



# **Inhibition of Cell Proliferation by *Houttuynia cordata* Extract on Gastric Cancer Cells via Induction of Apoptosis**

**Thanh Quang Nguyen<sup>a</sup>, Hoang Nhat Minh Nguyen<sup>b</sup>, Van Minh Le<sup>c</sup>,  
Dai-Hung Ngo<sup>d\*</sup> and Thanh Sang Vo<sup>b\*</sup>**

<sup>a</sup> Faculty of Technology, Van Lang University, Ho Chi Minh City, Vietnam.

<sup>b</sup> NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

<sup>c</sup> Research Center of Ginseng and Medicinal Materials, Ho Chi Minh City, Vietnam.

<sup>d</sup> Thu Dau Mot University, Binh Duong Province, Vietnam.

## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JPRI/2022/v34i35A36163

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/85433>

**Received 12 February 2022**

**Accepted 25 April 2022**

**Published 28 April 2022**

**Original Research Article**

## **ABSTRACT**

*Houttuynia cordata* Thunb. is an edible and medicinal plant that belongs to the family of Saururaceae and widely distributes in Eastern Asia countries. *H. cordata* have been reported as a promising anticancer agent against various cancer cells. This study was to determine anti-proliferative effect of *H. cordata* extract on gastric cancer cells using an in vitro experimental model. The cytotoxicity was examined via the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, while cell migration was conducted by wound healing assay. The gene expression level was measured by real time PCR. The result showed that butanol extract exhibited highest anti-proliferative activity against BGC-823 cells with IC<sub>50</sub> value of 193 µg/ml. Moreover, the wound healing assay determined the suppressive capacity of butanol extract on cell migration at concentration of 50 µg/ml. Notably, butanol extract treatment (50 µg/ml) was able to up-regulate the mRNA expression of apoptosis-mediated signaling molecules, including caspase-8, Bax, caspase-9, and caspase-3 in gastric cancer cells. These results indicate that butanol extract of *H. cordata* possesses inhibitory activity on tumor development and metastasis via induction of the process of programmed cell death.

\*Corresponding author: E-mail: [vtsang@ntt.edu.vn](mailto:vtsang@ntt.edu.vn), [hungnd@tdmu.edu.vn](mailto:hungnd@tdmu.edu.vn);

**Keywords:** *Houttuynia cordata*; gastric cancer cells; BGC-823 cells; apoptosis; caspase-9.

## 1. INTRODUCTION

Gastric cancer is among the most common cancer with high mortality worldwide. A high incidence of gastric cancer has been detected in East Asian countries such as Korea, Japan, China [1]. The risk factors affecting the development of gastric cancer were suggested due to diet, stomach diseases, and age. Gastric cancer is usually diagnosed at the late stage of metastasis due to the delay of symptoms such as indigestion and pain [2]. Therefore, the treatment of gastric cancer is mainly involved in surgical excision, followed by radiotherapy and chemotherapy [3]. However, these therapies still remain to be unsatisfactory and exhibit several adverse effects, causing various health problems for patients. As the result, novel approaches for improvement of efficacy and reduction of side effects are necessary for treatment of gastric cancer patients.

Dietary products have been considered as alternative therapeutics for prevention and management of gastric cancer [4]. *Houttuynia cordata* Thunb. is an edible and medicinal plant that belongs to the family of Saururaceae and widely distributes in Eastern Asia countries such as China, Japan, India, Thailand, Taiwan, and Vietnam [5]. *H. cordata* has been applied as a traditional medicine for treatment of skin diseases, cholera, hemorrhoids, blood deficiency, cancer, coughs, urination promotion, dysentery, toxin release, enteritis, and fever [6]. So far, *H. cordata* was found to contain various medicinal ingredients such as volatile oils, aristolactams, 5,4-dioxoaporphines, oxoaporphines, flavonoids, benzenoids, and steroids. Hence, numerous biological activities of *H. cordata* was evidenced in different experimental models [6]. Notably, *H. cordata* was also risen as a promising anticancer agent against the growth of various cancer cells such as colorectal cancer cells [7,8], hepatic cancer cells [9], leukemia cells [10], lung cancer cells [11], breast cancer cells [12], and cervical cancer cells [13]. In the same trend, the inhibitory activity of *H. cordata* on gastric cancer cells was further investigated in the present study on BGC-823 cells-mediated model.

