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CHEMICAL COMPOSITIONS AND BIOLOGICAL PROPERTIES OF ESSENTIAL OILS FROM *ZANTHOXYLUM RHETSA* (ROXB.) DC AND *ZANTHOXYLUM LIMONELLA* ALSTON

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Abstract

Background: *Zanthoxylum rhetsa* (Roxb.) DC and *Zanthoxylum limonella* Alston are spices for flavouring in indigenous Thai food. They are traditionally used as an aromatic, astringent, antimicrobial, antiseptic and antidiabetic agent. The purpose of this study is to examine their chemical compositions and evaluate antibacterial, antioxidant and anticancer properties of the essential oils.
Materials and Methods: The essential oils of *Z. rhetsa* and *Z. limonella* were analysed for phytochemical constituents by Gas chromatography–mass spectrometry (GC-MS). The antibacterial activity was determined against several bacteria using the microdilution method. Antioxidant capacity was determined by free radical scavenger 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) methods. The anticancer activity was determined with two breast cancer cell lines (MCF-7 and MDA-MB-231) and the normal African green monkey kidney epithelial (Vero) cell line and using MTT assay.
Results: Sabinene (22.51%) and terpinene-4-ol (32.33%) were found to be major components of *Z. rhetsa* essential oil while limonene (57.94%) and alpha-phelladrene (15.54%) were the major components of *Z. limonella* essential oil. Essential oil from *Z. limonella* exhibited broad spectrum antibacterial activity. *Z. rhetsa* and *Z. limonella* essential oils exhibited moderate antioxidant activity. The essential oil from *Z. rhetsa* possessed the ability to inhibit breast cancer cell (MCF-7 and MDA-MB-231) proliferation and cell viability.
Conclusion: This study suggest that the essential oils from *Z. limonella* and *Z. rhetsa* could be applied as safe antibacterial and antioxidant agents for food and have the potential for further development of new anticancer agents.

Keywords: *Zanthoxylum rhetsa, Zanthoxylum limonella*, GC-MS, antibacterial activity, antioxidant activity, anti-breast cancer activity

Introduction

Recent trends in the demand of natural products for healthcare are increasing due to side effects of synthetic chemicals medicine and people in developing countries being restricted in their access to essential medicines. Traditional medicines also have recently been promoted as a sustainable alternative for healthcare. Since Thailand is a country in tropical zone where it has variety of medicinal plants, many local plants have been studied for their biological activities. The plant genus *Zanthoxylum*, which is in the family of Rutaceae, is distributed worldwide (Tshin, 2011). Four species of *Zanthoxylum* have been reported in Northern Thailand (Suksathan et al., 2009). Two major species of *Zanthoxylum* found and sold in markets by local people are *Z. rhetsa* (Roxb.) DC and *Z. limonella* Alston. The plants have a pleasant odour and local people use the fruit rind as condiment in their indigenous food for flavouring. The fruit rind contains a mixture of volatile aroma compounds (Supabphol and Tangjitjareonkun, 2014). *Z. rhetsa* has long been used for medicinal uses as aromatic, astringent, antimicrobial, antiseptic and antidiabetic agent, as

well as used to treat inflammatory dermatosis, cholera, rheumatism, and toothache (Lalitharani et al., 2010; Reddy and Jose, 2011) . *Z. limonella* has been used as spice in traditional Thai food and used in traditional medicine. Its essential oils has anti-inflammatory, cytotoxic, antifungal, antibacterial and anesthetic properties (Nanasombat and Wimuttigosol, 2011). A previous study showed that sabinene (42.7%) and limonene (39.1%) were major components of *Z. limonella* fruit essential oil (Tangjitjareonkun et al., 2012a). A recent study showed that the major constituents presented in *Z. rhetsa* fruit essential oil were terpinen-4-ol (25.43%), sabinene (16.50%) and beta-pinene (10.40%) (Naik et al., 2015). The fruits are edible and widely used in traditional medicine for their analgesic, anticonvulsant, anthelmintic, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, antiparasitic and antitumor properties (Patiño et al., 2012).

Several reports are available on the composition and biological activity of the *Zanthoxylum* essential oils (Misra et al., 2013; Naik et al., 2015; Tangjitjareonkun et al., 2012a). However, composition differs according to geographical and environmental conditions. To provide scientific proof of their medicinal use, this present work reported the chemical components of the *Z. rhetsa* and *Z. limonella* essential oils collected from the North of Thailand and their antibacterial, antioxidation and anti-cancer activities.

