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ANTI-PROLIFERATIVE ACTIVITIES OF VIETNAMESE HERBS

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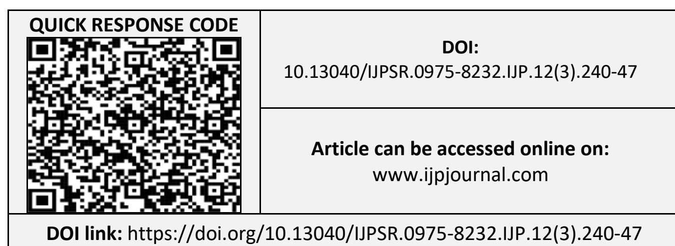
ABSTRACT: Cancer is a dangerous global disease and causes a heavy economic burden in low-income countries. Traditional medicinal plants play an important role in the research and development of cancer treatment drugs. Twenty-four traditional Vietnamese herbs were evaluated for their cytotoxic activities against nine cancer cell lines including HepG2, A549, MCF7, HT-29, HeLa, RD, LNCaP, HL-60 and NTERA-2. Cytotoxic activity was assessed following Monks' protocol (1991) by measuring total cellular protein content using sulforhodamine B (SRB) staining. The 70% ethanol extract of eight medicinal herbs including *Phyllanthus urinaria* L. (TD.MD1), *Solanum trilobatum* L. (TD.MD2), *Angelica sinensis* (Oliv.) Diels (TD.MD3), *Xanthium strumarium* L. (TD.MD11), *Scutellaria baicalensis* Georgi (TD.MD15), *Platycodon grandiflorus* (Jacq.) A.D.C. (TD.MD17), *Lycopodium clavatum* L. (TD.MD20) and *Ophiopogon japonicus* (Thunb.) Ker Gawl. (TD.MD21) showed cytotoxic activity against HepG2, A549, MCF7, HT-29, HeLa, RD, LNCaP, HL-60 cell lines with IC_{50} values ranging from 18.60 to 93.84 µg/mL. The hexane, ethyl acetate, *n*-butanol, water extract residues of the 70% ethanol extracts demonstrated cytotoxic activities against NTERA-2 cell line with IC_{50} values of 8.60 to 90.83 µg/mL. This is the first study to evaluate the proliferation inhibition potential of these medicinal herbs on cancer stem cells. The eight medicinal herbs have shown potential to aid in the treatment of various cancers and as raw materials for further research on secondary metabolites with cytotoxic activity.

INTRODUCTION: Cancer stands as a prominent contributor to global morbidity and mortality, with approximately 14 million new cases annually. Projections indicate a staggering 70% surge in new cases over the next two decades. It ranks as the second leading cause of death worldwide, accounting for nearly one in every six deaths. Alarmingly, about 70% of cancer-related fatalities transpire in low- and middle-income nations [1]. In 2020, approximately 10 million deaths related to cancer were reported, solidifying its position as one of the primary causes of death worldwide [2]. Cancer stem cells (CSCs) are identified as a small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population of tumor cells containing self-renewing and differentiating properties, they have the ability to initiate and proliferate tumors as well as metastasize and recur. More seriously, CSCs demonstrate drug resistance and overcome radiotherapy. It has been hypothesized that cancer stem cells (CSCs) are responsible for drug resistance and that targeting their treatment will lead to tumor regression [3].

Therefore, CSCs have become an important target for cancer treatment. Due to their high expression of stem signals such as Nanog homeobox, octamer-binding transcription factor 4, SRY-box 2, and teratocarcinoma-derived growth factor 1, NTERA-2 cells are widely utilized for assessing the anti-cancer stem cell (CSC) targeted activity of various compounds [4]. Human embryo carcinoma cell line NTERA-2 has been proposed as an *in-vitro* test system for developmental neurotoxicity [5].

Using medicinal plants to inhibit the proliferation of cancer cell lines has received special attention from scientists. Natural compounds have been included as part of approved anti-cancer treatments [6]. In this study, we evaluated the cytotoxic activities of 24 Vietnamese traditional medicinal herbs against 9 cancer cell lines including HepG2, A549, MCF7, HT-29, Hela, RD, LNCaP, HL-60 and NTERA-2. This study was conducted with the purpose of screening medicinal herbs with cytotoxic activity to support further research on herbal formulas supporting cancer treatment.

