Chemical Compositions and Anticancer Activity of Essential Oil from *Houttuynia cordata* Thunb.

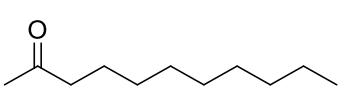
Sorachai Khamsan[1, *], Noppawan Intecha[1], Preeyaporn Inpeng[2], Waranyakorn Wongwan[2], Supawit Phintakul[2] and Chayanan Jitmanee[2, 3]

[1]Department of Chemistry, School of Science, University of Phayao, Phayao, 56000
[2]Demonstration School, University of Phayao, Phayao, 56000
[3]Department of Environment Science, School of Energy and Environment, University of Phayao, Phayao, 56000

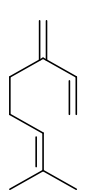
*Corresponding author. E-mail: sorabond@gmail.com

ABSTRACT

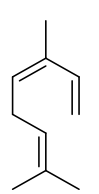
This work described the investigation of chemical compositions and *in vitro* evaluation of anticancer activity of the essential oil obtained from *Houttuynia cordata* Thunb. against HepG2 (hepatocarcinoma), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer). The volatile essential oil compositions of *H. cordata* were analyzed by GC and GC/MS techniques. The main constituents were 2-undecanone (48.61%), β-myrcene (11.94%), (*Z*)-β-ocimene (11.59%), 2-decanone (4.99%) and γ-terpinene (2.62%). The essential oil exhibited highest cytotoxic and selective activity against NCI-H187 cell lines with IC_{50} value of 17 µg mL^{-1}, but was non-cytotoxic to HepG2 and MCF-7 human cancer cell lines. Furthermore, the essential oil also showed cytotoxic effect to Vero cells with IC_{50} value of 42 µg mL^{-1}. Therefore, according to these results, we suggest that the essential oil from *H. cordata* might be another potential source for the discovery of new drugs to treat lung cancer and could be considered for the use of this plant.



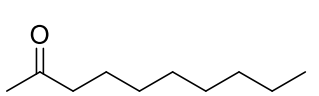
2-undecanone



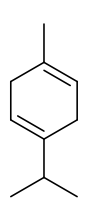
β-myrcene



(*Z*)-β-ocimene



2-decanone



γ-terpinene

Keywords: Anticancer activity, Cytotoxicity, Essential oil compositions, GC/GC-MS

Article history:
Received 26 June 2019; Received in revised form 1 April 2020;
Accepted 2 April 2020; Available online 2 June 2020

INTRODUCTION

Cancer is one of the major causes of death worldwide, with there being high mortality rates and the numbers of new cancer cases are expected to continue rising (Siegal et al., 2018). In Thailand, approximately 60% of cancer burden is due to five types cancer including breast, cervix, colorectal, liver and lung cancer (Ferlay et al., 2016). So, the continue searching for new and more effective anticancer drugs is urgently needed.

Essential oils exhibit a wide range of bioactivities, especially antibacterial, antioxidant, anti-inflammatory, antimalarial, anti-tumor, anti-mutagenic and anticancer activities against various cancer cells (Bakkali et al., 2008, Dhifi et al., 2016, Khamsan et al., 2011). Therefore, the biological activity screening of the essential oil from plants source is the focus of our research. It might be a potential source for drug discovery for treatment disease, especially cancer.

Many variety of plants, especially herb, can be found in the northern part of Thailand. Local people are used for consumption, processing into various products and also used as a traditional medicine such as antibiotics, anti-viral, inflammation, diabetes and anticancer activities (Mahidol et al., 2002).

Houttuynia cordata Thunb. belongs to Saururaceae family, *Houttuynia* genus. It is a flowering plant perennial herb native to Japan, Southern-China and Southeast Asia. This plant grows in moist shady places such as hillside, wayside and ridge of field with an altitude of 300 - 2600 m. *H. cordata* is extensively known by local people in the northern of Thailand as herb, vegetable for cooking and well known traditional medicine because of its medicinal properties (Jiangang et al., 2013). In Thailand, it has been used for immune stimulation and as an anticancer agent (Nuengchamnong et al., 2009).