Material and methods

Plant material and essential oil preparation

Fresh fruits of *Z. rhetsa* (Roxb.) DC were collected from November to December 2015 from Phayao province, Thailand (voucher No. 2559-010). *Z. limonella* Alston samples were collected in the same period from Chiang Mai province, Thailand (voucher No. 2559-020). They were authenticated and specimen vouchers were deposited at Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand. The fruits were hydrodistilled in a Clevenger-type apparatus for 6 hours. The essential oils were obtained, dried over anhydrous sodium sulphate and stored in a sealed, light protected bottle at -20°C prior to chemical and biological analyses.

Bacterial strains

The following bacterial strains were obtained from Department of Medical Sciences, Ministry of Public Health, Thailand: *Listeria monocytogenes* DMST 17303, *Bacillus cereus* DMST 5040, *Staphylococcus aureus* DMST 8840, *Salmonella* Typhi DMST 5784 and *Escherichia coli* DMST 4212. *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* and *Shigella enteritidis* group B were obtained and identified from the Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand. All strains were maintained on Brain Heart Infusion Agar (BHA) slant at 4°C, and were subcultured on fresh agar plate 24 h before antibacterial assays.

Identification of essential oil components

Gas chromatography–mass spectrometry (GC-MS) analyses were performed on a Agilent Technology apparatus (GC 6890, USA) equipped with a Hewlett Packard mass selective detector (MS 5973, USA) and HP-5MS 30m x 0.25 ID x 0.25 µm film thickness column (HP-5MS, USA). Oven temperature program was set to the following conditions: 70°C (0-3 min); 70-188°C (3°C/min); 188-280°C (20°C/min); 280°C (3 min), carrier gas: helium; gas flow rate: 1 mL/min; injection volume 1 µL (10 µL of essential oil was diluted with 490 µL dichloromethane). MS was connected to GC through transfer line which set the temperature at 150°C, and ion source temperature was 230°C. Identification of compound was confirmed by Wiley 275 and NIST98 library.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The MIC and MBC were determined by the broth dilution method described by the Clinical and Laboratory Standards Institute (CLSI) (Institute, 2014). The essential oils were first dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), then in Mueller Hinton Broth (MHB) (Criterion, USA) to the highest dilution of 512 mg/mL. Then serial two-fold dilutions were performed in 96-well microplate at a 50 µL final volume per well. The bacterial inoculum was adjusted to 0.5 McFarland Standard and diluted 100 times in MHB. Fifty microliter of bacterial suspension was added to each well. Tetracycline (Pacific Science, Thailand) was used as positive control. MHB culture media and DMSO were used as negative control. This experiment was carried out in triplicate. The microplate was incubated at 37°C for 24 h and examined for the growth of bacteria. The MIC is defined as the lowest concentration of the essential oil at which the bacteria did not demonstrate visible growth. The MBC was performed by inoculating 10 µL of each MIC tested well with no bacterial growth on BHA plates. The MBC was defined as the lowest concentration of the essential oil which gave no viable cells on the BHA plates.

DPPH radical scavenging activity

DPPH assay is well known in natural product studies (Fukumoto and Mazza, 2000). DPPH is a stable free radical which is purple in color. It will change its color to pale yellow when free radicals were scavenged by antioxidant compounds which can be determined by spectrophotometer. A DPPH (Sigma-Aldrich, Germany) solution

was prepared at a concentration of 0.2 mM in methanol. For antioxidant evaluation, 2 mL of DPPH solution was mixed with 1 mL of different concentrations of the essential oils in methanol. After 30 min of incubation in the dark at room temperature, the absorbance was measured at a wavelength of 517 nm. Tocopherol (Fluka, Switzerland) and beta-carotene (Sigma, Germany) were used as standard. Percentage of inhibition was calculated using following equation: % inhibition = $((A_{blank}-A_{sample})/A_{blank})) \times 100$. The antioxidant activity was calculated as IC_{50} where IC_{50} is concentration of essential oil that shows 50% of DPPH inhibition. The larger the antioxidant capacity, the lower IC_{50} will be observed.

