Agr. Nat. Resour. 53 (2019) 472–478

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Gas chromatography-mass spectrometry analysis and biological activities of hexane extract from *Boesenbergia xiphostachya* (Gagnep.) Loes. rhizome

Nungruthai Suphrom[a,*], Kamonlak Insumrong[a,†], Kornkanok Ingkaninan[b,†], Surat Boonphong[a,†]

[a] *Department of Chemistry, Faculty of Science and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand.*

[b] *Bioscreening Unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand.*

Article Info

Article history:
Received 29 March 2018
Revised 19 September 2018
Accepted 17 December 2018
Available online 31 October 2019

Keywords:
Biological activity,
Boesenbergia xiphostachya,
Cytotoxicity,
GC-MS,
phenylpropanoids

Abstract

Boesenbergia xiphostachya (Gagnep.) Loes. (Zingiberaceae) has been ethnomedically used in Thailand as a laxative and flatulence remedy. Only a few studies involving the phytochemical screening and anti-oxidant activity have been reported. The present work analyzed the volatile components using gas chromatography-mass spectrometry and investigated the antimalarial activity, neuraminidase inhibitory activity, antimycobacterial activity and antimicrobial activity of the hexane extract. The study was extended to investigate the cytotoxicity effects of the extract on both normal cells and cancer cells. The results indicated that many volatile components were present in the extract including phenylpropanoids (C_6–C_3 carbon skeleton), phenylpropanoid-related compounds (C_6–C_2) and benzenoids (C_6–C_1). The main components detected in the hexane extract were: 3,6-dimethoxy-2-ethylbenzaldehyde (38.43%), elemicin (15.36%), asarone (12.95%), *cis*-isoelemicin (4.32%), methyleugenol (4.20%), *trans*-isoelemicin (1.19%) and methylisoeugenol (1.14%). The extract exhibited weak inhibitory activity in all tested assays. Similar activity profiles were also observed in antimycobacterium tuberculosis, antibacterial and antifungal assays. Moreover, the hexane extract of *B. xiphostachya* showed weak activity against nine cell lines with half maximal inhibitory concentrations higher than 50 µg/mL. The results also showed a significant decrease in LNCaP cell viability at the highest concentration tested (1,000 µg/mL). It should be noted that the investigation of the volatile components, bioactivities and cytotoxicity studies was reported for the first time regarding the *B. xiphostachya* rhizome. The obtained data might be useful for further studies on other applications of the extract.

Introduction

Boesenbergia, a member of Zingiberaceae, is a genus of small rhizomatous herbs which is composed of approximately 80 species distributed throughout tropical Asia, and 19 species have been identified in Thailand (Sirirugsa, 1992; Techaprasan et al., 2006). *Boesenbergia xiphostachya* (Gagnep.) Loes., locally known in Thai as 'Ngon nark' or 'Ngon praya nark' is widely distributed in Indo-China and Northeastern Thailand (Sirirugsa, 1992; Chayamarit et al., 2014). Sirirugsa (1992) described as follows: a perennial ground herb that

† Equal contribution.

* Corresponding author.
E-mail address: suphrom.n1@gmail.com (N. Suphrom)

https://doi.org/10.34044/j.anres.2019.53.5.05

grows to 70 cm in height and has a brown rhizome; the leaf is simple with a lanceolate shape; the inflorescence is terminal with peduncle usually enclosed by the uppermost leaf-sheath and extending to 25 cm in length; calyx lobes are truncated whereas the corolla tubes are extended and dilated to the top; the anther is connective glandular-hairy, crest bilobed and not produced beyond the thecae; the ovary is glabrous and the placenta is free central.

The rhizomes of some *Boesenbergia* species have been widely used in Thai folk medicine for the treatment of several diseases (Kanathum, 2008). Moreover, *Boesenbergia* spp. also exerts various biological effects such as reduced platelet-activation (Jantan et al., 2005), an anti-inflammatory effect (Chahyadi et al., 2014; Sudsai et al., 2014), wound healing (Sudsai et al., 2013; Sudsai et al., 2016), an anti-allergic effect (Madaka et al., 2013), an anti-microbial effect (Chahyadi et al., 2014), an anti-ulcer effect (Abdelwahab et al., 2011), anti-cancer, anti-tumor activity (Eng-Chong et al., 2012), anti-oxidation and a tyrosinase inhibitory effect (Chan et al., 2008). Phytochemical studies of *Boesenbergia* species have reported the presence of flavonoid derivatives, chalcone derivatives, esters, kawains, and terpenoids (Eng-Chong et al., 2012; Madaka et al., 2013; Chahyadi et al., 2014; Sudsai et al., 2014; Sudsai et al., 2016).