The previous phytochemical studies on *H. cordata* revealed the possesses flavonoids (Meng et al., 2006), polyphenols (Meng et al., 2007), flavonoid glycosides (Nakamura et al., 1936) organic acids and fatty acids (Shoe et al., 1989), sterols (Takagi et al., 1996, Bauer et al., 1996), amino acids, alkaloids (Nishiya et al., 1988, Jong et al., 1993, Wang et al., 2007), as well as volatile oils (Bauer et al., 1996). The volatile oils of *H. cordata* were extracted by various methods such as steam distillation (Hao et al., 1995), supercritical CO_2 extraction, petroleum ether extraction (Zeng et al., 2003) and solid-phase microextraction (Zeng et al., 2005). The chemical compositions of essential oil from this species have been studied and terpenes, fatty acids, aldehydes, ketones were previously identified in the essential oil from *H. cordata* (Liu et al., 1979). Pharmacological activity studies of the essential oil from *H. cordata* were reported to exhibit anti-inflammatory activity (Li et al., 2013) antibacterial activity (Zhang et al., 2008) and antiviral activity (Hayashi et al., 1995).

However, the volatile components and anticancer activity of the essential oil from *H. cordata* growing in Thailand have rarely been studied. The pharmacological activity screening of essential oils from Thai medicinal plants for treatment of cancer is the focus of our ongoing research program. Therefore, the aims of this present work are to investigate the essential oil compositions using gas chromatography-mass spectrometry (GC-MS) and evaluate its anticancer activities.

METHODOLOGY

Plant materials and essential oil extraction

H. cordata were collected from Phayao province, Thailand. Amount 1200 g of the fresh aerial parts of plants were washed with distilled water, chopped into small pieces and subjected to hydrodistillation at the normal pressure for 3 hours in a modified Clevenger-type apparatus. The oil was collected, dried over with anhydrous Na_2SO_4. The essential oil was stored at 4°C for further analysis.

Gas Chromatography-Mass Spectrometry analysis of the essential oil

The analysis for volatile components of the essential oil from *H. cordata* were performed on a Hewlett-Packard (Agilent Tecnology GC 7890A) gas chromatograph equipped with HP-5 (HP 19091J-433E) fused silica capillary column 30 m X 0.25 mm, 0.25 µm film thickness (composed of 5% phenyl methyl polysiloxane) for the volatile components separation, temperature programmed as follows: 80°C held for 4 min, then to 260°C at 8°C /min for 20 min. High purity helium was used as the carrier gas with a constant flow rate at 1.00 mL/min; injector port and detector temperature were 250 and 280°C, respectively. Samples were injected by splitting and the split ratio was 100:1. GC/MS analysis was performed on Hewlett-Packard 6850GC coupled with a Hewlett-Packard 5973N mass selective detector under the same conditions as for GC. Significant quadrupole MS operating parameters: interface temperature 240°C; electron impact ionization at 70 eV with scan mass range of 35-550 m/z in full scan at a sampling rate of 1.0 scan/s. The sample solutions were prepared by dissolving 10 mg essential oil into 1 mL of dichloromethane (RCI Labscan Ltd, Thailand) and filtered prior to injection. Then, 1.0 µL of essential oil solution was injected into above GC-MS system. The volatile components were identified by comparison of their retention indices (RIs) which determined relatively to *n*-alkane series (C7-C30) with those given in the literature and their mass spectra authentic samples, which were compared with those libraries of mass spectral data (Wiely7n.1, the National Institute of Standards and Technology (NIST) mass spectral Database (2008) and W8NO8 library). The relative amounts of each components in the essential oil were calculated by normalization of peak areas as the percentage of total detected volatile components.

Determination of anticancer activity

The anticancer activities of the essential oil against the cancerous human-cell lines including MCF-7 (breast adenocarcinoma, ATCC HTB-22), NCI-H 187 (small cell lung carcinoma, ATCC CRL-5804) and HepG2 (hepatocarcinoma) cell lines were assayed employing the Resazurin microplate assay (REMA) as described by Brien et al. with suitable modification. In brief, cells at a logarithmic growth phase were harvested and diluted to $9x10^4$ cells/mL, in fresh medium. Successively, 5 µL of the essential oil diluted in 5% DMSO, and 45 µL of cell suspension were added to 384-well plates, incubated at 37ºC in 5% CO_2 incubator. After the incubation period (5 days), 12.5 µL of 62.5 µg/mL resazurin solution was added to each well, and the plates were then incubated at 37ºC for 4 hours. Fluorescence signal was measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 and 590 nm, respectively. Dose response curves were plotted from 6 concentrations of 2-fold serially diluted test compounds and

the sample concentrations that inhibit cell growth by 50% (IC_{50}) can be derived using the SOFTMax Pro software (Molecular Devices, USA). Ellipticine and doxorubicine were used as positive controls.