ABTS radical scavenging activity

Quantitative determination of ABTS assay is based on reaction of ABTS free radical which is reactive towards antioxidants and changes its color from deep blue to colorless. ABTS (Sigma-Aldrich, Germany) stock solution was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate (Ajax Finechem, Australia) solution at the ratio of 8:12 and kept in the dark for 16-18 hours. The working solution was prepared by diluting the stock solution with ethanol until the absorbance at 750 nm was 0.7±0.2. Then 200 µL of sample was mixed with 1,800 µL of working ABTS solution and the reaction tube was incubated in the dark for 5 min. The absorbance was measured at the wavelength of 750 nm. Trolox (Sigma-Aldrich, China) was used as a standard. Percentage of inhibition was calculated using following equation: $((A_{blank}-A_{sample})/A_{blank})) \times 100$. The antioxidant activity was expressed as Trolox equivalent antioxidant activity (TEAC).

Cytotoxicity and anticancer assay
Cell culture

African green monkey kidney epithelial Vero cells were obtained from Professor Sukhathida Ubol, Faculty of Sciences, Mahidol University, Thailand and breast cancer MCF-7 and MDA-MB-231 cell lines were provided by Professor Shaun McColl, The University of Adelaide, South Australia. Vero cells were grown in Dulbecco's Modified Eagle Medium (DMEM) while MCF-7 and MDA-MB-231 cell lines were cultured in Roswell Park Memorial Institute 1640 (RPMI1640) (Gibco-BRL, NY) at 37°C in a 5% CO_2 atmosphere. The medium was supplemented with 10% fetal bovine serum (FBS), 100 units penicillin and 100 µg/mL streptomycin/mL. (DMEM, RPMI 1640, FBS and penicillin/streptomycin; Gibco-BRL Biochemicals, Grand Island, NY, USA).

Determination of cell viability by 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide assay

In vitro cytotoxic activity of the essential oils obtained from *Z. rhetsa* and *Z. limonella* was tested by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay in Vero, MCF-7 and MDA-MB-231 cell lines. Cell suspension in growth medium of 200 µL ($2.5x10^5$ cells/mL) was seeded into a well in a 96-well microtiter plate and cultured for 16-18 h. The cells were washed once with PBS and treated with 1% DMSO-containing medium without sample and various concentrations of the essential oils diluted in the medium for 1or 24 h. The essential oils were removed carefully and cells were washed with PBS. MTT reagent (5 mg/mL), 50 µL, was added into a well and the plate was incubated for 1 h in 5% CO_2 incubator. After incubation, 150 µL of solubilising agent DMSO was added to each well and mixed well. Presence of viable cells was determined by the formation of formazan crystal visualized by development of purple color in the well. The plate was then measured for optical density (OD) by spectrophotometer at wavelength of 570 nm. Percentage of cell viability was calculated (Cell viability (%) = sample OD/control OD x 100). The concentration required for 50% inhibition (IC_{50}) of cell viability was analysed by GraphPad Prism 6.0 software (San Diego, CA, USA).

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as mean ± standard deviation (SD) or standard of the mean (SEM). The significant differences between groups were analyzed by unpaired student's t-test. *p* values less than 0.05 were considered statistically significant.

Results

Essential oils of *Z. rhetsa* and *Z. limonella* fruits were obtained by hydrodistillation. The essential oils were analysed for their compositions by GC-MS. The results are shown in Table 1. *Z. rhetsa* essential oil contained a mixture of 30 chemical components. Terpinen-4-ol and sabinene were major components (32.33 and 22.51%) followed by gamma-terpinene, decyl aldehyde and octanal (7.97, 5.97 and 5.35%). *Z. limonella* essential oil consisted of 21 components. Limonene and alpha-phellandrene were major components (57.94 and 15.54%) followed by trans-beta-ocimene (8.04%).

