous antioxidants in Swiss albino mice. Citronella, sweet basil, lemongrass, turmeric, kaffir lime, betel vine, hairy basil and guava leaf oils demonstrated anti-proliferative activity on human mouth epidermal carcinoma (KB) and murine leukemia (P388) cell lines. Moreover, clove oil also showed cytotoxicity effect on breast cancer (MCF-7 and MDA-MB-231), prostate cancer (DU-145), cervical cancer (HeLa), and esophageal cancer (TE-13) cell lines[15,16]. Although several essential oils have been reported on biological activity related to cancers, there are no present data on the essential oils on colorectal cancer. Hence, this study was aimed to examine chemopreventive activity of essential oils which were usually used for flavoring and fragrance.

2. MATERIALS AND METHODS

2.1. Plant essential oils and chemicals

All plant essential oils were purchased from Thai-China Flavours and Fragrances Industry Company, Limited. The plant essential oils were from (Table 1):

citronella (*Cymbopogon nadus* L.),
holy basil (*Ocimum tenuiflorum* L.),
sweet basil (*Ocimum basilicum* L.),
clove (*Syzygium aromaticum* L.),
lime (*Citrus aurantifolia* (Christm. et Panz.) Swing),
kaffir lime (*Citrus hystrix* L.),
hairy basil (*Ocimum americanum* L.),
lemongrass (*Cymbopogon citratus* (DC.) Stapf),
galangal (*Alpinia galanga* L.),
guava (*Psidium guajava*),
betel vine (*Piper betle* L.),
lesser galangal (*Boesenbergia rotun da* (L.) Mansf.),
turmeric (*Curcuma longa* L.)

The 3-methylthiopropionic acid ethyl ester (MTPE) from Japanese pickling melon *Cucumis melo* var. *conomon* was kindly provided by Professor Yasushi Nakamura, Kyoto Prefectural University, Japan.

Table 1. Specification of plant essential oils used in this study

Scientific name	Family	Common name	Part used	Color/ appearance	Chemical composition
Piper betel L.	PIPERACEAE	Betel vine	leaves	medium brown to bright yellow	Chavicol, chavibetol, cineol, eugenol
Cymbopogon nadus L.	POACEAE	Citronella	leaves	yellow to green	Citronellol, geraniol
Syzygium aromaticum L.	MYRTACEAE	Clove	buds	Pale yellow to yellowish brown	Eugenol, eugenyl acetate, beta-caryophyllene, alpha-humulene
Syzygium aromaticum L.	MYRTACEAE	Clove leaf	leaves	brown	Eugenol, eugenyl acetate, beta-caryophyllene, alpha-humulene
Alpinia galanga L.	ZINGIBERACEAE	Galangal	rhizomes	Yellow to olive brown	1,8-cineol, alpha-pinene, eugenol, camphor, methyl cinnamate, sesquiterpenes
Psidium guajava L.	MYRTACEAE	Guava leaf	leaves	pale yellow to yellow	Caryophyllene, cineol, tannin, sesquiterpenoids, triterpenoid compounds
Ocimum americanum L.	LAMIACEAE	Hairy basil	leaves	colorless to pale yellow	Limonene, 1,8-cineol, theta-cadinene, alpha-pinene, alpha-terpineol
Ocimum tenuiflorum L.	LAMIACEAE	Holy basil	leaves	pale yellow to yellow	Camphor, cineol, eugenol, limonene, rosmarinic acid
Citrus hystrix DC.	RUTACEAE	Kaffir lime	leaves	colorless to pale yellow	Beta-pinene, limonene, caryophyllene, sabinene, citronellol, 1,8-cineol
Cymbopogon citratus (DC.) Stapf	POACEAE	Lemongrass	leaves	reddish yellow to brownish red	Citral, myrcene, geraniol, nerol, farnesol, citronellol
Boesenbergia rotunda (L.) Mansf.	ZINGIBERACEAE	Lesser galangal	rhizomes	colorless to orange-yellow	Camphene, eucalyptol, ocimen, camphor, geraniol
Citrus aurantifolia (Christm. et Panz.) Swings	RUTACEAE	Lime	peel	light oilve	d-limonene, pinene, camphene, bergapten
Ocimum basilicum L.	LAMIACEAE	Sweet basil	leaves	yellowish to greenish	Estragole, linalool, 1,8-cineole
Curcuma longa L.	ZINGIBERACEAE	Turmeric	rhizomes	yellow to orange	Turmerone, ar-turmerone