From the local literature survey reported, *B. xiphostachya* rhizome has been ethnomedically used in Thailand as a laxative and flatulence remedy (Chuakul and Boonpleng, 2003). While the genus *Boesenbergia* has been the subject of previous studies, research involving the pharmacological activities and bioactive constituents of *B. xiphostachya* is limited. A few studies have been done on preliminary testing for phytochemical analysis and the extract has been reported to possess moderate 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Suphrom et al., 2018). The evaluation was reported of genetic variation, evolutionary relationships and phylogenetic relationships of *Boesenbergia* in Thailand using multilocus DNA fingerprints generated from amplified fragment length polymorphism analysis, with the molecular markers for identifying the species origin of these taxa also studied (Ngamriabsakul and Techaprasan, 2006; Techaprasan et al., 2006; Techaprasan et al., 2008). In continuation of previous studies on the chemical constituents and the other biological activities of hexane rhizome extract, the current work analyzed the volatile components using gas chromatography-mass spectrometry (GC-MS) and investigated antimalarial activity, neuraminidase inhibitory activity, antimycobacterial activity, and antimicrobial activity. The study was also extended to investigate the cytotoxicity effects of the extract on both normal cells and cancer cells.

Material and Methods

Plant material and extraction

Fresh rhizomes of *B. xiphostachya* (50 kg) were collected from Khao Kho district, Phetchabun province, Thailand (March 2014). The plant material was identified by Assistant Professor Pranee Nangngam. The voucher specimen (collection number: 004071) was registered at the Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand. The dried powder rhizomes (4.17 kg) were macerated for 3 d with hexane (20 L × 3 cycles) at room temperature. The filtrates were evaporated under reduced pressure to produce the crude hexane extract (82.72 g, 1.98% yield of dried weight).

Gas chromatography-mass spectrometry analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Hewlett Packard (Agilent Technologies, Palo Alto, CA, USA) model 6890 gas chromatograph equipped with a mass selective detector. The condition parameters were set for the method described by Suphrom et al. (2017) with some modifications. A fused silica capillary Hewlett Packard HP-5 (5% phenyl methyl siloxane) column (30 m × 0.25 mm × 0.25 µm film thickness) was used for the GC separation. High purity helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The injector was set at 250°C and performed in split mode with a split ratio of 10:1 volume per volume in 1 µL. The initial oven temperature was held at 70°C for 3 min, then programmed at 5°C/min to 280°C and finally held for 10 min. The temperature of the transfer line heater was set at 280°C. The mass scanning range was set as 50−550 amu in full scan. The extract was prepared by dissolving 50 mg extract into 1 mL of dichloromethane (RCI Labscan Ltd; Bangkok, Thailand) and filtering solution prior to injection. Then, 1.0 µL of the sample was injected into the GC-MS system. Retention indices (RIs) were determined by analyzing a solution containing the homologous series of *n*-alkanes (C_8−C_{33}, Fluka analytical, Germany) under the same chromatographic conditions and then calculated as described by van Den Dool et al. (1963). Identification of volatile components was performed by computer matching their recorded mass spectra fragmentation patterns with those stored on the wiley7n MS spectral library. Further identification was made by comparison of their mass spectra and their RIs relative to *n*−alkanes with those of the National Institute of Standards and Technology (NIST) Chemistry WebBook (Babushok et al., 2007; Linstrom and Mallard, 2016), or with the literature data. The relative contents of each component in the sample were also calculated based on the normalization of peak areas as the percentage of total detected volatile components.

Biological activity tests

The antimalarial assay was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain) using a microculture radio isotope technique (Desjardins et al., 1979). The inhibitory activity against neuraminidase was tested using fluorometric determination (MUNNA-base enzyme inhibition assay; Potier et al, 1979). The anti-herpes simplex virus type-1 (HSV-1) activity and antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra were assessed using the green fluorescent protein (GFP)-based microplate assay (Changsen et al., 2003). Antibacterial activity against *Bacillus cereus* and antifungal activity against *Candida albicans* were performed using the resazurin microplate assay (O'Brien et al., 2000). Antibacterial activities

against *Enterococcus faecium*, *Pseudomonas aeruginosa* (PAO1), *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Escherichia coli* were also performed using optical density microplate assay (OD600; Clinical and Laboratory Standards Institute, 2006).