Determination of cytotoxicity assay against Vero cell

The cytotoxicity against primate cell line (Vero) of the extract was assayed by using green fluorescent protein (GFP) detection described by Hunt et al. with suitable modification. In brief, the GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N-1 plasmid (Clontech). The cell line was maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/mL geneticin, at 37 ºC in a humidified incubator with 5% CO_2. The assay was carried out by adding 45 µL of cell suspension at 3.3x104 cells/mL to each well of 384-well plates containing 5 µL of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37ºC incubator with 5% CO_2. Fluorescence signals were measured by using SpectralMax M5 microplate reader (Molecular Devices, USA) in the bottom reading mode with excitation and emission wavelengths of 485 and 535 nm, respectively. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. The IC_{50} values were derived from dose-response curves, using 6 concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software (Molecular device). 0.5% DMSO was used as a negative control. Ellipticine and doxorubicine were used as positive controls.

RESULTS AND DISCUSSION

The yields of clear pale yellow essential oil of *H. cordata* were 0.015% v/w. The volatile composition of this oil was determined using GC (FID) and GC-MS. Table 1 shows the results of the qualitative and quantitative essential oil analyses listed in order of elution in the HP-5 capillary column. The relative amount of each components were calculated by peak-area normalization as list in Table 1. The main of the identified compounds consisted of ketones (54.80%), monoterpene hydrocarbons (28.19%) and oxygenated sesquiterpenes (3.29%) corresponding to 94.49% of the essential oil. In total, 26 compounds were identified, the most representative compounds containing 2-undecanone (48.61%), *β*-myrcene (11.94%), (*Z*)-*β*-ocimene (11.59%), 2-decanone (4.99%) and *γ*-terpinene (2.62%), respectively (Figure 1). The chemical compositions of essential oil of *H. cordata* have been studied. The detected compounds were corresponded to that of the previous reports. The analysis of essential oils from underground parts shows that 2-undecanone (23.96%) and *β*-myrcene (14.29%) were the major constituents (Lu et al., 2006). The essential oil from leaves revealed that the main constituents were found to contain *β*-myrcene (30.8%) and 2-undecanone (19.7%) (Do et al., 2015). It should be noted that, the chemical compositions in this study differed from previous reports, which can be attributed to the difference of plant parts used, the growing area, extraction condition method as well as instrumental analysis techniques.

The essential oil of *H. cordata* showed selective significant anticancer activity against NCI-H187 cell lines with IC_{50} value of 17 µg mL^{-1}, but was non-cytotoxic to

HepG2 and MCF-7 human cancer cell lines (Table 2). The cytotoxicity of some major constituents present in the essential oil has been reported in the literature that 2-undecanone inhibited effectively against lung tumorigenesis (Lou et al., 2019), while *β*-myrcene possesses strong against MDA-MB-231 human breast cancer cells (Lee et al., 2015). Furthermore, the pharmacological effect of the constituents has also reported that (*Z*)-*β*-ocimene was highly toxic on larvae of *Anopheles Stephensi* (Govindarajan et al., 2016) as well as *γ*-terpinene possesses anti-inflammatory activity (Ramalho et al., 2015).

The chemical compositions indicated that high sesquiterpene contents and structural diversity in the essential oil were responsible for its potent anticancer activity in particular, α-cadinol presenting in the essential showed selective toxicity against human colon adenocarcinoma cell lines HT-29 (He et al., 1997) and *β*-caryophyllene exhibited against cancer cell lines (Sylvestre et al., 2006). Hexadecanoic acid has shown selective cytotoxic activity to human leukemic cells (Harada et al., 2002), while methyl hexadecanoate possesses strong antitumor activity (Harada et al., 2002) and shows potent cytotoxicity in human gastric cancer cells (Lee et al., 1997). Furthermore, the essential oil also showed cytotoxic effect to Vero cells with IC_{50} value of 42 µg mL^{-1}. Findings from this study, the essential oil from *H. cordata* possessed potent anticancer activity. It might be another potential source for the discovery of new drugs to treat cancer especially in lung cancer treatment. This scientific supporting data might be helpful in their utilization as useful plant resources on traditional medicine herb, food supplement and pharmaceutical proposes.

