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Phenolic content and cytotoxic effect of Vietnamese endemic tea *Polyspora huongiana* evaluated on selected cancer cells

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ABSTRACT

Tea is prevalent in Asian culture with multiple uses, including health care and medical applications. *Polyspora huongiana* is a Vietnamese endemic tea first described in the 2010s. Due to its local distribution, scientists are challenged in approaching this tea; the academic evidence of the anti-cancer properties of this Polyspora has been limited. By using cytotoxic evaluation assays, such as the MTT assay and Trypan blue exclusion test, the anti-cancer capacity of the *P. huongiana* leaf extract was reported on leukemic, breast cancer, and hepatocellular carcinoma cells. The results showed a high amount of phenolic compounds (104.155 ± 11.32 mgGAE/g), including flavonoids (91.473 ± 17.52 mgQE/g), catechin (0.74 mg/g), and catechin derivatives (Epigallocatechin gallate with 1.06 mg/g and Epicatechin with 48.97 mg/g). *P. huongiana* expressed lethality in K562 (IC_{50} = 126.20 ± 15.92 µg/mL), MCF7 (IC_{50} = 184.48 ± 13.10 µg/mL), and HCC-J5 (IC_{50} = 151.03 ± 18.60 µg/mL). Besides, the extract also induced cell cycle dysregulation and transcriptomic modification in the treated cells. However, due to a suboptimal selectivity index, further research is required to enhance targeting efficiency.

Keywords: *Polyspora huongiana*, Phytochemicals, Catechin, Cytotoxic, Gene expression

Introduction

Asian tea culture is unique; Tea is commonly used in food and medicine (Eto et al., 2015). According to FAO (Food and Agriculture Organization of the United Nations), in 2021, the global tea market grew dramatically. The consumption rate increased by 3.5% compared to the previous decade; World Tea output in 2021 reached about 6.5 million tons of dried tea, which is expected to be about 17 billion USD in revenue from trade activities. Tea has been proven to contain natural compounds with high biological activity in terms of antioxidant, antibacterial, anti-inflammatory, and anti-cancer properties. Research on tea provides many important scientific arguments for its natural mechanism.

Camellia is an outstanding genus belonging to the Theaceae family, which has been reported for many bioactivities. Anticancer capacity has been proven in *Camellia* species, such as *Camellia sinensis*, *Camellia japonica*, and *Camellia nitidissima*. The proliferative inhibitory activities on cancer cells of *C. sinensis* have been explicitly reported on several cell lines consisting of MCF7, TH-29, and U2OS (Hajiaghaalipour et al., 2015; Er & Dikmen, 2017; Santos et al., 2021). The viability of MCF7, Calu-6, and SNU-601 cells declined under the impact of *C. japonica* (A. G. Pereira et al., 2022). *C. nitidissima* induces increased expression of caspase proteins, leading to apoptosis in A549 lung carcinoma cells (Zhang & Li, 2018). Besides, less popular tea species, such as endemic tea species, have also shown the ability to inhibit certain cancers, such as *Camellia cuongiana* on HCC-J5 and *Camellia kissi* on K562 (Linh et al., 2023; Xuan et al., 2023). The contribution of *Camellia* tea species in medicine is increasingly being clarified, leading to substantial medical applications.

On the other hand, the *Camellia*-close genus, *Polyspora*, has received little attention due to its rarity in terms of species number and distribution. *Polyspora* has about 40 species distributed mainly in Asia, such as Vietnam, Malaysia, China, Sri Lanka, Indonesia, Thailand, Laos, Myanmar, India, and the Philippines (Nguyet Hai Ninh et al., 2020). In Vietnam, 13 species of *Polyspora* have been described until now, including several endemic teas: *Polyspora cuongii*, *Polyspora bidoupensis*, and *P. huongiana* (Dung et al., 2016; Nguyet Hai Ninh et al., 2020; Orel et al., 2012). First described in the Tay Nguyen area, Vietnam, in 2012, *P. huongiana* is an evergreen tree; the height of a mature tree does not exceed 10m (Orel et al., 2012). The tree usually grows singly and upright and sometimes can be divided into branches. This tea variety is highly endemic, thus limiting scientific access (Orel et al., 2012). Currently, no scientific publications are related to the anti-cancer activity of this *Polyspora* species. The lack of scientific information slows down the application of this plant in medical applications. Therefore, research on *P. huongiana* plays a crucial role in the process of preserving and exploiting this species. This study aimed to evaluate the cytotoxic effect of the *P. huongiana* extract on selective cancer cell types.

Materials and Methods

Plant Extract Preparation

Polyspora Sweet tea, endemic to the Bidoup-Nui Ba National Park, *P. huongiana* Orel, Curry & Luu, was collected and identified by ecological conservation specialists Mr. Truong Quang Cuong and Mr. Phung My Trung, with voucher number 210622PHU, Center for Tourism and Science Services, Bidoup-Nui Ba National Park. The leaves were separated from the branches and immersed in distilled water for cleaning. The drained leaves were oven-dried in an oven at a steady 40ºC until thoroughly dried.

Tea powder was prepared by grinding the dehydrated leaves before soaking them in 100% methanol at a ratio of 1:3 (w/v). The filtrates were recovered three times after every 24 hours of maceration. The extraction process was performed in three independent replicates. A rotary evaporator was used for solvent removal from the extract; the crude pellet extract was obtained and dissolved in Dimethyl sulfoxide (DMSO, Merck, Germany) for stock at 400 mg/mL. The stock solutions were stored at -20°C during the study and abbreviated as PHE.

Cells and culturing. Cell lines were used in this study, including MCF7 (human breast cancer cells, ATCC, USA), HCC-J5 (hepatoblastoma cells, Cell Culture Center of the National Taiwan University, Taiwan), K562 (chronic myelogenous leukemia cells, ATCC, USA), and BJ (fibroblast, ATCC, USA). The MCF-7, HCC-J5, and fibroblast were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific, USA). In contrast, K562 cells were cultured in Roswell Park Memorial Institute (RPMI, Thermo Scientific, USA) 1640, and the medium was supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, USA) and 1% Penicillin-Streptomycin (100U/mL, Sigma-Aldrich, USA). The culture was maintained at 5% CO_2 and 37 °C, with subculturing after 72 hours. The mononuclear cells (MNC) were isolated from donated cord blood using Histopaque (Sigma-Aldrich, USA). MNC cells were cultured in RPMI supplemented with 5% active cord blood platelet-rich plasma.

Extract Background Description

Polyphenol content

The Folin-Ciocalteu reaction with the Gallic acid standard measured the total polyphenolic content. In detail, 200 µL of Gallic acid (Sigma-Aldrich, USA) at concentrations ranging from 0 to 200 µg/mL was reacted with 200 µL of Folin-Ciocalteu reagent 100% (Sigma-Aldrich, USA); meanwhile, the concentration of PHE used for this test ranged from 12.5 to 100 µg/mL. The blend was incubated at room temperature for 5 minutes before adding 1600 µL of sodium carbonate 5% and set for 20 minutes at 40°C. The polyphenolic content of PHE was linearly regressed based on Gallic acid's absorbance at 765 nm.