## 2. MATERIALS AND METHODS

### 2.1 Materials

*H. cordata* was purchased from local organic greengrocer in Ho Chi Minh city, Vietnam. BGC-

823 cells are human gastric cancer cells donated by Chinese Academy of Sciences (Shanghai, China). Reagents for real time PCR were purchased from Qiagen (Hilden, Germany). Primers for qPCR were purchased from Intergrated DNA Technologies, Iowa, USA. The others were purchased from Sigma-Aldrich (MO, USA).

### 2.2 Extraction

Dried powder of *H. cordata* was extracted by different solvents, including hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, ethanol, and butanol at a solid-to-liquid ratio of 1:8, (w/v) for 24h at room temperature. The process was repeated three times and the extracted solution was combined and subsequently evaporated to remove solvent under vacuum machine at 40°C. The extracts of *H. cordata* were obtained with the humidity level of less than 12% and stored at 4°C for further investigation.

### 2.3 Cytotoxic Assay

The cytotoxic effect of extracts on human gastric cancer cells was examined via MTT assay [14]. Briefly, BGC-823 cells were treated with 200 µg/ml of the extract for 24h. The MTT solution (0.5 mg/ml) was added and incubated for 4 h in CO<sub>2</sub> incubator. Finally, DMSO was added to solubilize formazan salt, and optical density was measured by a microplate reader (BioTek Instruments, USA) at 540 nm.

### 2.4 Inverted Microscope Assay

BGC-823 cells were treated with 200 µg/ml of extract for one day. Afterward, the treated cells were washed by PBS and the morphology was visualized under an inverted microscope with magnification of 10X (Oxion, Euromex, Netherlands).

### 2.5 Cell Migration Assay

The migration of the cells was examined according to Kwak and Ju [15]. Briefly, a monolayer of BGC-823 cells were scratched by a yellow tip. Subsequently, the cells were treated with extract at concentration of 50 µg/ml for 24 h. Gap area were visualized under an inverted microscope (Oxion, Euromex, Netherlands). Moreover, the relative gap area between 24h and

0 h in each group was measure by ImageJ and expressed as following formula:

$$\text{The relative gap area (\%)} = \frac{\text{Gap area of 24h}}{\text{Gap area of 0h}} * 100\%$$

## 2.6 Real Time PCR

BGC-823 cells were treated with 50 µg/ml of extract for 24 h. Total RNA from the treated cells was isolated by a commercial kit (Qiagen, Hilden, Germany). Subsequently, cDNA synthesis was performed according protocol of NEB (MA, USA). Each cycle of qPCR was conducted by using a master mix containing SYBR® Green as a detector of DNA under the conditions of denaturation of 94 °C (30 s), annealing of 60 °C (30 s), and extension of 72°C (30 s). The mRNA expression level of relative gene in the treated cells was respectively compared with that of the blank (without treatment). The sequences of the primers were designed as following Genecards and NCBI gene database. Primer for caspase-3 (4q35.1): F = TCGCTTTGTGCCATGCTGAA and R = ACTCAAATTCTGTTGCCACC; for caspase-8 (2q33.1): F = AATGGAACACACTTGGATGC and R = GCTCTACTGTGCAGTCATCG; for caspase-9 (1p36.21): F = TTGAGGACCTTCGACCAGCT and R = CAACGTACCAGGAGCCACTC); for Bax (19q13.33): F = CTGACGGCAACTTCAACTGG and R = CCAATGTCCAGCCCATGATG); for GAPDH (12p13.31): F = GGGCTCTCCAGAACATCATC and R = GGTCACCACTGACACGTTG. Experiments were performed in triplicate and repeated three times with similar results. The gene expression level was calculated as following formula (1), (2), and (3)

$$\Delta Cq = Cq (\text{Tar}) - Cq (\text{Ref}) \quad (1)$$

$$\Delta \Delta Cq = \Delta Cq (\text{Exp}) - \Delta Cq (\text{Con}) \quad (2)$$

$$2^{(-\Delta \Delta Cq)} \quad (3)$$

Where, Cq = quantification cycle; Tar = Target gene; Ref = Reference gene (GAPDH); Exp = Experimental; Con = Control