Cytotoxic activities against various cell-lines—oral human epidermoid carcinoma (KB), human breast cancer (MCF-7), human small-cell lung cancer (NCI-H187), human hepatocarcinoma (HepG2), human Caucasian colon adenocarcinoma (Caco2), mouse skin melanoma (B16-F10), mouse subcutaneous connective tissue (NCTC clone 929) and human dermal fibroblast (HDF)—were evaluated using the resazurin microplate assay (O'Brien et al., 2000). Cytotoxicity to African green monkey kidney fibroblasts (Vero cell) and androgen-sensitive human prostate adenocarcinoma (LNCaP) cell viability were performed using GFP-based microplate assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively (Hunt et al., 1999; Suphrom et al., 2012).

Statistical analysis

The experiments of biological activity tests were performed in triplicate, and the data were expressed as mean ± SD. Statistical significance was determined using analysis of variance and Student's t test. A test level of $p < 0.05$ denoted significance in all cases.

Results and Discussion

Volatile compositions

The analysis of hexane extract of *B. xiphostachya* rhizome was performed using GC-MS and the total ion chromatogram is illustrated in Fig. 1. The identifications of the compounds based on their MS spectra agreed with the RIs analyses. In total, 15 compounds in the extract were identified and are listed in Table 1, where the RIs of the volatile compounds in the sample are also presented. The relative amount (%) of the compositions was calculated based on peak-normalization. There has been no known previous report on the volatile constituents of *B. xiphostachya*. The current investigation of the hexane extract revealed the presence of different phytochemical components (monoterpenoids, sesquiterpenoids, phenylpropanoids, benzenoids and sterols). The major class of volatile compounds (78.65%) could be divided into phenylpropenes (C_6–C_3 carbon skeleton), phenylpropanoid-related compounds (C_6–C_2) and benzenoids (C_6–C_1). The main constituents found in the extract were 3,6-dimethoxy-2-ethylbenzaldehyde (38.43%), elemicin (15.36%), asarone (12.95%), *cis*-isoelemicin (4.32%), methyleugenol (4.20%), *trans*-isoelemicin (1.19%) and methylisoeugenol (1.14%). The other compounds were present in smaller percentages.

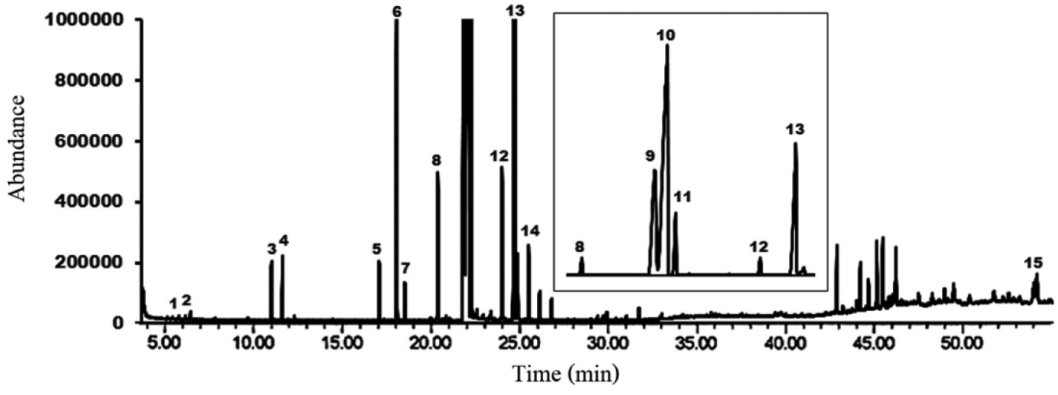

Fig. 1 Gas chromatography-mass spectrometry total ion chromatogram of hexane extract of *B. xiphostachya* rhizome

Table 1 Volatile compounds of *B. xiphostachya* rhizome extract identified using gas chromatography-mass spectrometry