Flavonoid content

The aluminum chloride colorimetric method with the Quercetin standard determined the flavonoid content. A volume of 500 µL Quercetin (Sigma-Aldrich, USA) at concentrations from 0 to 200 µg/ml and PHE ranging from 12.5 to 100 µg/mL was supplemented with 2000 µL distilled water and 150 µL of 5 % $NaNO_2$. Next, the mixture was incubated for 6 min at room temperature, and 150 µL $AlCl_3$ 10%. The mix was set for 6 min at room temperature before being loaded with 2000 µL of 4% NaOH and 200 µL distilled water. The absorbance was recorded at 510 nm, so the flavonoid content of PHE was deduced.

Catechin content

The catechin content of *P. huongiana* was analyzed using an UltiMate™ 3000 Rapid Separation (Syaratul et al.) HPLC system (Thermo Scientific, USA) with the column Phenomenex Synergi Polar-RP 4u 150x4.6 mm, 80 Å, and the mobile phase consisted of water with 0.1% formic acid (channel A) and Acetonitrile (channel B). The mobile phase was programmed as follows: 85% A - 15% B (minutes 0- 14), 75% A - 25% B (minutes 14- 15), and 100% B (minutes 15- 20). The wavelength of 280nm was used for monitoring, and the injection volume was 10 µL for the sample.

Cytotoxicity Evaluation

Cells were seeded at a density of $5x10^4$ cells/mL (10^5 cells/mL for K562 and MNC). After a 24-hour incubation, the medium supplemented with the PHE extract at different concentrations ranging from 25 to 400 µg/mL was added to the wells. The DMSO 0.01% (Dimethyl sulfoxide, Thermo Scientific, USA) was set as a negative control. The cells were treated for 72 hours before determining cell viability with the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, Thermo Scientific, USA) reagent for adherent cells and the Trypan Blue exclusion test for suspension cells (Chi et al., 2014). Cell proliferation was computed as the percentage difference between the absorbance of the trials and the negative control.

Apoptosis-Inducing Test

Cells were seeded at 10^5 cell/mL density in the 6-well plates and treated with PHE at a concentration of 100 µg/mL. After a 24-hour treatment, all cells were harvested and stained with PI and Annexin V following the ANNEX100B protocol (Bio-Rad, USA) (Quan et al., 2023). The events were gated by running the flow cytometer with unstained cells, PI-stained-alone cells, and Annexin V-stained-alone cells. The PI and Annexin V fluorescence represent the status of the cells; the distribution of events reflects the cell death modes (Kari et al., 2022).

Gene Expression Investigation

The cells at 10^5 cell/mL density were treated with 100 µg/mL PHE for 24 hours. The cells were harvested and then doubly washed with PBS 1X (TBR Co., Vietnam). Cells were suspended in TRIzol reagent (Thermo Scientific, USA). The total RNA of the samples was extracted using the RNA/DNA extraction kit (TBR Co., Vietnam). cDNA was complemented, and the amplicons were amplified with the oligo dT and the primers (ITD, USA) listed in Table 1 by using a combination of TaqPath™ 1-Step RT-qPCR Master Mix (Thermo Scientific, USA) and SensiFAST™ SYBR® No-ROX Kit (Meridian Bioscience, USA). Transcriptome gene expression was calculated as the $2^{-\Delta\Delta Ct}$ value using the housekeeping gene GAPDH (Quan et al., 2023).

Data Analysis

Data accumulation was performed at least three times. The data were computed using Microsoft Office 365 (Microsoft, USA) and GraphPad Prism (GraphPad, USA) software. The Student's T-test and one-way ANOVA method followed by Tukey's Honest Significant Difference test were applied to assess the significance of differences with the alpha value of 0.05.

Table 1. Primers used in gene expression analysis

Gene	(5' – 3') Sequence
TP53 (Lee et al., 2022)	TGTGGAGTATTTGGATGACA GAACATGAGTTTTTTATGGC
pRB (Burkhart et al., 2010)	ACTCCGTTTTCATGCAGAGACTAA GAGGAATGTGAGGTATTGGTGACA
Bcl-XL (Kumar et al., 2017)	TTGGACAATGGACTGGTTGA GTAGAGTGGATGGTCAGTG
Bcl-2 (Jaberipour et al., 2010)	AAGATTGATGGGATCGTTGC GCGGAACACTTGATTCTGGT
Fas (Oehm et al., 1992)	CACACTCACCAGCAACAC TCCTTTCTCTTCACCCAAAC
Survivin (Kim et al., 2019)	GTTGCGCTTTCCTTTCTGTC TCTCCGCAGTTTCCTCAAAT
Caspase 3 (Perveen et al., 2020)	GAACTGGACTGTGGCATTGA CCTTTGAATTTCGCCAAGAA
Caspase 8 (Jana et al., 2017)	CTGCTGGGGATGGCCACTGTG TCGCCTCGAGGACATCGCTCTC
Caspase 9 (Quan et al., 2023)	GGTGATGTCGGTGCTCTTGA CGACTCACGGCAGAAGTTCA
CDK-1 (Balasubramani et al., 2019)	GGGTCAGCTCGCTACTCAAC AAGTTTTTGACGTGGGATGC
CDK-4 (Balasubramani et al., 2019)	CATTGGGGACTCTCACACTCTC ATGGCTACCTCTCGATATGAG
CDK-2 (Quan et al., 2023)	TCATGGATGCCTCTGCTCTCAC TGGAGGACCCGATGAGAATGGC
GADPH (Chen et al., 2018)	GAAGGTGAAGGTCGGAGTC GAAGATGGTGATGGGATTTC

Results and Discussion

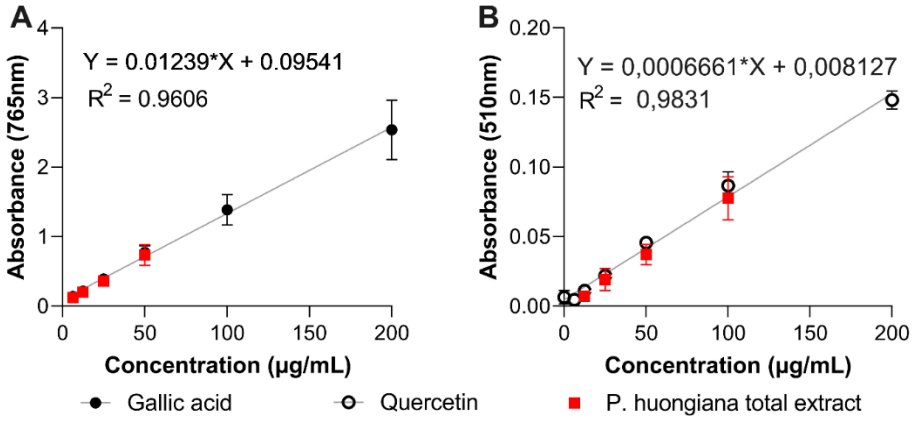
P. huongiana Extract Enriches Bioactive Compounds

The extraction yield of *P. huongiana* crude extract was calculated as 17.20 ± 1.15 %. The results from four independent points of PHE concentrations in Figure 1 reflected that the total phenolic content of PHE was 104.16 ± 11.32 mg/g by Gallic acid equivalent. Additionally, the total flavonoid content of PHE was calculated at 91.47 ± 17.52 mg/g by Quercetin equivalent. The HPLC results shown in Figure 2 indicated the presence of Catechin (0.74 mg/g), Epicatechin (48.97 mg/g), and Epigallocatechin gallate (1.06 mg/g). On the other hand, Epicatechin gallate and Epigallocatechin were not detected in the extract.