## 2.7 Statistical Analysis

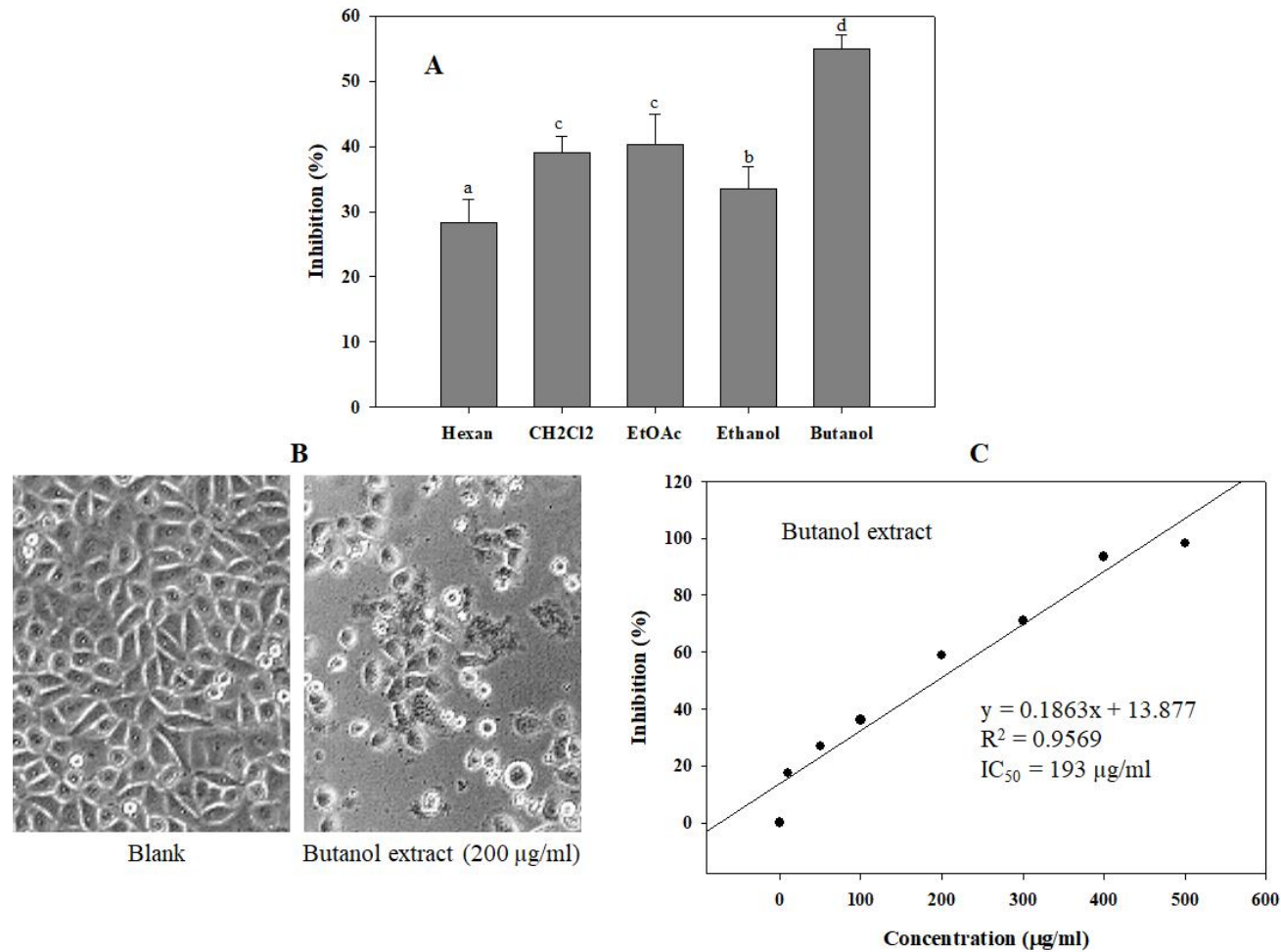
The ANOVA test of SPSS was used for analysis of data. Tukey's multiple range test was further

assessed to identify statistical differences among groups at p < 0.05.