No.	RT (min)	RI*	Identified compound	Classification of compound	Relative amount (%) †
1	5.68	972	Camphene	Monoterpenoids	0.03
2	6.34	987	β-Pinene	Monoterpenoids	0.05
3	10.91	1145	Camphor	Monoterpenoids	0.45
4	11.52	1166	Borneol	Monoterpenoids	0.49
5	16.99	1366	4-Ethenyl-1,2-dimethoxybenzene	Phenylpropanoid-related compounds	0.45
6	17.98	1403	Methyleugenol	Phenylpropanoid	4.20
7	18.44	1422	*trans*-Caryophyllene	Sesquiterpenoids	0.31
8	20.30	1496	Methylisoeugenol	Phenylpropanoids	1.14
9	21.79	1558	Elemicin	Phenylpropanoids	15.36
10	22.04	1569	3,6-Dimethoxy-2-ethylbenzaldehyde	Phenylpropanoid-related compounds	38.43
11	22.20	1576	*cis*-Isoelemicin	Phenylpropanoids	4.32
12	23.91	1650	*trans*-Isoelemicin	Phenylpropanoids	1.19
13	24.64	1683	Asarone	Phenylpropanoids	12.95
14	25.42	1718	Asaraldehyde	Benzenoids	0.61
15	54.08	–‡	Stigmasterol	Sterols	0.65

RT = retention time; RI = retention index.

* = retention indices were calculated using a homologous series of *n*-alkanes (C_8–C_{33}); † = results obtained by peak-area normalization; ‡ = not calculated.

For the main volatile constituents detected, 3,6-dimethoxy-2-ethylbenzaldehyde, which was a phenylpropanoid-related compound (C_6−C_2), had not been previously detected in *Boesenbergia* spp. Though it has been reported as a volatile composition found in the ethanolic extract of poplar propolis (Mişe Yonar et al., 2017). Elemicin is a natural organic compound which is known as phenylpropene and was discovered in the essential oil of *Canarium luzonicum*, which is known as elemi plant and is used to synthesize the alkaloid, mescaline (Villanueva et al., 1993). In addition, elemicin has been reported as a constituent of oleoresin and found in the rhizome essential oil of *Zingiber niveum* (Theanphong et al., 2015), *Myristica fragrans* (nutmeg) (Gopalakrishnan, 1992), *Laurus nobilis* (Bouzouita et al., 2009), *Petroselinum sativum* (De Vincenzi et al., 2004), and *Cymbopogon khasianus* (Lal et al., 2018). Asarone, which was one of the main volatile components in the current hexane extract, was reported to be also mainly present in the *Acorus* species and *Guatteria gaumeri* Greenman (Chellian et al., 2017). Other phenylpropanoids including methyleugenol, methylisoeugenol, *cis*- and *trans*-isoelemicin were found at less than 5% each in the current study. These compounds are common phenylpropanoids found in many plant species, particularly in spices and medicinal plants (Vahirua et al., 1993; Suarez et al., 2005; Tan and Nishida, 2012). Furthermore, methyleugenol can be converted into other useful phenylpropanoids such as elemicin or myristicin (Tan and Nishida, 2012). In the current study, asaraldehyde was detected as the only compound in the benzenoids group. This compound was also previously isolated from *Boesenbergia thorelii* (Madaka et al., 2013). Monoterpenes and sesquiterpene detected also corresponded to reports in the literature of essential oil in other species of *Boesenbergia* (Jantan et al., 2001; Sukari et al., 2008).

Biological activities

The *in vitro* antimalarial effects were evaluated against *P. falciparum*, neuraminidase inhibitory activity and anti-HSV-1 activity of hexane extract from *B. xiphostachya* rhizome. The extract exhibited weak inhibitory activity or was inactive (Table 2). In the antimycobacterial activity against *M. tuberculosis* test, the extract displayed weak inhibitory activity with a maximum effect of 28% at 50 µg/mL while the positive drugs—ofloxacin, ethambutol, streptomycin, and isoniazid—exhibited potent inhibitory activity with minimum inhibitory concentration (MIC) values of 0.391 µg/mL, 0.938 µg/mL, 0.313 µg/mL and 0.047 µg/mL, respectively. The antibacterial and antifungal activities of *B. xiphostachya* extract were tested against two Gram-positives—*B. cereus*, *E. faecium*—four Gram-negatives—*E. coli*, *P. aeruginosa*, *A. baumannii, K. pneumonia*—and a fungal strain—*C. albicans*. The results demonstrated that the extract was inactive. It exhibited weak inhibitory activity profiles against all tested organisms with maximum effects being lower than 10% at the highest tested concentration (50 µg/mL) while all positive drugs displayed potent inhibitory activity with MIC values and half maximal inhibitory concentration (IC_{50}) values as shown in Table 3. There are reports about the antibacterial effect on the same organisms (*E. coli, P. aeruginosa*, *K. pneumonia*) of extracts which contained some components detected in the current study such as β-asarone, methyleugenol and elemecin as well as isolated compounds and their MIC values were in the same range of concentration in milligrams per liter that were in agreement with the current study (McGaw et al., 2002; Goswami et al., 2017).