The phenolic results indicated a slightly lower amount compared to *C. sinensis* previously reported, with 144.52 ± 5.36 mg/g. Still, it was higher than the commercial green, black, and white tea products, which were reported at 55.40 mg/g, 36.28 mg/g, and 46.91 mg/g (Pereira et al., 2014; Şavşatli et al., 2021). Thus far, several Vietnam endemic teas have been investigated for their phenolic content, such as *Camellia tienii* (644.70 ± 116.20 mg/g), *Camellia tamdaoensis* (492.30 ± 129.10 mg/g), *Camellia kissi* (431,58 ± 71,54 mg/g), *Camellia dalatensis* (239.60 mg/g), *Camellia dormoyana* (181.3 mg/g), *Camellia thuongiana* (93.4 mg/g), *Camellia dilinhensis* (80.40 mg/g), and *Camellia langbianensis* (79.20 mg/g) (Diep, 2022; Ly, 2023; Nga et al., 2023). In juxtaposition to the other endemic teas, *P. huongiana* showed high polyphenol values. In addition, the flavonoid content of PHE is also considered high when compared to similar tea species. The diversity in flavonoid compound structures was described in the Theaceae family (Ngan et al., 2022; Tuyen et al., 2019). Tannins are outstanding compounds in medical use, especially catechins. The results exhibited the presence of Catechin and two of its derivatives, including Epicatechin and Epigallocatechin gallate (EGCG), in *P. huongiana* leaves. The absence of Epicatechin gallate (ECG) and Epigallocatechin (EGC) in the extract was clarified. The content of catechin compounds in *Polyspora* species was still unclear and lacked reporting. The Catechin amount was estimated in many related species, such as *C. sinensis*; however, this study was the first phytochemical report of *P. huongiana* (Deka et al., 2021). In various scientific papers, catechin-derived compounds have illustrated anti-cancer capacity against cancer types (Cheng et al., 2020). The mechanism of Catechin on tyrosine kinase receptors was indicated as the pathway in cancer cell death induction (Della Via et al., 2021; Ly & Chi, 2017, 2020).

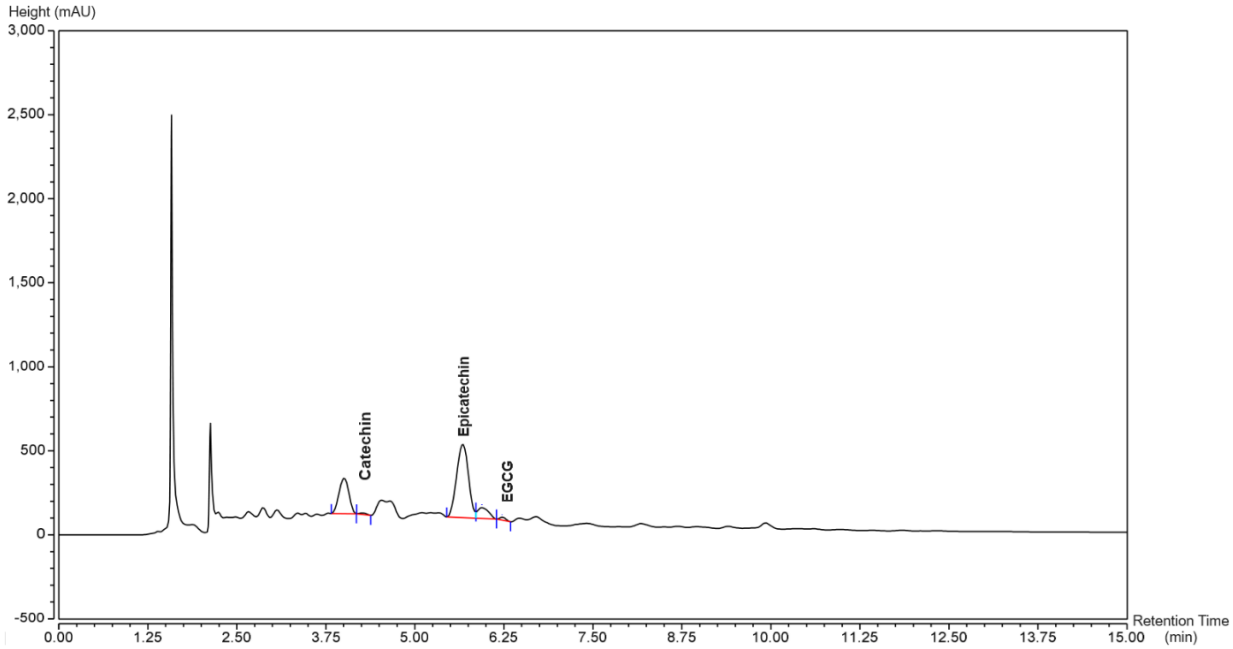
Selected Cancer Cells' Proliferation Under the P. Huongiana Extract Treatment

The PHE exhibited a cytotoxic effect on selected cancer cell types, with the recorded half-maximal inhibitory concentration (IC_{50}) values determined at 126.20 ± 15.92 µg/mL for K562, 151.03 ± 18.60 µg/mL for HCC-J5, and 184.48 ± 13.10 µg/mL for MCF7 (Figure 3). The toxicity of PHE was also clarified on reference cells, including MNC and BJ, and the IC_{50} values were computed at 35.15 ± 5.70 µg/mL and 66.84 ± 10.69 µg/mL for MNC and BJ, respectively (Figure 4A). There was a significant difference between the impact of PHE on cancer and reference cells, p-value = 0.0269, n=3. In Figure 4B, the selective index (SI) was calculated by dividing the IC_{50} value of PHE on non-malignant cell lines by the one on cancer cells (using BJ cells for MCF7 and HCC-J5 and MNC cells for K562). The SI values were sequentially indicated as 0.28 ± 0.04, 0,44 ± 0.05, and 0.36 ± 0.02 for K562, HCC-J5, and MCF7.