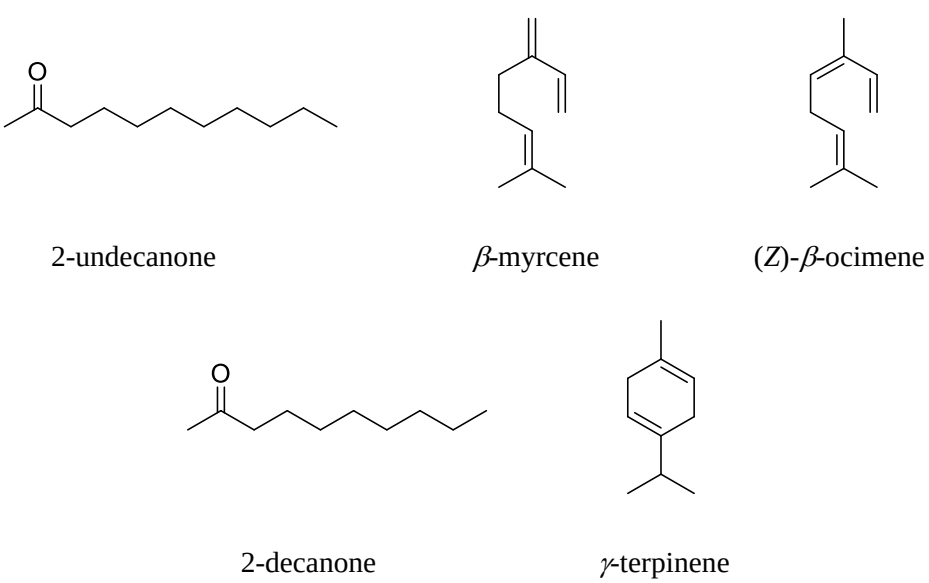


Figure 1 Major constituents of *H. cordata* essential oil

Table 1 Chemical constituents of the essential oil from *H. cordata*

Identified Compounds	RI[a]	percentage[b]	Identification[c]
Monoterpene hydrocarbons			
α-Pinene	935	0.68	RI, MS
Camphene	953	0.42	RI, MS
β-Pinene	979	0.52	RI, MS
β-Myrcene	990	11.94	RI, MS
Limonene	1032	0.21	RI, MS
(*Z*)-*β*-Ocimene	1042	11.59	RI, MS
(*E*)-*β*-Ocimene	1044	0.21	RI, MS
γ-Terpinene	1063	2.62	RI, MS
Esters			
Octyl acetate	1132	0.33	RI, MS
Oxygenated monoterpene			
Terpen-4-ol	1175	0.41	RI, MS
Ketones			
2-Decanone	1190	4.99	RI, MS
Aldehydes			
Decanal	1209	1.76	RI, MS
Ketones			
Piperitone	1240	0.88	RI, MS
2-Undecanone	1291	48.60	RI, MS
Sesquiterpene hydrocarbons			
β-Caryophyllene	1415	0.87	RI, MS
α-Humulene	1452	0.82	RI, MS
Oxygenated sesquiterpenes			
(*E*)-*β*-Farnansene	1454	0.23	RI, MS
Aldehydes			
1-Dodecanal	1469	0.48	RI, MS
Oxygenated sesquiterpenes			
β-Selinene	14$_{85}$	1.17	RI, MS
Ketones			
2-Tridecanone	1497	0.33	RI, MS
Oxygenated sesquiterpenes			
(*E*)-Nerolidol	1564	0.22	RI, MS
Caryophyllene oxide	1572	0.26	RI, MS
α-Cadinol	1657	1.41	RI, MS
Esters			
Methyl hexdecanoate	1929	1.06	RI, MS
Carboxylic acids			
Hecadecanoic acid	1940	1.57	RI, MS
Others			
(*Z*)-9-Octadecenamide	2398	0.91	RI, MS
Monoterpene hydrocarbons		28.19	
Oxygenated monoterpene		0.41	

Sesquiterpene hydrocarbons	1.69
Oxygenated sesquiterpenes	3.29
Ketones	54.80
Aldehydes	2.24
Esters	1.39
Carboxylic acids	1.57
Others	0.91
Total	94.49

[a]RI retention indices; relative to n-alkane series (C7-C30)
[b]Results obtained by peak area normalization
[c]Methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature

Table 2 Cytotoxicity of *H. cordata* essential oil on human cancer cell lines

	IC_{50}[a] (µg mL^{-1})			
Sample	**Vero cells**	**MCF-7**	**NCI-H187**	**HepG2**
essential oil	42	-[b]	17	-
Tamoxifen[c]	-	7.8	-	-
Doxorubicine[d]	-	9.7	0.3	-
Ellipticine[e]	1.2	-	4.3	3.7

[a]Concentration that killed 50% of cell lines
[b] - Inactive at 50 µg mL^{-1}
[c, d, e]Anticancer drugs used as positive controls