MATERIALS AND METHODS:

Cell Lines: The NTERA-2, A549, and various cancer cell lines (HepG2, A549, MCF7, HT-29, Hela, RD, LNCaP, HL-60) were provided as a gift by Dr. Prof. Wongtrakoongate from Mahidol University, Thailand, and Prof. J. Maier from Milan University, Italy.

Chemicals and Herbal Materials: Chemicals used in the cytotoxicity assay (sulforhodamine B, dimethyl sulfoxide, trichloroacetic acid, ellipticine) were purchased from Sigma-Aldrich. The solvents (*n*-hexane, ethanol, ethyl acetate, *n*-butanol) and other chemicals met analytical standards and were purchased from XILONG Scientific, China.

The fresh herbs were provided by Thai Duong Joint Stock Company and were botanically identified by Dr. Do Ngoc Dai **Table 1**. Specimens of medicinal herbs have been kept and preserved at the Department of Biology of Vinh University.

TABLE 1: LIST OF MEDICINAL HERBS USED IN THIS STUDY

Voucher specimen	Science name	Traditional medicine name	Part
TD.MD1	*Phyllanthus urinaria* L.	Diệp hạchâu	Aerial parts
TD.MD2	*Solanum trilobatum* L.	Cà gai leo	Aerial part
TD.MD3	*Angelica sinensis* (Oliv.) Diels	Đương quy	Root
TD.MD4	*Codonopsis pilosula* (Franch.) Nannf.	Đảng sâm	Root
TD.MD5	*Rehmannia glutinosa* (Gaertn.) DC.	Sinh địa	Rhizome
TD.MD6	*Panax notoginseng* (Burkill) F.H.Chen	Tam thất	Rhizome
TD.MD7	*Poria cocos* (Schw.) Wolf	Bạch Linh	Fruit body
TD.MD8	*Atractylodes macrocephala* Koidz.	Bạch truật	Rhizome
TD.MD9	*Paeonia lactiflora* Pall.	Bạch thược	Rhizome
TD.MD10	*Schefflera heptaphylla* (L.) Frodin	Ngũ gia bì	Tree bark
TD.MD11	*Xanthium strumarium* L.	Ké đầu ngựa	Fruit
TD.MD12	*Smilax glabra* Roxb.	Thổ phục linh	Rhizome
TD.MD13	*Catharanthus roseus* (L.) G.Don	Dừa cạn	Aerial parts
TD.MD14	*Cratoxylum cochinchinense* (Lour.) Blume	Thành ngạnh	Leaf
TD.MD15	*Scutellaria baicalensis* Georgi	Hoàng cầm	Root
TD.MD16	*Scrophularia ningpoensis* Hemsl.	Huyền sâm	Rhizome
TD.MD17	*Platycodon grandiflorus* (Jacq.) A.DC.	Cát cánh	Root
TD.MD18	*Fritillaria cirrhosa* D. Don	Xuyên bối mẫu	Bulbs
TD.MD19	*Lilium brownii* F.E.Br. ex Miellez	Bách hợp	Bulbs
TD.MD20	*Lycopodium clavatum* L.	Thạch tùng	Aerial parts
TD.MD21	*Ophiopogon japonicus* (Thunb.) Ker Gawl.	Mạch môn	Rhizome
TD.MD22	*Prunella vulgaris* L.	Hạ khô thảo	Flower
TD.MD23	*Zingiber officinale* Rose	Can khương	Rhizome
TD.MD24	*Curcuma longa* L.	Nghệ vàng	Rhizome

Preparation of the Extraction Residue: The dried medicinal herbs were ground, then soaked in 70% ethanol solvent for 14 days. The 70% ethanol extract was evaporated using a vacuum rotary evaporator under low pressure to obtain the 70% ethanol extract residue. The anti-proliferative activities of the ethanol extract residues were assessed and residues with IC_{50} values < 100 µg/mL underwent further extraction with *n*-hexane, ethyl-acetate, and *n*-butanol solvents. The 70% ethanol extraction residue was completely distributed in distilled water, then subjected to liquid-liquid extraction with the solvents *n*-hexane, ethyl acetate, and *n*-butanol, each extraction solvent repeated four times. The extracts of *n*-hexane, ethyl acetate, *n*-butanol, and aqueous solution were evaporated using a vacuum rotary evaporator at low pressure to obtain residues *n*-hexane (H), ethyl acetate (E) extraction, *n*-butanol (B), and water (W), respectively **Table 2.**