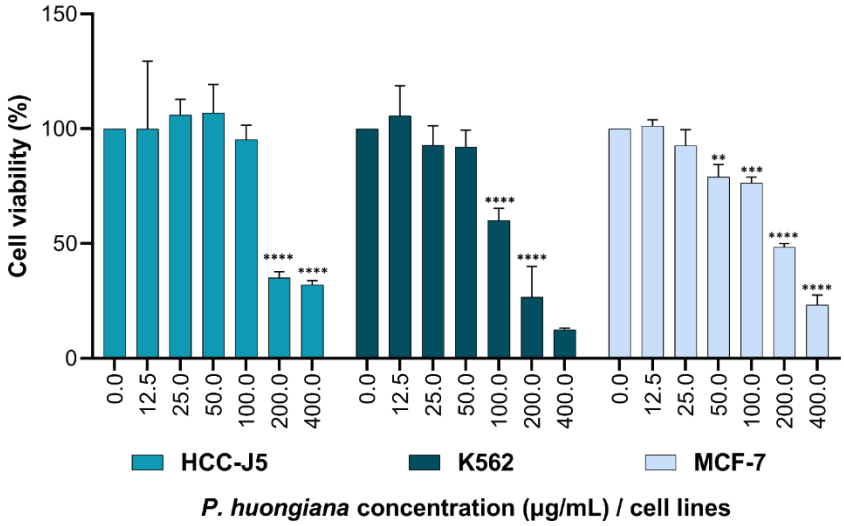
The Folin-Ciocalteu and the aluminum chloride colorimetric reactions were performed with Gallic acid and Quercetin standards. Measurement results were subjected to non-linear regression at four independent concentration points

Figure 1. The phenolic (A) and flavonoid (B) content of the *Polyspora huongiana* extract



The HPLC with catechin standards was conducted on an UltiMate™ 3000 Rapid Separation HPLC System. The presence of Catechin and two of its derivatives, including EC and EGCG, was recorded. The results indicated that *P. huongiana* included 0.74 mg/g of Catechin, 1.06 mg/g of EGCG, and 48.97 mg/g of EC.

Figure. 2. The Catechin compounds present in *P. huongiana*



The cytotoxicity assays were performed on selected cancer cells for 72 hours of treatment with the *P. huongiana* extract at different concentrations. The results indicated an extract dose-dependent inhibition in the experimental cells. The IC_{50} value was calculated at 151.03 ± 18.60 µg/mL, 126.20 ± 15.92 µg/mL, and 184.48 ± 13.10 µg/mL for HCC-J5, K562, and MCF7, respectively. (**),(***),(****) for p-value <0.01, <0.002,<0.001, n=3

Figure 3. *P. huongiana* extract illustrated the inhibitory ability against HCC-J5, K562, and MCF7 cells

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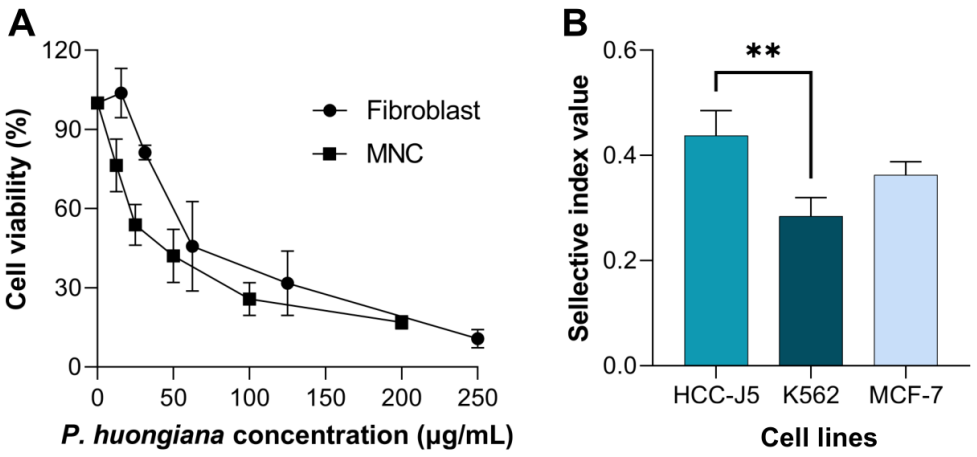


The MNC and fibroblast BJ cells were exposed to the extract ranging from 0 to 250 µg/mL for 72 hours. The results showed that the IC50 values of the extract were 35.15 ± 5.70 µg/mL and 66.84 ± 10.69 µg/mL for MNC and BJ, respectively. The selective indexes were 0.44 ± 0.05, 0.28 ± 0.04, and 0.36 ± 0.02 for HCC-J5, K562, and MCF7. (**) for p-value <0.01, n=3

Figure 4. Toxicity of *P. huongiana* extract on non-malignant cells (A) and the selectivity indices (B)