## 3. RESULTS AND DISCUSSION

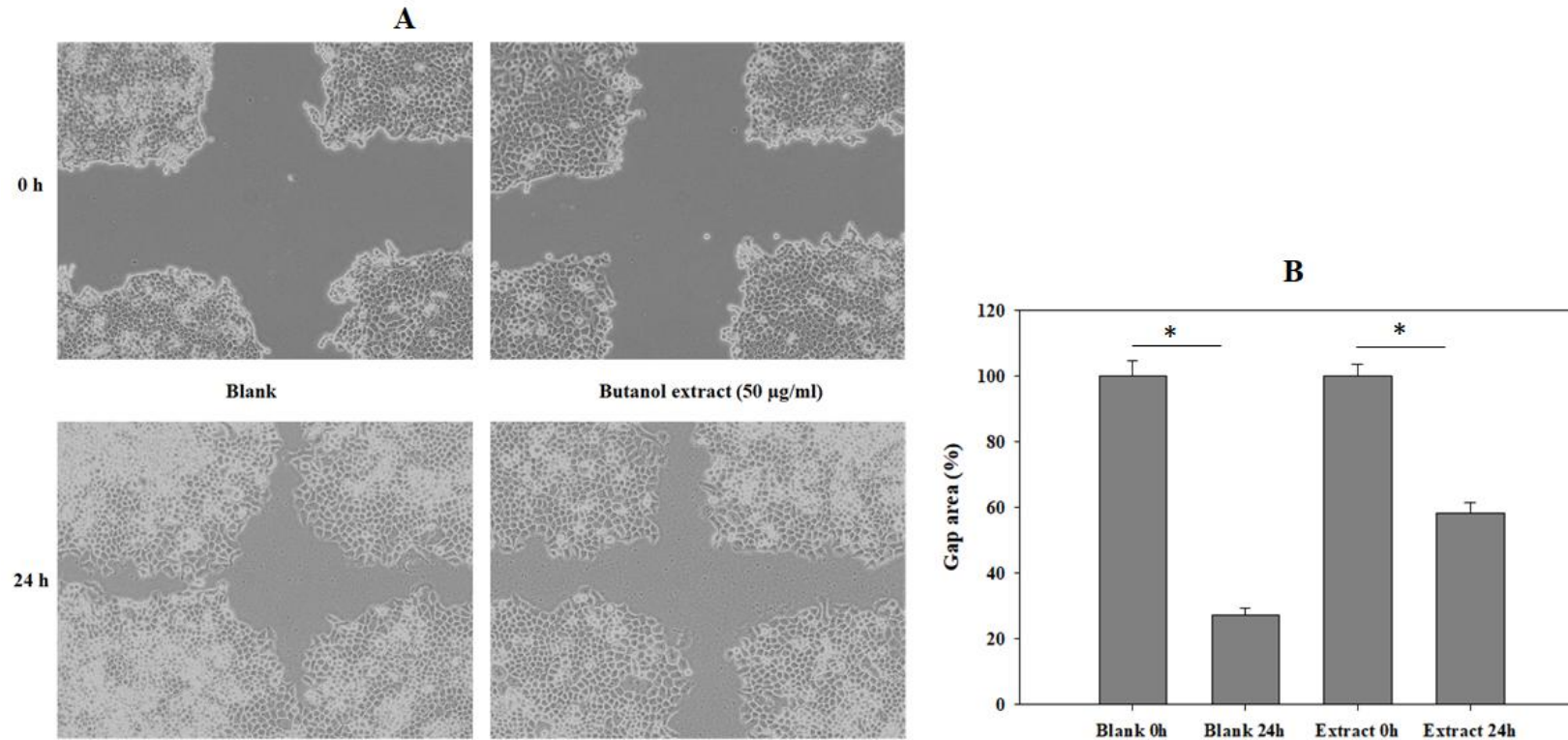
### 3.1 Cytotoxic Effect of *H. cordata* Extracts

*In vitro* assay has been developed as a useful, reliable and rapid method for identifying the cytotoxic activity of natural products toward different target cancer cells [16]. In this study, the cytotoxic effect of *H. cordata* extracts on human gastric cancer cells (BGC-823) was examined by MTT assay. The result of this assay showed that the rate of cell death was identified in a range of 28.2 – 55.1% as the cells treated with 200 µg/ml of hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, ethanol, and butanol extracts (Fig. 1A). Among them, butanol extract exhibited the highest cytotoxic effect against BGC-823 cell proliferation. Accordingly, butanol extract with high anti-proliferation effect was preferred to be used for the further investigation. The inverted microscopy assay showed that the morphological changes occurred in butanol-treated cells (Fig. 1B). The cells were observed to be round-shaped, reduced cell size, disrupted boundaries, and irregular surfaces as compared with the blank cells. Moreover, the inhibition of cell proliferation in dose-dependent manner was also investigated by butanol extract (Fig. 1C). Whereby, IC<sub>50</sub> value of butanol extract on cell growth inhibition was determined up to 193 µg/ml. Notably, the suppression of butanol extract of *H. cordata* on gastric cancer cell growth is higher than that of aqueous extracts of ziziphora, ginger, saffron, aloe vera [17], dandelion (*Taraxacum* spp.) [18], and *Curcuma manga* [19]. So far, *H. cordata* has been determined as a rich source phenolic compounds such as quercetin, rutin, hyperin, afzelin, isoquercitrin, apigenin, kaempferol, vanillic acid, and protocatechuic acid [20]. Numerous studies have reported that phenolic compounds possess the inhibitory activity on tumor generation, and thus have capacity for the prevention and treatment of cancer [21,22]. As the result, the high inhibition of butanol extract of *H. cordata* on the proliferation of gastric cancer cells could be suggested due to its phenolic content.