2.2. Cell cultivation

Human rectum adenocarcinoma cells (RCM-1) is a colon cancer cell line was derived from a 73-year-old female human that was diagnosed as a well-differentiated rectum adenocarcinoma provided by Professor Yasushi Nakamura, Kyoto Prefectural University, Japan. RCM-1 cells were maintained in 45% RPMI 1640 medium with 45% Ham's F12 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% streptomycin and penicillin G. The cells were incubated at 37˚C under a humidified in 5% CO_2.

2.3. Assay for differentiation-inducing effect

All essential oils were dissolved in dimethyl sulfoxide (DMSO) to make the stock solutions. RCM-1 cell line was seeded into 96-well flat-bottom culture plates before adjusted to 1×10^5 cells/well. The cell line was incubated overnight or 90% exhibited contact-insensitive growth at 37˚C in 5% CO_2 before treated with each essential oils in triplicates at various concentrations from 0 - 500 µg/ml. 3-methylthiopropionic acid ethyl ester (MTPE) from Japanese Pickling Melon *Cucumis melo* var. *conomon* was used as the positive control at the concentrations of 0 - 1 mM/L. After 72 hours, the results were determined by two major criteria: duct formation numbers and the size of ducts[17].

3. RESULTS AND DISCUSSION

The concept of differentiation therapy has been proposed due to toxicity and non-specificity of current cancer therapy. This approach is based on the assumption that specific cancer cells exhibit aberrant patterns of differentiation. Some compounds can induce tumor reprogramming resulting in a loss in proliferative capacity and induction of terminal differentiation[17]. Many essential oils are considered on the U.S. Food and Drug Administration's (FDA) Generally Recognized as Safe (GRAS) list, which permits the use of certain essential oils for use in food products. Therefore, this study was aimed to examine on essential oils because of safety issue since essential oils have been regularly used in culinary.

In this study, essential oils were evaluated on chemopreventive activity using human rectum adenocarcinoma cells (RCM-1) as a study model for cell differentiating induction. The effects of essential oils were compared with MTPE, a positive control from Japanese pickling melon *Cucumis melo* var. *conomon*. MTPE has been reported in previous studies to inhibit or prevent the activity of a carcinogen properties by duct induced differentiation of RCM-1 cells at 0.25 to 2 mM[18]. Later, the new compound has found in fully ripened Japanese pickling melon, methylthioacetic acid ethyl ester (MTAE), has been demonstrated to be anticarcinogen by inducing duct formation with ED_{50} at 0.61mM[19]. This study used MTPE as the positive control which could induce the fractions of duct formation index at 2.13 ± 1.33. The results demonstrated that essential oils possessed some abilities in inducing cell differentiation (Figure 1). All essential oils could increase the numbers of duct formation as shown in Table 2. Compared to MTPE, all essential oils could produce higher fraction numbers with the exception of lemongrass and lime oils. Among those, three essential oils which were hairy basil oil, holy basil oil and guava leaf oil exhibited the highest activity. The fraction numbers were 4.71 ± 0.20, 4.03 ± 0.26, and 3.81 ± 1.05, respectively. When varied the concentrations of three essential oils, there were obviously no dose-dependent pattern (Figure 2). On the other hand, the pure compound, MTPE has shown the activity depending upon the concentration. It could be possibly because of a variety of compounds in essential oils. Hairy basil, holy basil, and guava leaf oils showed the highest differentiation-inducing effect in human colon cancer cells at 31.25, 62.5, and 15.63 ug/ml, respectively. It is noteworthy that hairy basil yielded the greatest activity compared to other tested oils and the positive control.