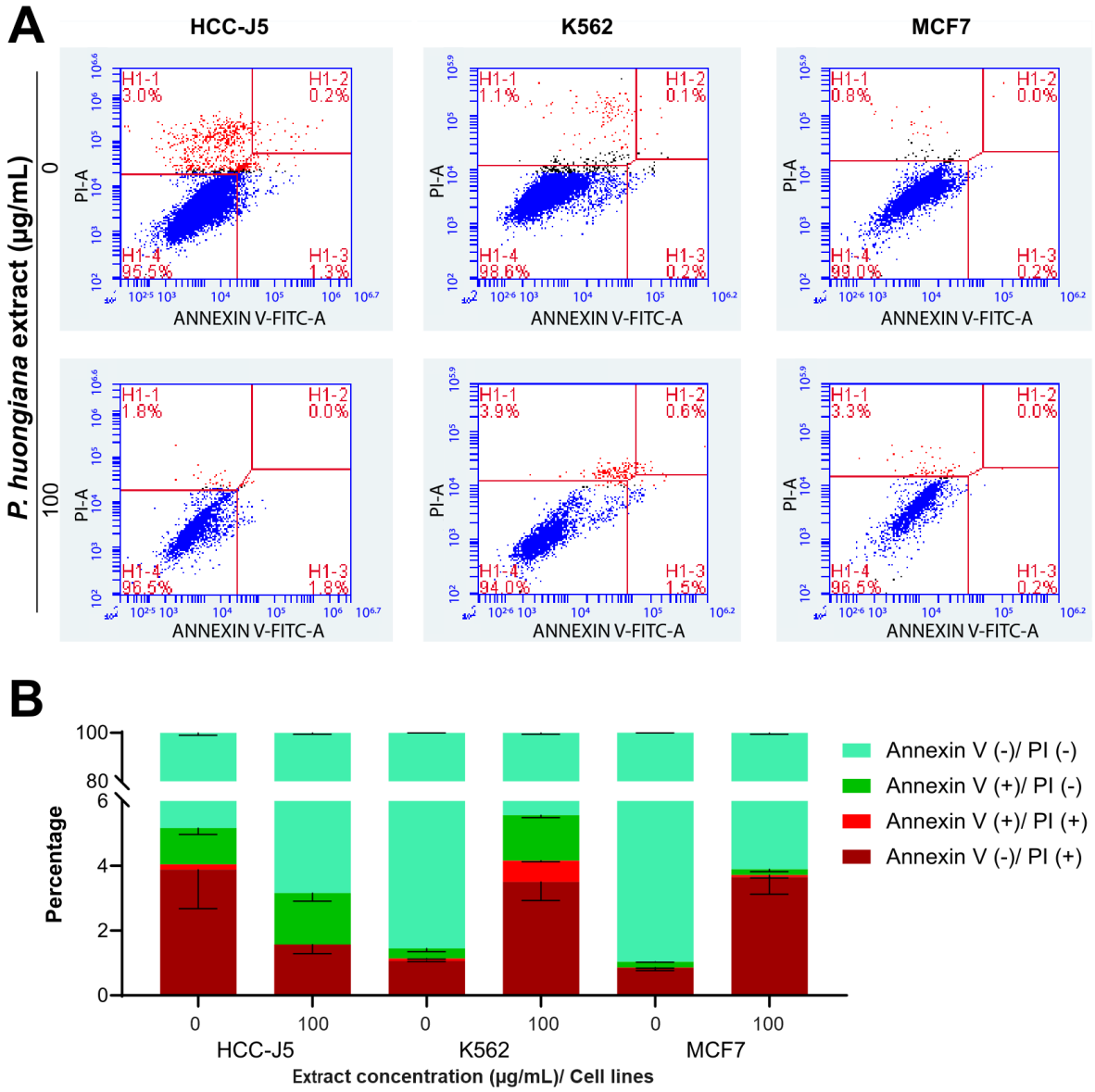
Thus far, tea extracts have been shown to exhibit cytotoxicity in various cancer types. In a previous study, *P. huongiana* extract demonstrated a strong inhibitory effect on A549 lung cancer cells (Quan et al., 2026). Although studies on *Polyspora* species remain highly limited, this activity has been widely documented in the closely related genus *Camellia*. *C. japonica* illustrated the inhibited effect on A375 cells (IC_{50} = 118.1 µg/mL), HCT116 cells (IC_{50} = 148.4 µg/mL), MCF7 cells (IC_{50} = 139.9 µg/mL), Hela cells (IC_{50} = 127.1 µg/mL), and HepG2 cells (IC_{50} = 84.2 µg/mL) (Ji & Min, 2022). *C. cuongiana* inhibited the growth of HCC-J5 with an IC_{50} greater than 100 µg/mL (Linh et al., 2023). *C. kissi* showed an IC_{50} of 40.01 ± 3.12 µg/mL on K562 (Xuan et al., 2023). This study determined the anti-proliferation capacity of *P. huongiana* on K562, MCF7, and HCC-J5 cells; the effect was classified as a weak impact, as the IC_{50} was greater than 100 µg/mL (Indrayanto et al., 2021). The results showed equivalence in effects when compared with similar teas. On the other hand, a strong cytotoxic effect was found on both MNC and BJ cells, which kept the SI values below 1 (Indrayanto et al., 2021). In contrast to drinkable tea, the high toxicity of *P. huongiana* on non-malignant cell lines is an issue that needs to be considered during the medical application of this endemic tea species (Er & Dikmen, 2017).

Cell Death and Cell Cycle Analysis

Cells were stained with PI and Annexin V for flow cytometry analysis after 24 hours of treatment. The results ambiguously reflected the impact of the extract on cell lines (Figure 5A). An increase in the number of PI-permeable cells was observed in K562 and MCF7 cells after 24 hours of exposure to PHE (Figure 5B). In addition, an increase in cells positive for both PI and Annexin V dyes was found only in K562 cells.

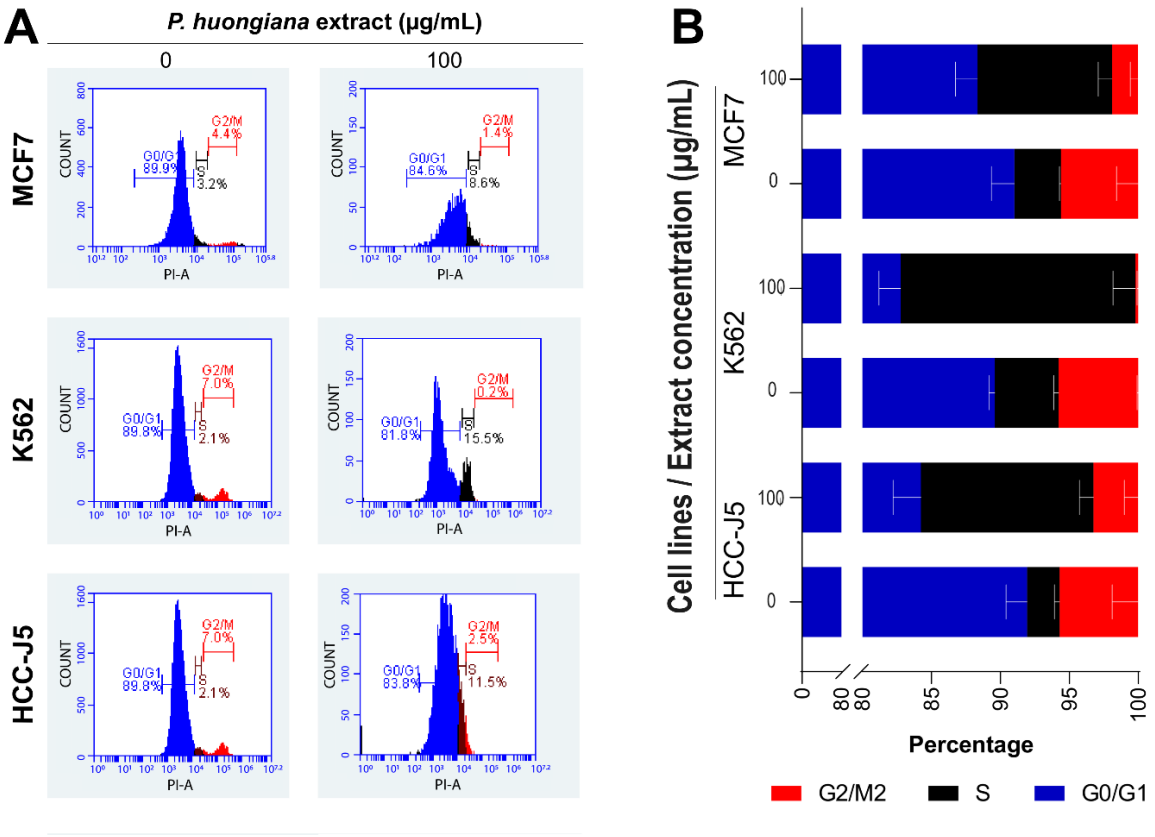
P. huongiana causes disturbances in the cell cycle. The histogram chart of PI-positive cells was illustrated in Figure 6. The disorganization of the cell cycle was recorded under the impact of PHE at 100 µg/mL. The cell cycle progression of MCF7 and HCC-J5 was disrupted after exposure to PHE, and the cells failed to progress through the cell cycle phases. In K562 cells, cell arrest occurred at the S phase.