Table 1: Essential oils chemical compositions from fruit of *Z. rhetsa* and *Z. limonella*

RT[a]	Compound	Peak area. %[b] (Data from other authors are included in brackets for comparisons)	
		Z. rhetsa	***Z. limonella***
3.95	Alpha-thujene	**0.61** (0.74[d])	**0.09**
4.10	Alpha-pinene	**0.68** (3.87[c], 4.33[d])	**2.86**
5.02	Sabinene	**22.51** (47.12[c], 16.50[d])	**0.22** (9.13[e], 42.73[f])
5.08	1-beta-pinene	**0.82** (3.87[c], 10.40[d])	-
5.39	beta-Myrcene	**1.18** (0.74[c], 0.68[d])	**3.05**
5.76	Octanal	**5.35**	-
5.81	Alpha-phellandrene	- (0.48[c])	**15.54** (0.7[f])
5.93	Delta, 3-carene	- (0.05[c])	**0.24** (2.70[f])
6.13	Alpha-terpinene	**4.40** (3.45[c])	-
6.38	Para-cymene	**2.65** (3.08[c], 2.45[d])	-
6.57	1, 8-cineole	**1.90**	-
6.59	Limonene	- (4.06[c])	**57.94** (31.09[e], 39.05[f])
6.76	Cis-ocimene	-	**1.57**
7.10	Trans-beta-ocimene	- (0.42[c])	**8.04** (0.1[f])
7.45	Gamma-terpinene	**7.97** (3.64[c], 5.64[d])	-
7.81	Trans-sabinene	**0.20** (0.34[d])	-
7.99	1-octanol	**2.44** (0.67[c], 0.20[d])	**0.25**
8.41	Terpinolene	**1.84** (0.71[c], 1.48[d])	**0.12**
8.97	Linalool	**1.41** (0.76[c], 3.25[d])	**3.24**
9.75	2-cyclohexen-1-ol	**1.48**	**0.34**
10.46	1-terpineol	**0.99**	-
10.71	Cyclohexene	**0.17**	-
12.05	Terpinen-4-ol	**32.33** (6.61[c], 25.43[d])	**0.27** (13.94[e], 5.40[f])
12.52	1-alpha-terpineol	**2.05** (7.73[c], 7.63[d])	**1.65**
12.65	Cis-piperitol	**0.42**	-
12.97	Decyl aldehyde	**5.97** (0.36[d])	**1.28**
13.20	Trans-piperitol	**0.66**	-
13.24	Octyl acetate	-	**1.50**
15.88	Cyclooctane	**0.45**	-
15.94	3-hexyne-2, 5-diol	**0.28**	-
16.63	2-undecanone	-	**0.60**
20.38	Lavandulyl acetate	-	**0.75**
21.34	Dodecanal	**0.19**	-
21.54	Beta-caryophyllene	**0.17** (0.63[c])	-
24.04	Germacrene	**0.20**	-
24.85	2-tridecanone	-	**0.10**
25.76	Delta-cadinene	**0.11** (0.33[c])	-
30.85	Alpha-cadinol	**0.19**	-
46.53	9-octadecenamide	**0.40**	**0.35**

[a]RT, (Retention time, min); [b]peak area obtained by GC-MS of the present study; [c](Jirovetz et al., 1998); [d](Naik et al., 2015); [e] (Itthipanichpong et al., 2002); [f](Tangjitjareonkun et al., 2012a)

The essential oils were tested for antibacterial susceptibility against pathogenic bacteria by microdilution method (Table 2). Both essential oil showed broad spectrum antibacterial activity. *Z. limonella* essential oil presented lower MIC/MBC values of 8-128/8-128 mg/mL while *Z. rhetsa* essential oil showed higher MIC/MBC values of 256/256 mg/mL. *Z. limonella* essential oil showed strongest antibacterial activity (4-8/8 mg/mL) against *B. cereus, S. aureus* and *E. coli.*

Table 2: Essential oil susceptibility test by microdilution

Bacteria	MIC/MBC (mg/mL)		
	Z. rhetsa	***Z. limonella***	**Tetracycline**
Gram positive bacteria			
Listeria monocytogenes DMST 17303	256/256	64/64	0.002/0.004
Bacillus cereus DMST 5040	256/256	8/8	0.004/0.008
Staphylococcus aureus DMST 8840	256/256	4/8	0.001/0.004
Gram negative bacteria			
Salmonella Typhi DMST 5784	256/256	8/8	0.016/0.016
Shigella enteritidis group B	256/256	128/128	0.016/0.016
Escherichia coli DMST 4212	256/256	8/8	0.002/0.008

Pseudomonas aeruginosa	256/256	16/64	0.032/0.064
Vibrio parahaemolyticus	256/256	16/16	0.008/0.016

The DPPH and ABTS radical scavenging activities of essential oils from *Z. rhetsa* and *Z. limonella* fruits are shown in Table 3. Both essential oils demonstrated similar DPPH (25 and 24 mg/mL, respectively) and ABTS radical scavenging activities (16.35 and 13.6 mg/mL, respectively).