The study was extended to assess the cytotoxicity of the hexane extract against cancer cell lines (KB, MCF-7, NCI-H187, HepG2, Caco2, B16-F10) and nonmalignant Vero cells, NCTC 929 and HDF cells. The cytotoxicity results of the samples and three positive controls are shown in Table 4. The extract exhibited weak activities against the tested cell lines with IC_{50} values higher than 50 µg/mL for KB, MCF-7, NCI-H187, and Vero cells, while similar profiles of cytotoxicity were observed on other five cell lines (IC_{50} > 100 µg/mL). The hexane extract had the same level in micrograms per liter of inhibitory effect to that of some *Boesenbergia* spp. in the literature (Zaeoung et al., 2005; Jing et al., 2010). Moreover, the extract was also subjected to an in-house bioassay protocol to evaluate the cytotoxic effect of the hexane extract on LNCaP cell viability. Specifically, the cells were cultured for 24 hr with various concentrations of the extract, and the cell viability was determined using MTT assay. The result showed that hexane extract had no cytotoxic effect on LNCaP cells at concentrations of 0.01–100 µg/mL (Fig. 2). It should be noted that the hexane extract significantly decreased cell viability at the highest concentration tested (1,000 µg/mL).

Table 2 Antimalarial, neuraminidase inhibition, anti-herpes simplex virus type-1 (anti-HSV-1) and antimycobacterium tuberculosis (Anti-TB) activities of *B. xiphostachya* hexane extract

Sample	Antimalarial (IC_{50}, ng/mL)	Neuraminidase inhibition (IC_{50}, ng/mL)	Anti-HSV-1 (IC_{50}, µg/mL)	Anti-TB (MIC, µg/mL)
Extract	Inactive	Inactive	Inactive	> 50
Dihydroartemisinine*	0.60	n.d.	n.d.	n.d.
Mefloquine*	23.63	n.d.	n.d.	n.d.
Oseltamivir carboxylate†	n.d.	0.27	n.d.	n.d.
Acyclovir‡	n.d.	n.d.	7.82	n.d.
Ofloxacin§	n.d.	n.d.	n.d.	0.39
Ethambutol§	n.d.	n.d.	n.d.	0.94
Streptomycin§	n.d.	n.d.	n.d.	0.31
Isoniazid§	n.d.	n.d.	n.d.	0.05

IC_{50} = half maximal inhibitory concentration; MIC = minimum inhibitory concentration; n.d. = Not Determined.

* = positive control for antimalarial assay; † = positive control for neuraminidase inhibition assay; ‡ = positive control for anti-HSV-1 assay; § = positive control for antimycobacterium tuberculosis assay.

Table 3 Antibacterial and antifungal activities of *B. xiphostachya* hexane extract

Sample	Activity of samples against tested organisms (MIC or IC_{50}, μg/mL)*						
	B. cereus	*E. faecium*	*E.coli*†	*P. aeruginosa*	*A. baumannii*†	*K. pneumonia*†	*C. albicans*
Extract	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Vancomycin	2.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rifampicin	n.d.	3.13	n.d.	n.d.	n.d.	n.d.	n.d.
Tetracycline HCl	n.d.	0.10	n.d.	n.d.	n.d.	n.d.	n.d.
Amikacin	n.d.	n.d.	0.50	0.78	3.13	0.25	n.d.
Ofloxacin	n.d.	n.d.	0.03	0.39	0.39	1.00	n.d.
Amphotericin B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13

IC_{50} = half maximal inhibitory concentration; MIC = minimum inhibitory concentration; n.d. = Not Determined.

* results are expressed as MIC values except the tested for *C. albicans* which was expressed as IC_{50} values.

† MIC value required to inhibit the growth of 90% of organisms (MIC_{90}) value.