Apoptosis is a frequently reported pathway for the effects of tea extract on malignant cells (Er & Dikmen, 2017; Gao et al., 2020; He et al., 2019). Apoptosis signs were not evident in PHE-affected cells, but signs of necrosis were obvious in K562 and MCF7 cells. The cell cycle dysregulation was described under the treatment, and S-phase accumulation was detected. The cell arrest by tea extract was reported previously, such as trapping the G1 phase by C. nitidissima in Eca109, trapping the G2 phase by C. sinensis in E6-1, trapping the G0 phase by C. oleifera in HL-60 (Dai et al., 2016; Syaratul et al., 2022; Zhang & Li, 2018; Zong et al., 2016). Disturbances in gene expression were observed as an effect of PHE, and this modification partially strengthened the effector pathway. The kinases contributing to cell cycle regulation diminished after the extract treatment. The increase in TP53 in MCF7 after treatment induced a decrease in CDK family mRNA.



Cells were treated with 100 µg/mL *P. huongiana* extract for 24 hours. Cells were stained with PI and Annexin V and analyzed on a flow cytometer. The results suggested a necrosis-like mode of cell death

Figure 5. PI- and Annexin V-positive cells under *P. huongiana* treatment analysis in dot blot (A) and stacked column (B) charts



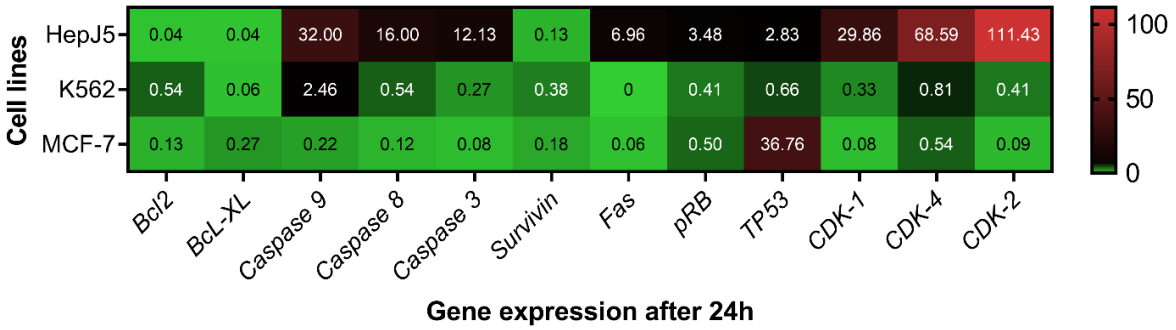
Cells were treated with 100 µg/mL P. huongiana extract for 24 hours. PI-positive cells were analyzed for the brightness of the fluorescent signal representing the chromosome content. The results showed that cell cycle dysregulation occurred, and the S-phase cell population increased.

Figure 6. Cell cycle dysregulation induced by *P. huongiana* extract histogram (A) and stacked column (B) charts

Transcriptomic Regulation Induced by P. huongiana

Nondirectional mRNA expression changes were recorded in cells after PHE exposure (Figure 7). The augmentation in cell cycle regulator-associated mRNA was found in HCC-J5 cells. Besides the induction of apoptosis-inducing elements, the anti-apoptotic genes, such as *Bcl-2* and *Bcl-XL*, declined after PHE treatment. The collapse in transcription was detected in most selected genes in K562 and MCF7. *Caspase 9* was the only gene that increased under treatment in K562 cells, and TP53 was the one that increased in MCF7 cells.

The increased caspase mRNA and decreased *Bcl-2* and *Bcl-XL* suggest apoptosis in HCC-J5. Although information on *Polyspora* is not yet available, information on the regulation of cancer cell gene expression by *Camellia* extracts has been shown previously (Zeng et al., 2015). Understanding the principles of some metabolic pathways is a good sign in studying the effects of plant extracts on cancer cells (Boroughs & DeBerardinis, 2015). In the A549 cell model, P. huongiana extract modulated the expression of genes involved in apoptosis induction and cell cycle arrest (Quan et al., 2026). This study provided basic information about the effectiveness of *P. huongiana* on some cancer cell lines. In-depth studies need to be developed to clarify the pathways of the impact of this Vietnamese endemic tea on cancer.



Cells were extracted for RNA after a 24-hour treatment with *P. huongiana* extract at 100 µg/mL. The RT-qPCR and ΔΔCt calculations were computed to determine gene expression. The gene-expressing re-regulation was observed in cells under the treatment.

Figure 7. The gene expression ($2^{-\Delta\Delta Ct}$ values) of the cancer cells under the extract treatment

Conclusion

Due to endemism, the scientific data of *Polyspora huongiana* have been overlooked. This is the first study to report on phenolic components, including flavonoids and catechins, and the activity of inhibiting cell proliferation in leukemia, liver cancer, and breast cancer of this plant. Since the selectivity index remains suboptimal, the scope of future research should be expanded to include intensive fractionation and purification processes to enhance targeting efficiency. Furthermore, changes in the cell cycle and mRNA expression were preliminarily described. However, the toxicity of the extract needs to be scrutinized, and extensive research is needed to clarify the pathway of the extract's impact on cells.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: The data will be made available upon request by the author(s).

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References

Balasubramani, S.P., Rahman, M., & Basha, S. (2019). Synergistic action of stilbenes in muscadine grape berry extract shows better cytotoxic potential against cancer cells than resveratrol alone. *Biomedicines*, 7(4), 96. https://doi.org/10.3390/biomedicines7040096

Boroughs, L.K., & DeBerardinis, R. (2015). Metabolic pathways promoting cancer cell survival and growth. *Nature Cell Biology*, 17(4), 351–359. https://doi.org/10.1038/ncb3124

Burkhart, D.L., Ngai, L.K., Roake, C.M., Viatour, P., Thangavel, C., Ho, V.M., . . . Sage, J. (2010). Regulation of RB transcription in vivo by RB family members. *Molecular and Cellular Biology*, 30(7), 1729-1745. https://doi.org/10.1128/MCB.00952-09

Chen, Q., Kirk, K., Shurubor, Y. I., Zhao, D., Arreguin, A.J., Shahi, I., . . . D'Aurelio, M. (2018). Rewiring of glutamine metabolism is a bioenergetic adaptation of human cells with mitochondrial DNA mutations. *Cell Metabolism*, 27(5), 1007-1025.e1005. https://doi.org/10.1016/j.cmet.2018.03.002