**Fig. 1. The cytotoxic effect of *H. cordata* extracts on gastric cancer cells**

Each determination was made in three independent experiments, and the data are shown as means  $\pm$  SD. Different letters (a–d) indicate significant difference among groups ( $p < 0.05$ ) by Tukey's multiple range test



**Fig. 2. The suppressive effect of butanol extract on cell migration**

BGC-823 cells were seeded on 6-well plates before a gap line was made by a yellow pipette tip. The cells were then treated with butanol extract for 24 h. (A) The images were captured at 0 h and 24 h treatment by an inverted microscope (10x magnification). (B) Moreover, the relative gap area between 24h and 0 h in each group was measure by ImageJ. Differences compared to the blank group were considered significant at  $*p<0.05$

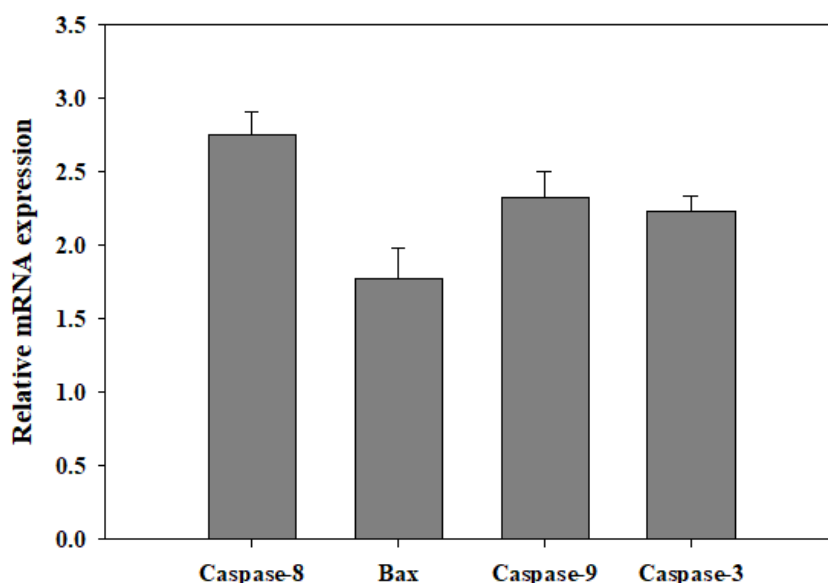
### 3.2 The Inhibition of Butanol Extract on Cancer Cell Migration

A crucial capacity of cancer cells is known as metastasis that is due to the migration of cancer cells from the primary tumor to secondary sites, causing mortality for many patients [23]. Thus, the inhibition of cell migration is considered to be as important as suppression of cell proliferation. Herein, a wound-healing assay was conducted to determine the inhibitory activity of butanol extract on gastric cancer cell migration [24]. It was shown that the treatment of *H. cordata* extract at concentration of 50 µg/ml significantly suppressed the spreading of gastric cancer cells to the gap area as compared with untreated group (Fig. 2). These results supported that butanol extract suppressed not only cell proliferation but also cell migration of gastric cancer cells, suggesting its role in prevention of cancer cell growth and metastasis. However, the effect of *H. cordata* extract on anti-proliferative activity-related intracellular signaling should be further investigated in gastric cancer cells.