TABLE 2: EXTRACTION YIELDS OF DIFFERENT SOLVENTS OF HERBS

Code	Yield (g)				
	Ethanol extract residue (g)	**(*n*-Hexane)**	**Ethyl acetate**	***n*-Butanol**	**Water**
TD.MD1	35.2	0.1	1	11.5	22.6
TD.MD2	33	2	0.2	22	8.8
TD.MD3	20.9	0.1	0.7	10.1	10
TD.MD11	39.6	1.7	0.7	2.2	35
TD.MD15	57.5	0.1	0.4	35	22
TD.MD17	19.4	0.2	0.3	14.9	4
TD.MD20	8.1	1.3	1.4	1.4	4
TD.MD21	42.1	1.1	17	16	8

Cytotoxicity Test: The cytotoxic activities were conducted following the protocol outlined by Monks (1991) [7, 8]. The evaluation aimed to determine the total cellular protein content by measuring the optical density (OD) when the cell protein composition was stained with sulforhodamine B (SRB). The OD value, directly proportional to the SRB bound to protein molecules, increased with more cells (more protein). To initiate the test, a solution of the extracted residue or pure compound (10 µL) was diluted in 10% dimethyl sulfoxide (DMSO) and placed in a 96-well tray to create concentrations of 100 µg/mL, 20 µg/mL, 4 µg/mL, 0.8 µg/mL, and 0.16 µg/mL.

Cells, adjusted to a density of 1×10^5 cells/mL through trypsinization and counting, were added to the test wells (190 µL medium, 6000 cells/well). A separate 96-well tray with cancer cells (190 µL) served as a day 0 control. After 1 hour, the day 0 control cells were fixed with trichloroacetic acid (TCA). Following the growth period in the CO_2 incubator, cells in the test wells were fixed to the well bottom with TCA for 1 hour and stained with 0.4% SRB for 30 minutes at 37 °C. Subsequently, SRB was removed from the test wells, which were then washed three times with acetic acid and air-dried at room temperature. The bound SRB was dissolved using 10 mM unbuffered Tris base, and the protein molecules were stained. After gentle shaking for 10 minutes on a plate shaker, an ELISA Plate Reader (ELx800, BioTek Instruments Inc., Winooski, VT, USA) was employed to read the color content of the SRB dye through the absorption spectrum at 515 nm. The viability of cells in the presence of the extracted residue or pure compound was determined using the formula:

$$\text{Percentage of cell viability (\%)} = ([\text{OD (reagent)} - \text{OD (day 0)}] \times 100) / (\text{OD (negative control)} - \text{OD (day 0)})$$

The assays were repeated three times for accuracy. Ellipticine served as a positive control and was tested at concentrations of 10 µg/mL, 2 µg/mL, 0.4 µg/mL, and 0.08 µg/mL, while a 10% DMSO solution served as the negative control.

RESULTS: The results of testing the cytotoxicity of the medicinal herbs are presented in Table 3. The 70% ethanol extraction residue of eight medicinal samples including TD.MD1, TD.MD2, TD.MD3, TD.MD11, TD.MD15, TD.MD17, TD.MD20, and TD.MD21 showed anti-proliferative activities with IC_{50} values ranging from 18.60 to 93.84 µg/mL. The *n*-hexane, ethyl acetate, *n*-butanol, and water extract residues of these medicinal herbs demonstrated strong cytotoxic activities against nine cancer cell lines, for NTERA-2 cell line with IC_{50} values of 8.60 to 90.83 µg/mL **Table 4.**