Cheng, Z., Zhang, Z., Han, Y., Wang, J., Wang, Y., Chen, X., . . . Wu, Z. (2020). A review on anti-cancer effect of green tea catechins. *Journal of Functional Foods*, 74, 104172. https://doi.org/10.1016/j.jff.2020.104172

Chi, H. T., Ly, B. T. K., Vu, H. A., Sato, Y., Dung, P. C., & Xinh, P. T. (2014). Down-regulated expression of NPM1 in IMS-M2 cell line by (−)-epigallocatechin-3-gallate. *Asian Pacific Journal of Tropical Biomedicine*, 4(7), 570–574.

https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0177

Dai, L., Li, J. L., Liang, X. Q., Li, L., Feng, Y., Liu, H. Z., . . . Zhang, L. T. (2016). Flowers of Camellia nitidissima cause growth inhibition, cell-cycle dysregulation, and apoptosis in a human esophageal squamous cell carcinoma cell line. *Molecular Medicine Reports*, 14(2), 1117–1122. https://doi.org/10.3892/mmr.2016.538

Deka, H., Barman, T., Dutta, J., Devi, A., Tamuly, P., Kumar Paul, R., & Karak, T. (2021). Catechin and caffeine content of tea (*Camellia sinensis* L.) leaf significantly differ with seasonal variation: A study on popular cultivars in North East India. *Journal of Food Composition and Analysis*, 96, 103684. https://doi.org/10.1016/j.jfca.2020.103684

Della Via, F.I., Shiraishi, R. N., Santos, I., Ferro, K.P., Salazar-Terreros, M.J., Franchi Junior, G.C., . . . Torello, C.O. (2021). (–)-Epigallocatechin-3-gallate induces apoptosis and differentiation in leukaemia by targeting reactive oxygen species and PIN1. *Scientific Reports,* 11(1), 9103. https://doi.org/10.1038/s41598-021-88478-z

Diep, T. (2022). Yellow Camellias: a review of chemical constituents and biological activities. *The Dalat University Journal of Science*, 12(3), 117-144. https://doi.org/10.37569/DalatUniversity.12.3.977(2022)

Dung, L., Lieu, N., Cuong, T., & Thanh, N. (2016). *Polyspora microphylla* Luong, Nguyen et Truong: A new species of tea family (Theaceae) in Vietnam. *Journal of Science: Natural Sciences and Technology*, 32(2).

Er, S., & Dikmen, M. (2017). Camellia sinensis increased apoptosis on U2OS osteosarcoma cells and wound healing potential on NIH3T3 fibroblast cells. *Cytotechnology*, 69(6), 901-914. https://doi.org/10.1007/s10616-017-0105-4

Eto, H., Dey, N., I-chun, L., Mahujchariyawong, P., & Roy, P. (2015). Comprehensive study of tea culture and its possible contribution to creativity education in locals. *International Journal of Sociology and Anthropology Research*, 1, 54–64.

Gao, X., Li, X., Ho, C.-T., Lin, X., Zhang, Y., Li, B., & Chen, Z. (2020). Cocoa tea (*Camellia ptilophylla*) induces mitochondria-dependent apoptosis in HCT116 cells via ROS generation and PI3K/Akt signalling pathway. Food Res Int, 129, 108854. https://doi.org/10.1016/j.foodres.2019.108854

Hajiaghaalipour, F., Kanthimathi, M.S., Sanusi, J., & Rajarajeswaran, J. (2015). White tea (*Camellia sinensis*) inhibits proliferation of the colon cancer cell line, HT-29, activates caspases, and protects DNA of normal cells against oxidative damage. *Food Chemistry*, 169, 401-410. https://doi.org/10.1016/j.foodchem.2014.07.005

He, D., Wang, N., Sai, X., Li, X., & Xu, Y. (2019). *Camellia euphlebia* protects against corticosterone-induced apoptosis in differentiated PC12 cells by regulating the mitochondrial apoptotic pathway and PKA/CREB/BDNF signalling pathway. *Food and Chemical Toxicology*, 96126, 211-222. https://doi.org/10.1016/j.fct.2019.02.028

Indrayanto, G., Putra, G.S., & Suhud, F. (2021). Validation of in vitro bioassay methods: Application in herbal drug research. Profiles of Drug Substances, Excipients and Related Methodology, 46, 273–307. https://doi.org/10.1016/bs.podrm.2020.07.005

Jaberipour, M., Habibagahi, M., Hosseini, A., Abbasi, M., Sobhani-Lari, A., Talei, A., & Ghaderi, A. (2010). Detection of B cell lymphoma 2, tumor protein 53, and FAS gene transcripts in blood cells of patients with breast cancer. *Indian Journal of Cancer,* 47(4), 412–417. https://doi.org/10.4103/0019-509x.73576

Jana, S., Hsieh, A.C., & Gupta, R. (2017). Reciprocal amplification of caspase-3 activity by nuclear export of a putative human RNA-modifying protein, PUS10, during TRAIL-induced apoptosis. *Cell Death & Disease*, 8(10), e3093-e3093. https://doi.org/10.1038/cddis.2017.476

Ji, H.K., & Min, Y.K. (2022). Phytochemicals, antioxidant and anticancer properties of *Camellia japonica* L. mistletoe Extracts. *Journal of Tropical Life Science*, 12(2), 269–274.

Kari, S., Subramanian, K., Altomonte, I.A., Murugesan, A., Yli-Harja, O., & Kandhavelu, M. (2022). Programmed cell death detection methods: A systematic review and a categorical comparison. *Apoptosis*, 27(7-8), 482–508. https://doi.org/10.1007/s10495-022-01735-y

Kim, S.I., Yeo, S.G., Gen, Y., Ju, H.R., Kim, S. H., & Park, D.C. (2019). Differences in autophagy-associated mRNAs in peritoneal fluid of patients with endometriosis and gynecologic cancers. *European Journal of Obstetrics & Gynecology and Reproductive Biology: X*, 2, 100016.

https://doi.org/10.1016/j.eurox.2019.100016

Kumar, S., Sharma, V.K., Yadav, S., & Dey, S. (2017). Antiproliferative and apoptotic effects of black turtle bean extracts on human breast cancer cell line through extrinsic and intrinsic pathways. *Chemistry Central Journal,* 11(1), 56. https://doi.org/10.1186/s13065-017-0281-5

Lee, K.P., Baek, S., Yoon, M.S., Park, J.S., Hong, B.S., Lee, S.J., . . . Moon, B.S. (2022). Potential anticancer effect of aspirin and 2'-hydroxy-2,3,5'-trimethoxychalcone-linked polymeric micelles against cervical cancer through apoptosis. *Oncology Letters,* 23(1), 31. https://doi.org/10.3892/ol.2021.13149

Linh, P., Ly, B., & Chi, H. (2023). Evaluate the effect of Camellia cuongiana methanol extract collected in Bidoup - nui ba national park in hepatocellular carcinoma HCC-J5 cancer cells. *The University of Danang - Journal of Science and Technology,* 21(5), 78–81.