### 3.3 Effect of Butanol Extract on Apoptotic Signaling Molecules

It is the fact that butanol extract of *H. cordata* caused cytotoxicity on gastric cancer cells, bring about suppression of cell growth and migration. It should be clear that whether its cytotoxic effect was due to necrosis or apoptosis on gastric cancer cells. Certainly, a natural product is

considered as a potential anticancer agent as it has capacity of apoptotic induction in the cancer cells [25,26]. Apoptosis is a caspase-mediated programmed cell death, leading to chromosome condensation, nuclear fragmentation, and membrane blebbing [27]. It has been evidenced that apoptotic induction triggers the up-regulation of caspase-8, Bax, caspase-9, and caspase-3 activation in cancer cells, leading to the process of cell death. In this study, the apoptotic effect of *H. cordata* butanol extract was investigated via measuring the expression levels of caspase-8, Bax, caspase-9, and caspase-3 in BGC-823 cells. Notably, butanol extract treatment significantly up-regulated the gene expression of apoptotic signaling molecules including caspase-8, Bax, caspase-9, and caspase-3 (Fig. 3). The gene expression levels of caspase-8, Bax, caspase-9 and caspase-3 in the treated cells were up to 2.8-fold, 1.8-fold, 2.3-fold, and 2.2-fold higher than that of the untreated cells, respectively. Likewise, several anticancer agents derived from plant extracts, such as *Brucea javanica*, *Camellia sinensis*, *Cinnamomum kanehirai* Hayata, *Corni Fructus*, *Cucurbita ficifolia*, *Cyperus rotundus* L. have been evidenced as apoptotic inducers due to up-regulating caspase-8, Bax, caspase-9, and caspase-3 in cancer cells [28]. This result indicated that *H. cordata* butanol extract possessed anticancer activity via induction of apoptosis, leading to the inhibition of proliferation and migration of gastric cancer cells.



**Fig. 3. Butanol extract of *H. cordata* induced apoptosis in BGC-823 cells**

Each determination was made in three independent experiments, and the data are shown as means ± SD

#### 4. CONCLUSION

*H. cordata* butanol extract can be concluded to possess a selective antitumor agent for gastric cancer due to inhibiting cell proliferation, inducing apoptosis and suppressing cell migration. These results suggested that crude extract of *H. cordata* can be developed as a novel component of potential products that is used for the treatment of gastric cancer. However, the mechanism of action of *H. cordata* extract on inhibition of cell migration and metastasis should be further investigated in the next studies.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### ACKNOWLEDGEMENT

This study was supported by Van Lang University and Nguyen Tat Thanh University.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer Manag Res.* 2018;10:239-48.
2. de Vries AC, Kuipers EJ. Epidemiology of premalignant gastric lesions: implications for the development of screening and surveillance strategies. *Helicobacter.* 2007; 12 Suppl 2:22-31.
3. Waddell T, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D. Gastric cancer: ESMO-ESSO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2013; 24 Suppl 6:vi57-63.
4. Ahmad R, Khan MA, Srivastava AN, Gupta A, Srivastava A, Jafri TR, Siddiqui Z, Chaubey S, Khan T, Srivastava AK. Anticancer Potential of Dietary Natural Products: A Comprehensive Review. *Anticancer Agents Med Chem.* 2020;20(2):122-236.
5. Rathi R, Roy S, Misra A, Singh S. Ethnobotanical notes on *Houttuynia cordata* Thunb. in North-eastern region of India. *Indian J Nat Prod Resour;* 2013.
6. Kumar M, Prasad SK, Hemalatha S. A current update on the phytopharmacological aspects of *Houttuynia cordata* Thunb. *Pharmacogn Rev.* 2014;8(15):22-35.
7. Lai KC, Chiu YJ, Tang YJ, Lin KL, Chiang JH, Jiang YL, Jen HF, Kuo YH, Agamaya S, Chung JG, Yang JS. *Houttuynia cordata* Thunb extract inhibits cell growth and induces apoptosis in human primary colorectal cancer cells. *Anticancer Res.* 2010;30(9):3549-56.
8. Tang YJ, Yang JS, Lin CF, Shyu WC, Tsuzuki M, Lu CC, Chen YF, Lai KC. *Houttuynia cordata* Thunb extract induces apoptosis through mitochondrial-dependent pathway in HT-29 human colon adenocarcinoma cells. *Oncol Rep.* 2009;22(5):1051-6.
9. Yang L, Ji W, Zhong H, Wang L, Zhu X, Zhu J. Anti-tumor effect of volatile oil from *Houttuynia cordata* Thunb. on HepG2 cells and HepG2 tumor-bearing mice. *RSC Advances.* 2019;9(54):31517-26.
10. Chanprasert S, Pawinwongchai JJPM. Induction of apoptosis and cytokines secretion of *Houttuynia cordata* Thunb. flavonoids in human leukemic cells. *Planta Med.* 2014;80(16):P1C6.
11. Chen Y, Chang W, Tsai HY, Lee M, Wu TS. Bioactivities and action mechanisms of active compounds from *Houttuynia cordata* THUNB on human lung cancer cells. *Biomedicine.* 2021;11(2):40-6.
12. Subhawa S, Chewonarin T, Banjerdpongchai R. The effects of *Houttuynia cordata* Thunb and *Piper*