Table 4 Cytotoxicity of *B. xiphostachya* hexane extract on nine cell lines

Sample	Cytotoxicity (IC_{50}, μg/mL)								
	KB	MCF-7	NCI-H187	HepG2	Caco2	B16-F10	NCTC	HDF	Vero
Extract	> 50	> 50	> 50	> 100	> 100	> 100	> 100	> 100	> 50
Ellipticine	2.79	n.d.	4.56	2.13	16.39	3.05	1.96	3.12	1.01
Doxorubicin	0.70	7.51	0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tamoxifen	n.d.	6.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = Not Determined.

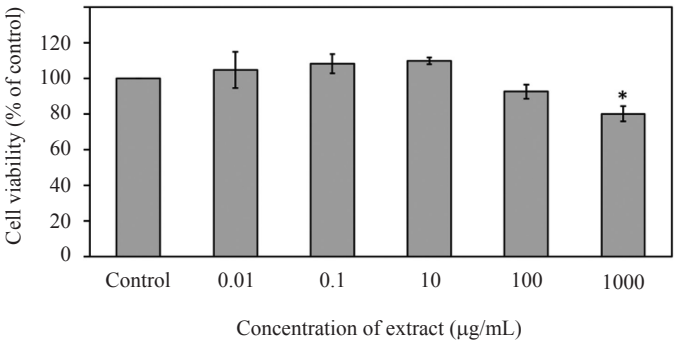

Fig. 2 Effect of *B. xiphostachya* hexane extract on LNCaP cell viability after treatment for 24 hr, shown as mean ± SE bar (n = 3) and * = $p < 0.05$ comparison to the control group

This study investigated several biological activities of *B. xiphostachya* hexane rhizome extract. However, no promising activities were obtained. These results differed from the data reported in the literature mentioned above where *Boesenbergia* spp. exerted various biological effects. Moderate polar and nonpolar solvents for extraction were previously applied. Polar solvents can be used to extract a broad range of metabolites from samples and the presence of several phytochemical components such as flavonoids derivatives, chalcone derivatives and terpenoids has been previously reported in *Boesenbergia* species (Eng-Chong et al., 2012; Madaka et al., 2013; Chahyadi et al., 2014; Sudsai et al., 2014; Sudsai et al., 2016)). Interestingly, the *B. xiphostachya* rhizome in the current study was extracted with a nonpolar solvent. The results demonstrated that the extract contained a different classification of compounds and proportions of components mainly containing phenylpropanoids and traces of mono- and sesquiterpenoids. It also exhibited weak biological effects when compared to other *Boesenbergia* spp. Therefore, its weak biological effects could have been due to the different polarity in solvent extraction.

In conclusion, the hexane extract of *B. xiphostachya* rhizome was investigated for volatile components, biological activities and cytotoxicity effects for the first time. The obtained data may be useful for further studies on the other application of the extract. Further studies on the isolation and identification of chemical constituents are still needed.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was financially supported by Naresuan University, Thailand (Grant number R2559B044). The Cosmetics and Natural Products Research Center, Faculty of Pharmaceutical Sciences, Naresuan University allowed access to their cell culture facility. We are indebted to the Bioassay Research Facility of the National Center for Genetic Engineering and Biotechnology for bioactivity tests. Assistant Professor Dr Pranee Nangngam helped in the identification of plant material.

References

Abdelwahab, S.I., Mohan, S., Abdulla, M.A., et al. 2011. The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property *in vivo*: Possible involvement of indirect antioxidant action. J. Ethnopharmacol. 137: 963−970.

Babushok, V.I., Linstrom, P.J., Reed, J.J., Zenkevich, I.G., Brown, R.L., Mallard, W.G., Stein, S.E. 2007. Development of a database of gas chromatographic retention properties of organic compounds. J. Chromatogr. A. 1157: 414−421.

Bouzouita, N., Abdelfateh, E., Faten, K., Paul, J.W., Michel, M., Moncef, C.M. 2009. Chemical composition and antioxidant activity of *Laurus nobilis* floral buds essential oil. J. Essent. Oil Bear. Plants. 12: 694−702.

Chahyadi, A., Hartati, R., Wirasutisna, K.R., Elfahmi. 2014. *Boesenbergia pandurata* Roxb., An Indonesian medicinal plant: phytochemistry, biological activity, plant biotechnology. Procedia Chem. 13: 13−37.