CONCLUSIONS

In conclusion, the chemical constituents of the essential oil of *H. cordata* was analyzed by GC and GC-MS techniques and contained ketones (54.80%), monoterpene hydrocarbons (28.19%) and oxygenated sesquiterpenes (3.29%) and 2-undecanone (48.61%), *β*-myrcene (11.94%) were the major constituents. The essential oil possessed selective cytotoxic activity against NCI-H187 cell lines, but was non-cytotoxic to HepG2 and MCF-7 human cancer cell lines. The cytotoxicity of the essential oil against Vero cells. This scientific finding could be helpful for the use of this plant.

ACKNOWLEDGMENTS

The authors gratefully acknowledge School of Science and Demonstration School University of Phayao, University of Phayao for laboratory facilities and some financial support.

REFERENCES

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, *46*, 446–475.

Bauer, R., Proebstle, A., & Lotter, H. (1996). Cyclooxygenase inhibitory constituents from *Houttuynia cordata*. *Phytomedicine*, *2*, 305–308.

Brien, J.O., Wilson, I., Orton, T., & Pognan, F. (2000). Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European journal of biochemistry*, *267*(17), 5421–5426.

Choe, K.H., Kwon, S. J., & Lee, K.C. (1989). Chemical composition of Saururaceae growing in Korea (3). on fatty acids and amino acids of *Houttuynia cordata* and *Saururus chinensis*. *Punsok Kwahak*, *2*, 285–292.

Dhifi, W., Bellili, S., Jazi , S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, *25*(3), 1–16.

Do, N.D., Tran, D.T., AbdulRazaq, O., Olanrewaju, I.E., & Isiaka, A.O. (2015) Chemical constituents of essential oils from the leaves of *Tithonia diversifolia*, *Houttuynia cordata* and *Asarum glabrum* grown in Vietnam. *American Journal of Essential Oils and Natural Products*, *2*, 17–21.

Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., … Bray, F. (2016). Globocan 2012 cancer incidence and mortality worldwide. Web site: http://globocan.iarc.fr/

Govindarajan, M., & Benelli G. (2016). Artemisia absinthium-borne compounds as novel larvicides: effectiveness against six mosquito vectors and acute toxicity on non-target aquatic organisms. *Parasitology Research*, *115*(12), 4649–4661.

Hao, X., Li, L., Ding, Z., & Yi, Y. (1995). Analysis of essential oil from *Houttuynia cordata* in Guizhou. *Acta Botanica Yunnanica*, *17*, 350–352.

Harada, H,. Yamashita, U., Kurihara, H., Fukushi, E., Kawabata, J., & Kamei, Y. (2002). Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer Research*, *22*, 587–2590.

Hayashi, K., Kamiya, M., & Hayashi, T. (1995). Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV. *Planta Medica*, *61*, 237–241.

He, K., Zeng, L., Shi, G., Zhao, G.-X., Kozlowski, J.F., & McLaughlin, J.L. (1997). Bioactive compounds from *Taiwania cryptomerioides*. *Journal of Natural Products*, *60*, 38–40.

Jang, D.S., Kim, J.M., Lee,Y.M., Yoo, J.L., Kim, Y.S., Kim, J.-H., & Kim, J.S. (2006). Flavonols from *Houttuynia cordata* with protein glycation and aldose reductase inhibitory activity. *Natural Product Sciences*, *12*, 210–213.

Jiangang, F., Ling, D., Zhang L., & Hongmei, L. (2013). *Houttuynia cordata* Thunb.: A review of phytochemistry and pharmacology and quality control. *Chinese Medicine*, *4*, 101–123.

Jong, T. T., & Jean, M. Y. (1993). Alkaloids from *Houttuynia cordata*. *Journal of the Chinese Chemical Society*, *40*, 301–303.

Khamsan, S., Liawruangrath, B., Liawruangrath, S., Teerawutkulrag, A., Pyne, S.G., & Garson, M.J. (2011). Antimalarial, anticancer, antimicrobial activities and chemical constituents of essential oil from the aerial parts of *Cyperus kyllingia* Endl. *Records of Natural Products*, *5*(4), 324–327.

Lee, J.H., Lee, K., Lee, D.H., Shin, S.Y., Yong, Y., & Lee, Y.H., (2015). Anti-invasive effect of beta-myrcene, a component of the essential oil from *Pinus koraiensis* cones, in metastatic MDA-MB-231 human breast cancer cells. *Journal of the Korean Society for Applied Biological Chemistry*, *58*, 563–569.