Ly, B. (2023). Investigation of phytochemicals from *Camellia kissi* leaves. *Thu Dau Mot University Journal of Science,* 3(64), 30–38. https://doi.org/10.37550/tdmu.VJS/2023.03.434

Ly, B., & Chi, H. (2017). The potential effects of green tea (-)-epigallocatechin-3-gallate on overcoming imatinib resistance in chronic myeloid leukemia bearing *BCR-ABL. Ho Chi Minh City University of Education Journal of Science,* 14(9), 134. https://doi.org/10.54607/hcmue.js.14.9.2207(2017)

Ly, B., & Chi, H. (2020). Effects of green tea epigallocatechin-3-gallate on transcription factors regulating expression of FLT3. *Journal of BUON,* 25(1), 549–553.

Nga, N., Oanh, N., & Linh, N. (2023). Evaluation of the antioxidant activity of two endemic golden Camellia species (*Camellia tamdaoensis* Ninh Et Hakoda and *Camellia tienii* Ninh) in Vietnam. *TNU Journal of Science and Technology*, 228(5), 333–340. https://doi.org/10.34238/tnu-jst.7010

Ngần, N.T., Tuấn, N.N., Trinh, N.T.N., Thuần, N.N., & Mai, D.S. (2022). Flavonoid glycosides from the leaves of *Andrographis paniculata* Burm. F. in Vietnam. Journal of Science and Technology - IUH, 53. https://doi.org/10.46242/jstiuh.v53i05.4189

Nguyet Hai Ninh, L., Luong, V., Nguyen Van, C., Pham Thi, T.D., Luu, T., & Pham, T. (2020). An updated checklist of Theaceae and a new species of Polyspora from Vietnam. Taiwania, 65, 216-227. https://doi.org/10.6165/tai.2020.65.216

Oehm, A., Behrmann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., . . . et al. (1992). Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily, sequence identity with the Fas antigen. *Journal of Biological Chemistry,* 267(15), 10709–10715. https://doi.org/10.1016/S0021-9258(19)50076-X

Orel, G., Wilson, P., Curry, A., & Luu, H.T. (2012). *Polyspora huongiana* sp nov (Theaceae) from Vietnam and notes on related species. *Nordic Journal of Botany,* 30, 47–52. https://doi.org/10.1111/j.1756-1051.2011.01330.x

Pereira, A.G., Garcia-Perez, P., Cassani, L., Chamorro, F., Cao, H., Barba, F.J., . . . Prieto, M. A. (2022). *Camellia japonica*: A phytochemical perspective and current applications facing its industrial exploitation. *Food Chemistry X*, 13, 100258. https://doi.org/10.1016/j.fochx.2022.100258

Pereira, V.P., Knor, F.J., Vellosa, J.C., & Beltrame, F. (2014). Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Camellia sinensis* (L.) Kuntze, Theaceae. *Revista Brasileira de Plantas Medicinais,* 16(490-498. https://doi.org/10.1590/1983-084X/13_061

Perveen, S., Ashfaq, H., Shahjahan, M., Manzoor, A., & Tayyeb, A. (2020). *Citrullus colocynthis* regulates de novo lipid biosynthesis in human breast cancer cells. *Journal of Cancer Research and Therapeutics*, 16(6), 1294–1301. https://doi.org/10.4103/jcrt.JCRT_206_20

Quan, N., Ly, B., & Thanh Chi, H. (2023). The cytotoxic effect of *Vernonia amygdalina* Del. extract on myeloid leukemia cells. *Biomedical Research and Therapy*, 10, 5855–5863. https://doi.org/10.15419/bmrat.v10i8.827

Quan, N. T., Thi, T., Thi Kim Ly, B., & Thanh Chi, H. (2026). The augmentation in apoptosis and cell cycle-associated transcriptional gene expression in A549 cells induced by *Polyspora huongiana* leaves methanol extract. *International Journal of Secondary Metabolite*, 13(2), 565–574. https://doi.org/10.21448/ijsm.1788049

Santos, R.A., Andrade, E.D.S., Monteiro, M., Fialho, E., Silva, J.L., Daleprane, J.B., & Ferraz da Costa, D.C. (2021). Green Tea (*Camellia sinensis*) Extract Induces p53-Mediated Cytotoxicity and Inhibits Migration of Breast Cancer Cells. *Foods*, 10(12), 901–914. https://doi.org/10.3390/foods10123154

Şavşatli, Y., Özcan, A., & Civelekoğlu, O. (2021). Variation of antioxidant activity and total phenolic content of tea (*Camellia sinensis* L. O. Kuntze) genotypes. *KSU Journal of Agriculture and Nature*, 24(1), 40–48. https://doi.org/10.18016/ksutarimdoga.vi.744662

Syaratul, D., Endang, K., Mursidah, M., Yap, R., Norsyahida, M., & Affidah, S. (2022). Camellia sinensis and *Phyllanthus amarus* ethanol extracts induced apoptosis and cell cycle arrest on human leukemic cell lines. *Sains Malaysiana*, 51(8). https://doi.org/10.17576/jsm-2022-5108-20

Tuyen, N., Hieu, T., Dien, P., Ninh, T., Hung, N., & Vu, H. (2019). A new sexangularetin derivative from *Camellia hakodae*. *Natural Product Communications*, 14(9), 1934578X1987620. https://doi.org/10.1177/1934578X19876209

Xuan, N., Tin, N., Trung, P., Chi, H., & Ly, B. (2023). Investigating the anti-cancer effects of Camellia kissi methanol extract on K562 leukemia cells. *The University of Danang - Journal of Science and Technology*, 21(5), 73–77.

Zeng, J., Chen, S., Li, N., Chen, L., Su, J., Niu, G., . . . Liang, Y. (2015). Sasanquasaponin from *Camellia oleifera* Abel. induces apoptosis via Bcl-2, Bax and caspase-3 activation in HepG2 cells. *Molecular Medicine Reports,* 12(2), 1997-2002. https://doi.org/10.3892/mmr.2015.3666

Zhang, Z.-h., & Li, P. (2018). Anti-proliferation and apoptosis effects of *Camellia nitidissima* C.W. Chi extract on A549 lung cancer cells. *Tropical Journal of Pharmaceutical Research*, 17(395-400. https://doi.org/10.4314/tjpr.v17i3.2

Zong, J., Wang, D., Jiao, W., Zhang, L., Bao, G., Ho, C., . . . Wan, X. (2016). Oleiferasaponin C6 from the seeds of Camellia oleifera Abel.: A novel compound inhibits proliferation through inducing cell-cycle arrest and apoptosis on human cancer cell lines in vitro. *RSC Advances*, 6(94), 91386–91393. https://doi.org/10.1039/C6RA14467E