Table 3: DPPH and ABTS of the *Z. rhetsa* and *Z. limonella* essential oil

Essential oil	IC_{50} (mg/mL)	TEAC (µM)
Z. rhetsa	25±5	16.35±0.04
Z. limonella	24±3	13.6±0.2
Tocopherol	3.67±0.09	0.3±0.2
Beta-carotene	108±5	108.7±0.2

IC_{50}: the concentration of essential oil at DPPH radical scavenging activity 50%; TEAC: Trolox equivalents antioxidant capacity

The essential oils were initially tested for their cytoxicity with Vero cells as well as breast cancer MCF-7 and MDA-MB-231 cell lines. After 24 hours exposure, the cell viability assay demonstrated that IC_{50} of the *Z. rhetsa* and *Z. limonella* essential oils tested with all cell lines were less than 0.82 µg/mL. According to the American National Cancer Institute guidelines that set the limit of activity for crude extract at IC_{50} of proliferation less than 30 mg/ml (Suffness and Pezzuto 1990), the extracts were tested for anti-cancer activity in MCF-7 and MDA-MB-231 and normal Vero cell lines. As shown in Table 4, after 1 hour of exposure to the essential oils, MTT assay indicated that the concentrations at which 50% cells death occur (IC_{50}) of *Z. rhetsa* extract tested in Vero cells (3.75 ± 0.72 µg/mL) was significantly 2 times greater than those tested in MCF-7 (1.98 ± 0.23 µg/mL) and MDA-MB-231 cells (1.96 ± 0.24 µg/mL) (p<0.0001), while there is no significant difference of the IC_{50} of *Z. Limonella* extract tested between those in Vero, and in MCF-7 or MDA-MB-231 cells (p > 0.5).

Table 4: Inhibitory concentration 50 (IC_{50}) of the *Z. rhetsa* and *Z. limonella* essential oil tested in Vero, MCF-7 and MDA-MB-231 cells

Essential oil	IC_{50} (Mean ± SD) (µg/mL)		
	Vero cells	MCF-7 cells	MDA-MB-231 cells
Z. rhetsa	3.75 ± 0.72	1.98 ± 0.23	1.96 ± 0.24
Z. limonella	8.53 ± 2.17	5.40 ± 1.37	3.80 ± 1.03

IC_{50}: half-maximal inhibitory concentration

Discussion

In the present study, the chemical compositions of essential oils obtained from *Z. rhetsa* and *Z. limonella* was investigated. It was found that terpinene and sabinene (32.33 and 22.51%, respectively) were the major components in *Z. rhetsa* while the major components in *Z. limonella* were limonene and alpha-phellandrene (57.94 and 15.54%, respectively). Components from *Z. rhetsa* differ from two reports where the plant material was collected from India (Jirovetz et al., 1998; Naik et al., 2015). Sabinene (47.12%) was found to be a major component from *Z. rhetsa* seed (Jirovetz et al., 1998) but terpinen-4-ol, sabinene and 1-beta-pinene (25.43, 16.50 and 10.40%, respectively) were found to be the major components (Naik et al., 2015). Components from *Z. limonella* from this study differ from 2 other reports even though the plant was collected from Thailand (Itthipanichpong et al., 2002; Tangjitjareonkun et al., 2012a). From this study, limonene was found to be the major component from *Z. limonella* (57.94%) which was almost 2 times higher than that was reported from another study (31.09%) (Itthipanichpong et al., 2002). This study is also in contrast with Tangjitjareonkun et al, (2012) where it was reported that sabinene was the major component followed by limonene (42.73 and 39.05%). In a given species of plant, variation of chemical compositions and content may occur as a result of genetic or environmental factors such as age of plant, season, phase of plant development, geographical location and bioclimate distribution (Eiter et al., 2010; ElHadj Ali et al., 2010; Nezhadali et al., 2014; Zouari et al., 2012).