Figure 1. Differentiation- inducing assay on RCM-1 cell line. RCM-1 cells were seeded into a 96-well plate before adjusted to 1×10^5 cells/well and incubated overnight or 90% exhibited contact-insensitive growth at 37˚C in 5% CO_2. Each essential oils at various concentrations from 0 - 500 µg/ml were added into each well. 3-methylthiopropionic acid ethyl ester (MTPE) from Japanese pickling melon *Cucumis melo* var. *conomon* was used as the positive control at the concentrations of 0 - 1 mM/L. After 72 hours, the results were determined by two major criteria: duct formation numbers and the size of ducts.

Table 2. Fractions of duct formation after treated with a variety of essential oils compared to MTPE.

Essential oils	Fractions of duct formation index
Betel vine	2.87 ± 1.19
Citronella	3.33 ± 1.48
Clove	3.59 ± 1.33
Clove leaf	3.15 ± 0.39
Galangal	3.33 ± 1.74
Guava leaf	3.81 ± 1.05
Hairy basil	4.71 ± 0.20
Holy basil	4.03 ± 0.26
Kaffir lime	2.74 ± 1.82
Lemongrass	2.00 ± 1.06
Lesser galangal	2.99 ± 0.17
Lime	1.56 ± 0.20
Sweet basil	2.65 ± 0.97
Turmeric	2.44 ± 0.24
MTPE	2.13 ± 1.33

Values expressed are mean ± SD of three parallel measurements.

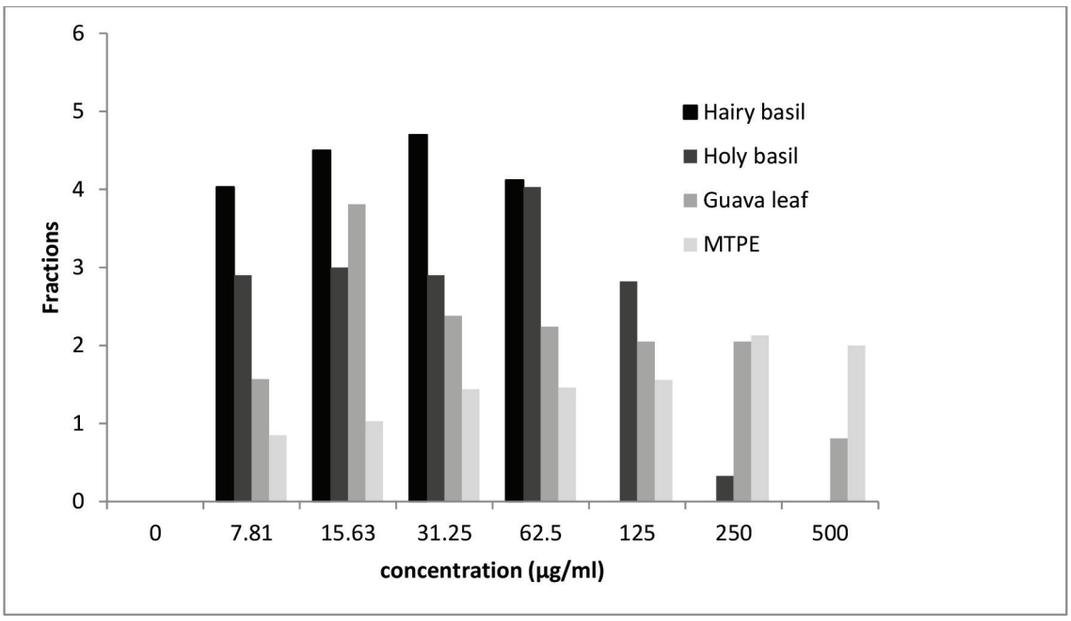

Figure 2. Effect of potential essential oils at various concentrations on colon cancer cell differentiation.