TABLE 3: CYTOTOXIC ACTIVITIES (IC_{50}, MG/ML) OF 70% ETHANOL EXTRACT RESIDUES OF MEDICINAL HERBS

Code	Extract residue	Yield	HepG2	A549	MCF7	HT-29	Hela	RD	LNCaP	HL-60
TD.MD1	35.2	17.6	50.35±3.43	55.93±4.45	54.46±4.79	61.27±2.08	56.88±2.92	66.72±6.97	55.02±6.86	71.09±8.14
TD.MD2	33	16.5	26.67±1.85	30.02±2.20	23.59±3.35	23.49±1.01	34.81±1.14	44.62±1.67	29.27±3.06	18.60±1.77
TD.MD3	20.9	10.5	64.21±4.90	64.13±7.25	61.10±3.28	71.89±3.77	58.59±5.22	52.31±6.13	80.29±8.93	93.84±6.72
TD.MD4	13	6.5	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD5	24.1	12.05	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD6	48.7	24.35	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD7	48.5	28.25	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD8	25	12.5	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD9	30	15	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD10	19.2	9.6	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD11	39.6	19.8	38.29±2.04	46.17±3.18	32.29±2.38	30.97±2.49	46.42±3.93	26.20±2.08	40.06±3.57	25.66±2.18
TD.MD12	36.6	12.3	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD13	53.3	26.65	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD14	49.7	24.85	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD15	57.5	28.75	45.90±3.13	48.99±3.85	57.72±4.14	46.62±5.22	53.91±3.33	42.21±2.08	42.62±2.15	63.09±5.57
TD.MD16	36.4	18.2	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD17	19.4	9.7	60.95±2.36	61.27±3.90	59.26±6.07	56.88±6.16	62.64±7.18	59.92±2.05	72.50±5.60	77.68±8.11
TD.MD18	27.8	13.9	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD19	42	21	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD20	8.1	4.55	49.23±3.14	50.01±5.26	66.48±2.71	64.47±3.17	50.62±5.32	50.16±6.21	52.88±3.01	48.87±2.08
TD.MD21	24.1	12.05	89.93±2.47	70.86±3.23	77.39±3.84	91.96±4.60	69.56±4.74	69.32±4.46	77.81±4.58	89.65±3.46
TD.MD22	29.5	14.75	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD23	40	20	>100	>100	>100	>100	>100	>100	>100	>100
TD.MD24	59.4	29.7	>100	>100	>100	>100	>100	>100	>100	>100

HepG2: human hepatoma cells; A549: human lung cells; MCF7: human breast cancer cells; HT-29: human colon cancer cells; HeLa: human cervical cancer HeLa cells; RD: Rhabdomyosarcoma (RD) cells; LNCaP: human prostate cancer LNCaP cells; HL-60: human leukemia HL-60 cancer cell.

TABLE 4: CYTOTOXIC ACTIVITIES (IC_{50}, MG/ML) OF DIFFERENT EXTRACT RESIDUES (*N*-HEXANE, ETHYL ACETATE, *N*-BUTANOL, WATER) OF MEDICINAL HERBS