Z. rhetsa and *Z. limonella* have been reported with uses as spices and medicine for infectious diseases (Patiño et al., 2012). The results from this study showed that essential oils from both species had broad spectrum antibacterial activity to Gram positive and negative bacteria. This is in accordance with other studies that showed broad spectrum antibacterial activity from these oils (Naik et al., 2015; Supabphol and Tangjitjareonkun, 2014; Tangjitjareonkun et al., 2012a). In this study, *Z. rhetsa* essential oil showed similar MIC/MBC level against all tested bacteria at 256/256 mg/mL while the essential oil from *Z. limonella* showed greater antibacterial activity with variable MIC/MBC levels ranging from 4-128/8-128.

A previous study (Naik et al., 2015) reported antibacterial activity of *Z. rhetsa* oil, fractions and pure compound (terpinen-4-ol) against 3 bacteria: *S. aureus* ATCC 6538a, *E. coli* ATCC 8739 and *Klebsiella pneumonia.* The essential oil showed greater antibacterial activity against Gram positive bacteria (*S. aureus*) than Gram negative bacteria (*E. coli* and *K.* pneumonia). The MIC level of the crude essential oil and terpinen-4-ol against *S. aureus* were 35 µg/mL while the MIC levels of the fractions were 70-140 µg/mL. This suggested a synergistic effect of active constituents contained in the crude oil. In this study, terpinen-4-ol was the major component in *Z. rhetsa* oil. It is suggested that purified components and the synergistic effect of the components should be further investigated.

A previous study (Tangjitjareonkun et al., 2012a) presented antibacterial activity of from fruit of *Z. limonella* against 4 bacteria; *B. subtilis* ATCC 6633, *S. aureus, E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The crude oil had stronger antibacterial activity than the pure compounds (sabinene and terpinen-4-ol) but limonene had no antibacterial activity. The results of this study showed limonene and alpha-phelandrene as major components (57.94 and 15.54%). Supportive data from Iscan et al (2012) (Iscan et al., 2012) showed that alpha-phellandrene and its biotransformation metabolites had antibacterial activity. It has been suggested that alpha-phellandrene acts as active antibacterial agent. In addition, a metabolite from alpha-phellandrene (5-p-menthene-1,2-diol) gave stronger antibacterial activity than phellandrene. This suggested that not only active components and synergistic effects should be investigated further, but also the chemical or biological modification of the active compounds should be further investigated to study the subsequent antibacterial activity.

The essential oils from *Z. rhetsa* and *Z. limonella* presented similar antioxidant activity (Table 3). A previous study (Nanasombat and Wimuttigosol, 2011) reported strong antioxidant activity of the fruit of *Z. limonella* essential oil with IC_{50} value of 5.66 mg/mL. Tangjitjareonkun, Supabphol, Cavasiri (2012a) (Tangjitjareonkun et al., 2012b) reported DPPH antioxidant assay of *Z. limonella* fruit essential oil with IC_{50} value of 5,764 µg/mL and the TEAC value was 7.1 µM. They showed mixture compounds of *Z. limonella* fruit essential oil with DPPH scavenging effect on thin layer chromatography (TLC).

The cell viability assays indicated cytotoxicity of these essential oils in all cell lines that was relatively selective to the breast cancer cells. The inhibitory effect of *Z. rhetsa* essential oil on cell viability of breast cancer cells suggested potential ability of the essential oil to inhibit cancer cell proliferation and cell survival. A previous study reported cytotoxic effect of *Z. rhetsa* bark constituents against melanoma cancer cells (B16-F10) but is non-toxic to normal skin cell lines and suggested that lignans and alkaloids were responsible for the cytotoxic properties (Santhanam et al., 2016). Another *Zanthoxylum* species (*Z. Schinifolium*) was also reported to be toxic to HepG2 human hepatoma cell lines (Paik et al., 2005). However, in this study, the *Z. rhetsa* essential oil showed the relatively lower selective ability to cancer cells than those observed in the previous studies possibly due to differences in the extraction methods, parts of plant and chemical compositions in the extracts. Additionally, the anti-cancer activities of the essential oils should be further clarified in relation to standard cancer drugs such as a doxorubicin and mechanisms underlying the potential anti-cancer activities is required more investigation.

The findings of this study suggest the possibility of these essential oils as safe antibacterial and antioxidant agents for food and have the potential for further development of new anticancer agents. More study on active components with their synergistic effects should be undertaken. The area of investigation of the chemical or biological modification of active compounds to enhance biological effects and mechanism of the active compounds on those biological evaluations is also in need of further work.

Conflict of interest statement: We declare that we have no conflict of interest.

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