Chan, E.W.C., Lim, Y.Y., Wong, L.F., et al. 2008. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. Food. Chem. 109: 477–483.

Changsen, C., Franzblau, S.G., Palittapongarnpim, P. 2003. Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamidase promoter. Antimicrob. Agents. Chemother. 47: 3682−3687.

Chayamarit, K., Pooma, R., Pattharahirantricin, N. 2014. A checklist of plants in Thailand Volume I. Office of Natural Resources and Environmental Policy and Planning. Bangkok, Thailand.

Chellian, R., Pandy, V., Mohamed, Z. 2017. Pharmacology and toxicology of α- and β-asarone: A review of preclinical evidence. Phytomedicine. 32: 41−58.

Chuakul, W., Boonpleng, A. 2003. Ethnomedical uses of Thai Zingiberaceous plant (1). Thai J. Phytopharm. 10: 33−39.

Clinical and Laboratory Standards Institute (CLSI). 2006. Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically. Approved Standard, M7–A7. Clinical and Laboratory Standards Institute. Wayne, PA, USA.

De Vincenzi, M., De Vincenzi, A., Silano, M. 2004. Constituents of aromatic plants: elemicin. Fitoterapia. 75: 615–618.

Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents. Chemother. 16: 710−718.

Eng-Chong, T., Yean-Kee, L., Chin-Fei, C., et al. 2012. *Boesenbergia rotunda*: from ethnomedicine to drug discovery. J. Evidence-Based Complementary Altern. Med. 2012: 1−25.

Gopalakrishnan, M. 1992. Chemical composition of nutmeg and mace. J. Spices Arom. Crops 1: 49−54.

Goswami, P., Verma, S.K. Chauhan, A., et al. 2017. Chemical composition and antibacterial activity of *Melaleuca bracteata* essential oil from India: A natural source of methyl eugenol. Nat. Prod. Commun. 12: 965−968.

Hunt, L., Jordan, M., De Jesus, M., Wurm, F.M. 1999. GFP-expressing mammalian cells for fast, sensitive, noninvasive cell growth assessment in a kinetic mode. Biotechnol. Bioeng. 65: 201−205.

Jantan, I., Basni, I., Ahmad Abu, S., Mohd Ali Nor, A., Ahmad Abdul, R., Ibrahim, H. 2001. Constituents of the rhizome oils of *Boesenbergia pandurata* (Roxb.) Schlecht from Malaysia, Indonesia and Thailand. Flavour Fragrance J. 16: 110−112.

Jantan, I., Rafi, I.A.A., Jalil, J. 2005. Platelet-activating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants. Phytomedicine. 12: 88−92.

Jing, L.J., Mohamed, M., Rahmat, A., Bakar, M.F.A. 2010. Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*). J. Med. Plants Res. 4: 27−32.

Kanathum, N. 2008. Medicinal and lucky plant of Thailand. Baanlaesuan. Bangkok, Thailand.

Lal, M., Dutta, S., Munda, S., Pandey, S.K. 2018. Novel high value elemicin-rich germplasm of lemon grass *Cymbopogon khasianus* (Hack) Stapf (ex Bor) from North East India. Ind. Crops. Prod. 115: 98−103.

Linstrom, P.J., Mallard, W.G. 2016. NIST Chemistry WebBook. NIST Standard Reference Database Number 69. Gaithersburg, MD, USA. http://webbook.nist.gov/chemistry, 31 August 2017.

Madaka, F., Wattanapiromsakul, C., Sudsai, T., Tewtrakul, S. 2013. Anti-allergic activity of compounds from *Boesenbergia thorelii*. Songklanakarin J. Sci. Technol. 35: 197−201.

McGaw, L.J., Jäger, A.K., Staden, J.V. 2002. Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* in South Africa. S. Afr. J. Bot. 68: 31−35.

Mişe Yonar, S., Köprücü, K., Yonar, M.E., Silici, S. 2017. Effects of dietary propolis on the number and size of pleopadal egg, oxidative stress and antioxidant status of freshwater crayfish (*Astacus leptodactylus* Eschscholtz). Anim. Reprod. Sci. 184: 149−159.

Ngamriabsakul, C., Techaprasan, J. 2006. The phylogeny of Thai *Boesenbergia* (Zingiberaceae) based on petA-psbJ spacer (chloroplast DNA). J. Sci. Technol. 28: 49−57.