4. CONCLUSIONS

Most essential oils especially hairy basil oil, holy basil oil and guava leaf oil could induce differentiation of the RCM-1 well-differentiated human colon cancer cells. Our findings indicated that these three essential oils could potentially possess the chemopreventative properties. Further investigation should be pursue to identify the major active compounds of potential essential oils.

5. ACKNOWLEDGEMENT

The author wish to thank Miss Pornchanok Monkaewmiss, Miss Patchara Promsurin and staff in the Department of Microbiology, Mahidol University for their assistance.

REFERENCES

1. Liu S, Zheng R, Zhang M, Zhang S, Sun X, Chen W. Incidence and mortality of colorectal cancer in China, 2011. Chin J Cancer Res. 2015;27(1):22-8.
2. Garcia-Albeniz X, Chan AT. Aspirin for the prevention of colorectal cancer. Best Pract Res Clin Gastroenterol. 2011;25(0): 461-72.
3. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138(6):2101-14.e5.
4. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147(2):275-92.
5. Umar A, Viner JL, Hawk ET. The future of colon cancer prevention. Ann N Y Acad Sci. 2001;952:88-108.
6. Jacob BP, Salky B. Laparoscopic colectomy for colon adenocarcinoma: an 11-year retrospective review with 5-year survival rates. Surg Endosc. 2005;19(5):643-9.
7. Kahnamoui K, Cadeddu M, Farrokhyar F, Anvari M. Laparoscopic surgery for colon cancer: a systematic review. Can J Surg. 2007;50(1):48-57.
8. Jolfaie NR, Mirzaie S, Ghiasvand R, Askari G, Miraghajani M. The effect of glutamine intake on complications of colorectal and colon cancer treatment: A systematic review. J Res Med Sci. 2015;20(9):910-8.
9. Wang CZ, Yuan CS. Potential role of ginseng in the treatment of colorectal cancer. Am J Chin Med. 2008;36(6):1019-28.
10. Rajamanickam S, Agarwal R. Natural products and colon cancer: current status

and future prospects. Drug Dev Res. 2008; 69(7):460-71.
11. Aggarwal B, Prasad S, Sung B, Krishnan S, Guha S. Prevention and treatment of colorectal cancer by natural agents from mother nature. Curr Colorectal Cancer Rep. 2013;9(1):37-56.
12. Silva GL, Luft C, Lunardelli A, Amaral RH, Melo DA, Donadio MV, et al. Anti-oxidant, analgesic and anti-inflammatory effects of lavender essential oil. An Acad Bras Cienc. 2015;87(2 Suppl):1397-408.
13. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol. 2003;89 (2-3):277-83.
14. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine. 2002;9(8):721-6.
15. Manosroi J, Dhumtanom P, Manosroi A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett. 2006; 235(1):114-20.
16. Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: A mechanistic view. Biomed Res Int. 2014:154106.
17. Leszczyniecka M, Roberts T, Dent P, Grant S, Fisher PB. Differentiation therapy of human cancer: basic science and clinical applications. Pharmacol Ther. 2001;90(2-3): 105-56.
18. Nakamura Y, Nakayama Y, Ando H, Tanaka A, Matsuo T, Okamoto S, et al. 3-Methyl-thiopropionic acid ethyl ester, isolated from Katsura-uri (Japanese pickling melon, *Cucumis melo* var. *conomon*), enhanced differentiation in human colon cancer cells. J Agric Food Chem. 2008;56(9):2977-84.
19. Nakamura Y, Watanabe S, Kageyama M, Shirota K, Shirota K, Amano H, et al. Antimutagenic; differentiation-inducing; and antioxidative effects of fragrant ingredients in Katsura-uri (Japanese pickling melon; *Cucumis melo* var. *conomon*). Mutat Res. 2010;703(2):163-8.