Code	HepG2	A549	MCF7	HT-29	HeLa	RD	LNCaP	HL-60	NTERA-2
H-TD.MD1	64.02±4.80	67.76±7.11	75.58±4.50	54.86±4.32	55.40±3.52	78.89±4.81	72.13±3.58	76.94±4.15	61.15±1.23
E-TD.MD1	84.11±4.51	82.53±5.52	69.10±7.09	69.39±6.09	60.96±2.31	79.60±3.15	79.79±3.50	92.28±3.42	53.25±1.09
B-TD.MD1	59.35±2.89	64.04±3.09	56.10±3.63	69.47±5.34	61.58±2.91	77.20±7.82	56.33±3.27	73.55±3.82	41.15±1.25
W-TD.MD1	58.74±5.24	67.49±7.21	45.24±4.29	52.56±3.40	45.10±4.57	66.07±5.22	65.58±3.99	77.16±4.92	32.61±2.02
H-TD.MD2	28.36±1.83	35.75±3.20	23.72±2.36	23.49±0.39	39.20±3.23	20.02±1.50	22.74±2.86	21.65±1.39	46.13±4.82
E-TD.MD2	61.35±3.14	43.54±3.76	52.10±3.99	52.45±3.11	53.14±6.04	45.98±2.13	54.28±2.08	65.07±4.70	6,.43±0.79
B-TD.MD2	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	>100
W-TD.MD2	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	>100
H-TD.MD3	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	90.83±1.88
E-TD.MD3	61.35±3.14	43.54±3.76	52.10±3.99	52.45±3.11	53.14±6.04	45.98±2.13	54.28±2.08	65.07±4.70	37.35±2.37
B-TD.MD3	51.26±6.09	40.94±4.27	39.29±2.36	39.97±3.01	62.14±5.43	51.29±3.76	50.26±4.36	66.20±2.11	22.61±2.41
W-TD.MD3	58.89±2.98	63.72±6.78	53.41±4.01	73.09±4.54	75.72±5.58	55.26±1.05	82.23±5.34	67.65±2.77	74.51±7.02
H-TD.MD11	8.81±0.76	12.65±1.43	8.69±0.66	7.53±0.93	18.14±1.11	16.44±1.12	13.85±1.41	21.46±1.39	12.69±1.22
E-TD.MD11	16.01±0.64	23.78±1.93	14.79±1.60	17.58±0.89	16.71±1.14	16.57±1.79	13.23±1.41	22.07±1.87	8.60±0.54
B-TD.MD11	59.31±1.78	59.33±2.76	43.95±1.28	48.03±4.77	62.57±4.61	38.86±3.92	43.57±2.86	56.97±4.96	11.95±0.90
W-TD.MD11	86.74±5.11	75.50±3.72	59.66±2.49	65.12±3.40	88.32±3.89	68.93±4.50	61.98±2.63	77.83±6.07	61.36±3.28
H-TD.MD15	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	46.84±4.69
E-TD.MD15	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	30.87±1.71
B-TD.MD15	50.613.91	49.71±2.90	43.59±5.17	48.11±4.83	45.15±4.88	56.20±2.37	40.62±3.94	46.08±2.98	35.67±3.31
W-TD.MD15	61.88±4.71	58.07±6.25	49.33±3.85	62.91±5.07	68.80±4.94	52.31±5.13	50.06±3.64	61.06±4.92	76.62±6.66
H-TD.MD17	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	51.61±5.53
E-TD.MD17	49.42±5.54	40.26±4.45	44.10±4.09	43.54±2.11	45.41±2.89	54.46±5.90	49.46±5.15	58.29±2.46	15.22±1.46
B-TD.MD17	42.63±2.46	46.11±2.64	36.56±2.97	25.50±2.74	35.88±4.26	29.55±2.98	31.71±2.30	51.80±5.27	18.29±2.27
TD.MD17	74.62±6.67	71.53±5.17	58.07±3.02	56.69±5.24	53.91±3.33	74.86±2.15	67.68±6.14	72.88±5.49	56.12±2.30
H-TD.MD20	60.33±5.87	78.22±1.63	67.09±7.14	73.62±3.23	58.78±5.30	64.04±2.87	74.48±3.58	81.59±4.91	56.25±2.09

E-TD.MD20	16.16±2.05	22.21±2.48	13.19±1.39	18.81±1.58	11.97±1.46	23.68±2.63	15.48±1.56	22.94±2.43	11.35±2.49
B-TD.MD20	50.82±2.91	65.44±1.85	59.55±5.94	66.19±5.31	45.24±2.97	51.73±1.82	79.51±4.37	54.45±3.13	51.25±2.23
W-TD.MD20	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	>100
H-TD.MD21	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	32.54±4.07
E-TD.MD21	74.40±3.46	55.89±4.48	71.25±5.87	62.72±3.63	51.01±5.63	61.51±6.67	61.01±4.21	76.40±3.47	12.89±0.79
B-TD.MD21	69.84±4.24	54.40±5.31	58.22±4.74	47.78±5.27	70.78±5.12	77.20±5.92	70.92±2.80	74.24±3.83	37.72±3.54
W-TD.MD21	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	>100
Ellipticine	0.41± 0.04	0.38± 0.04	0.36± 0.02	0.40± 0.04	0.46± 0.05	0.45± 0.05	0.45± 0.05	0.52± 0.05	0.51±0.05

H: *n*-Hexane. E: Ethyl acetate. B: *n*-butanol. W: water.

DISCUSSION: The aqueous and methanolic extracts of *P. urinaria* were evaluated for antiproliferative activities against different cancer cell lines, the results indicated that the methanol extract exhibited stronger activities than the water extract [3, 9–13]. Several proliferation inhibition mechanisms of *P. urinaria* extracts have been reported: through inhibition of cellular mobility, invasion, and migration of cells (Saos-2 cell line) [14]; through a mitochondria-associated intrinsic pathway [15] (Lewis lung carcinoma cells), through a ceramide-related pathway (HL-60 cells, human osteosarcoma 143B cells) [13], *via* activation of Fas/FasL (human osteosarcoma 143B cells) 16; modulation of cell cycle and induces apoptosis through caspase activation in melanoma and prostate cancer cells [17]. The aqueous extract demonstrated a dose-dependent anti-tumor effect at the *in-vivo* level [18]. The compounds lignan hypophyllanthin, neonirtetralin and helio-buphthalmin lactone demonstrated cytotoxic activities against CHO (Chinese hamster ovary) and J774 (Murine macrophage) cell lines with IC_{50} values in the range of 6.00–41.30 μM [19].