Lee, K.I., Rhee, S.H., & Park, K.Y. (1997). Anticancer activity of phytol and eicosatrienoic acid identified from Perilla leaves. *Han'guk Sikp'um Yongyang Kwahak Hoechi*, *28*, 1107–1112.

Li, W., Fan, T., Zhang, Y., Fan, T., Zhou, P., Niu, X., & He, L. (2013). *Houttuynia cordata* Thunb. volatile oil exhibited antiinflammatory effects in vivo and inhibited nitric oxide

and tumor necrosis factor-α production in LPS-stimulated mouse peritoneal macrophages in vitro. *Phytotherapy Research*, *27*, 1629–1639.

Liu, Y.L., & Deng, Z.F. (1979). Investigation of the chemical constituents of the essential oil of *Houttuynia cordata* Thunb. *Acta Botanica Sinica*, *21*, 244–249.

Lou, Y., Guo, Z., Zhu, Y., Kong, M., Zhang, R., Lu, L., … Wu, J. (2019). *Houttuynia cordata* Thunb. and its bioactive compound 2-undecanone significantly suppress benzo(a)pyrene-induced lung tumorigenesis by activating the Nrf2-HO-1/ NQO-1 signaling pathway. *Journal of Experimental & Clinical Cancer Researc*, *38*, 242.

Lu, H., Wu, X., Liang, Y., & Zhang. J. (2006). Variation in chemical composition and antibacterial activities of essential oils from two species of *Houttuynia Thunb*. *Chemical and Pharmaceutical Bulletin*, *54*, 936–940.

Mahidol, C., Prawat, H., Prachyawarakorn, V., & Ruchirawat, S. (2002). Investigation of some bioactive Thai medicinal plants. *Phytochemistry Reviews*, *1*, 287–297.

Meng, J., Dong, X.P., Jiang, Z.H., Leung, S.Y., & Zhao Z.Z. (2006). Study on chemical constituents of flavonoids in fresh herb of *Houttuynia cordata*. *China Journal of Chinese Materia Medica*, *31*(16), 1335–1337.

Meng, J., Dong, X.P., Zhou, Y.S., Jiang, Z.H., Leung, S.Y., & Zhao, Z.Z. (2007). Studies on chemical constituents of phenols in fresh *Houttuynia cordata*. *China Journal of Chinese Materia Medica*, *32*(10), 2007, 929–931.

Nakamura, H., Ota, T., & Fukuchi, G. (1938). The constituents of diuretic drugs. II. The flavonol glucoside of *Houttuynia cordata* Thunb. *Journal of the Pharmaceutical Society of Japan*, *56*,1936, 68.

Nishiya, H., Ishiwata, K., Komatsu, K., Nakata, O., Kitamura, K., & Fujii, S. (1988). Platelet aggregation inhibitors from Jyu-Yaku (Houttuyniae Herb). *Chemical & Pharmaceutical Bulletin*, *36*, 1902–1904.

Nuengchamnong, N., Krittasilp, K., & Ingkaninan, K. (2009). Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* Using LC-ESIMS Coupled with DPPH Assay. *Food Chemistry*, *117*, 750–756.

Ramalho, T.R., Oliveira, M.T., Lima, A.L., Bezerra-Santos, C.R., & Piuvezam, M.R. (2015). Gamma-terpinene modulates acute inflammatory response in mice. *Planta Medica*, *8*,1248–1254.

Siegal, R.L., Miller, D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*, *68*, 7–30.

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F., & Legault, J. (2006). Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *Journal of Ethnopharmacology*, *103*(1), 99–102.

Takagi, S., Yamaki, M. Masuda, K., & Kubota, M. (1978). On the constituents of the terrestrial part of *Houttuynia cordata* Thunb. *Shoyakugaku Zasshi*, *32*,123–125.

Wang, L., Zhao, Y., Zhou, L., & Zhou, J. (2007). Chemical constituents of *Houttuynia cordata*. *Chinese Traditional and Herbal Drugs*, *38*, 1788–1790.

Zeng, H., Jiang, L., & Zhang, Y. (2003). Chemical constituents of volatile oil from *Houttuynia cordata* Thunb. *Journal of Plant Resources and Environment*, *12*, 50–52.

Zhang, W., Lu, F., Pan, S., & Li, S. (2008). Extraction of volatile oil from Houttuynia cordata and its antibiotic and anti-virus activities. *Practical Preventive Medicine*, *15*, 312–316.