- ribesoides* wall extracts on breast carcinoma cell proliferation, migration, invasion and apoptosis. *Molecules*. 2020;25(5):1196.
13. Senawong T, Khaopha S, Misuna S, Komaikul J, Senawong G, Wongphakham P, Yunchalard S, JSA. Phenolic acid composition and anticancer activity against human cancer cell lines of the commercially available fermentation products of *Houttuynia cordata*. *ScienceAsia*. 2014;40(1):420-7.
  14. Hong SC, Eo H, Song H, Woo S, Kim M, Lee J, Seo J, Park S, Eom J, Koo J. *Houttuynia cordata* Thunberg exhibits anti-tumorigenic activity in human gastric cancer cells. *Korea J Herbol*. 2013;28(6):155-60.
  15. Kwak Y, Ju J. Inhibitory activities of *Perilla frutescens* britton leaf extract against the growth, migration, and adhesion of human cancer cells. *Nutr Res Pract*. 2015;9(1):11-6.
  16. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res*. 1987;47(4):936-42.
  17. Ghazanfari T, Yaraee R, Shams J, Rahmati B, Radjabian T, Hakimzadeh H. Cytotoxic effect of four herbal medicines on gastric cancer (AGS) cell line. *Food Agric Immunol*. 2013;24(1):1-7.
  18. Han H, Chen GZ, Zhou SK, Xu RR, Wu CL. *In vitro* anti-tumor activity in SGC-7901 human gastric cancer cells treated with dandelion extract. *Trop J Pharm Res*. 2018;17(1):65-70.
  19. Liu Y, Nair MG. Labdane diterpenes in *Curcuma mangga* rhizomes inhibit lipid peroxidation, cyclooxygenase enzymes and human tumour cell proliferation. *Food Chem*. 2011;124(2):527-32.
  20. Jiangang F, Ling D, Zhang L, Hongmei L. *Houttuynia cordata* Thunb: A review of phytochemistry and pharmacology and quality control. *Chinese Medicine*. 2013;4:101-23.
  21. Sharma A, Kaur M, Katnoria JK, Nagpal AK. Polyphenols in food: Cancer prevention and apoptosis induction. *Curr Med Chem*. 2018;25(36):4740-57.
  22. Zhou Y, Zheng J, Li Y, Xu DP, Li S, Chen YM, Li HB. Natural polyphenols for prevention and treatment of cancer. *Nutrients*. 2016;8(8):515.
  23. Krakhmal NV, Zavyalova MV, Denisov EV, Vtorushin SV, Perelmuter VM. Cancer invasion: Patterns and mechanisms. *Acta naturae*. 2015;7(2):17-28.
  24. Eccles SA, Box C, Court W. Cell migration/invasion assays and their application in cancer drug discovery. *Biotechnol Annu Rev*. 2005;11:391-421.
  25. Safarzadeh E, Sandoghchian Shotorbani S, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. *Adv Pharm Bull*. 2014;4(Suppl 1):421-7.
  26. Wang Y, Zhong J, Bai J, Tong R, An F, Jiao P, He L, Zeng D, Long E, Yan J, Yu J, Cai L. The application of natural products in cancer therapy by targeting apoptosis pathways. *Curr Drug Metab*. 2018;19(9):739-49.
  27. Chen Q, Kang J, Fu C. The independence of and associations among apoptosis, autophagy, and necrosis. *Signal Transduct Target Ther*. 2018;3:18.
  28. Rajabi S, Maresca M, Yumashev AV, Choopani R, Hajimehdipoor H. The most competent plant-derived natural products for targeting apoptosis in cancer therapy. *Biomolecules*. 2021;11(4):534.

© 2022 Nguyen et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

The peer review history for this paper can be accessed here:  
<https://www.sdiarticle5.com/review-history/85433>