O'Brien, J., Wilson, I., Orton, T., Pognan, F. 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267: 5421−5426.

Potier, M., Mameli, L., Belisle, M., Dallaire, L., Melancon, S.B., 1979. Fluorometricassay of neuraminidase with a sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) substrate. Anal. Biochem. 94: 287–296.

Sirirugsa, P. 1992. A revision of the genus *Boesenbergia* Kuntze (Zingiberaceae) in Thailand. Natural History Bulletin of the Siam Society. 40: 67−90.

Suarez, A.I., Vasquez, L.J., Manzano, M.A., Compagnone, R.S. 2005. Essential oil composition of *Croton cuneatus* and *Croton malambo* growing in Venezuela. Flavour. Frag. J. 20: 611−614.

Sudsai, T., Prabpai, S., Kongsaeree, P., Wattanapiromsakul, C., Tewtrakul, S. 2014. Anti-inflammatory activity of compounds from *Boesenbergia longiflora* rhizomes. J. Ethnopharmacol. 154: 453−461.

Sudsai, T., Wattanapiromsakul, C., Nakpheng, T., Tewtrakul, S. 2013. Evaluation of the wound healing property of *Boesenbergia longiflora* rhizomes. J. Ethnopharmacol. 150: 223−231.

Sudsai, T., Wattanapiromsakul, C., Tewtrakul, S. 2016. Wound healing property of isolated compounds from *Boesenbergia kingii* rhizomes. J. Ethnopharmacol. 184: 42−48.

Sukari, M.A., Mohd Sharif, N.W., Yap, A.L.C.T., et al. 2008. Chemical constituents variations of essential oils from rhizomes of four Zingiberaceae species. MJAS. 12: 638−644.

Suphrom, N., Insumrong, K., Sriboon, P., Sonyot, W., Ingkaninan, K., Boonphong, S. 2018. Chromatographic fingerprint and free radical scavenging activity of *Boesenbergia xiphostachya* (Gagnep.) Loes rhizome extract. NU. Int. J. Sci. 15: 1−8.

Suphrom, N., Pumthong, G., Khorana, N., Waranuch, N., Limpeanchob, N., Ingkaninan, K. 2012. Anti-androgenic effect of sesquiterpenes isolated from the rhizomes of *Curcuma aeruginosa* Roxb. Fitoterapia. 83: 864−871.

Suphrom, N., Sonyot, W., Insumrong, K., Sawangsup, P., Sutamuang, P., Ingkaninan, K. 2017. GC-MS analysis and *in vitro* anti-androgenic activity of *Kaempferia rotunda* Linn extract. NUJST. 25: 34−43.

Tan, K.H., Nishida, R. 2012. Methyl eugenol: Its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. Int. J. Insect Sci. 12: 56.

Techaprasan, J., Klinbunga, S., Jenjittikul, T. 2008. Genetic relationships and species authentication of *Boesenbergia* (Zingiberaceae) in Thailand based on AFLP and SSCP analyses. Biochem. Syst. Ecol. 36: 408−416.

Techaprasan, J., Ngamriabsakul, C., Klinbunga, S., Chusacultanachai, S., Jenjittikul, T. 2006. Genetic variation and species identification of Thai Boesenbergia (Zingiberaceae) analyzed by chloroplast DNA polymorphism. J. Biochem. Mol. Biol. 39: 361−370.

Theanphong, O., Jenjittikul, T., Mingvanish, W. 2015. Essential oil compositions from root and rhizome of *Zingiber niveum* Mood & Theilade from Laos. Biol. Chem. Res. 2: 157−160.

Vahirua, I.L., François, P., Menut, C., Lamaty, G., Bessiere, J.M. 1993. Aromatic Plants of French Polynesia. I. Constituents of the essential oils of rhizomes of three Zingiberaceae: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koenig and *Etlingera cevuga* Smith. J. Essent. Oil Res. 5: 55−59.

van Den Dool, H., Dec. Kratz, P. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. A. 11: 463−471.

Villanueva, M.A., Torres, R.C., Baser, K.H.C., Ozek, T., Kurkcuogl, M. 1993. The composition of Manila elemi oil. Flavour Fragr. J. 8: 35−37.

Zaeoung, S., Plubrukarn, A., Keawpradub, N. 2005. Cytotoxic and free radical scavenging activities of Zingiberaceous rhizomes. Songklanakarin J. Sci. Technol. 27: 799−812.