In this study, 70% ethanol extract of *Solanum trilobatum* showed cytotoxic activities with IC50 values of 16.5 to 44.62 μg/mL. The H-TD.MD2 and E-TD.MD2 extracts demonstrated strong proliferation inhibition, whereas the B-TD.MD2 and W-TD.MD2 extracts did not (IC_{50}> 100 μg/mL). The petroleum ether (PE), chloroform (C), ethyl acetate (EA), and ethanol (E) extracts demonstrated cytotoxic activity against the L929 cell line with IC_{50} values of 7.0, 16.0, 36.0 and 18.5 (μg/mL), respectively [20]. Thus, less polar components were responsible for the antiproliferative activities of *S. trilobatum*. The sobatum compound isolated from petroleum ether extract demonstrated strong cytotoxicity against L929 and Vero cells with LC^{50} values of 7.0 μg and 7.5 μg, respectively [20].

Acetone, chloroform, methanol, and CO2 extracts of *A. sinensis* all exhibit strong cytotoxic activities. Phthalide compounds are responsible for the antiproliferative activities of *Angelica sinensis* [5, 21, 22]. The compounds n-butylidenephthalide (BLP), senkyunolide A (SKA), and Z-ligustilide (LGT) inhibited the proliferation of HT-29 cell line with IC_{50} values of 54.17 ± 5.10, 60.63 ± 6.79, and 236.90 ± 18.22 μM, respectively [23]. The compounds riligustilide, tokinolide A, and tokinolide C have shown proliferation inhibitory activities of A549, HCT-8, and HepG2 cell lines with IC^{50} values of 6.79-13.82, 30.92-55.84, and 27.97 -34.34 μM, respectively [24]. BLP demonstrated cytotoxic activities against brain tumor cell lines (DBTRG-05MG, GBM8401, GBM8901, G5T/VGH, RG2, SK-N-AS, N18) and other cancer cell lines (A549, B16/F10, J5, PA-1, BCM-1, HL-60) with IC50 values between 15.5 to 67.4 [25]. Phthalide compounds have been isolated from extracts of less polar solvents such as n-hexane, pentane, petroleum ether, methanol, 70% ethanol and dichloromethane [26]. In this study, the n-hexane extract did not show cytotoxic activities against cancer cell lines, whereas the aqueous extract showed strong activity. The cytotoxic activity of the aqueous extract may have been related to the responsible polysaccharide [27].

The xanthanolide sesquiterpene lactone compounds may have been involved in the cytotoxic activities of *X. strumarium*. Two compounds, 8-epi-xanthatin and 8-epi-xanthatin epoxide, inhibited the proliferation of cell lines A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nervous system) and HCT-15 (colon) through inhibiting the farnesylation process of human lamin-B by farnesyltransferase [28]. Xanthatin fraction (xanthatin and 8-epi-xanthatin) demonstrated anti-proliferative activity of CT26WT cells through interference with the mitotic apparatus with an IC_{50} value of 8.3 μM [29].

Xanthatin and xanthinosin demonstrated cytotoxic activities against WiDr and MDA-MB-231 cell lines with IC_{50} values of 6.15, 13.9 and 2.65, 4.8, respectively [30]. The n-hexane and ethyl acetate extracts in this study showed stronger activities than the n-butanol and water extracts, consistent with previous studies 6–8, which is consistent with the relatively low polarity of sesquiterpene lactone compounds. However, the study of Ly (2021) reported that an ethanolic extract rich in polyphenols exhibited cytotoxic activity against HepG2 cancer cells with an IC_{50} value of 81.69 μg/mL [31].

The free flavones baicalein, wogonin, chrysin, and oroxylin A were isolated from n-butanol extracts that showed significant cytotoxic activity against HepG2, SW480, and MCF7 cells at a concentration of 10 μM32. In the present study, n-butanol and water extracts demonstrated strong cytotoxic activities, while n-hexane and ethyl acetate extracts did not.

The triterpenoid saponin compounds in the roots of *P. grandiflorum* may be responsible for the antiproliferative activities against cancer cell lines. The platycoside-containing n-butanol fraction of *P. grandiflorum* inhibited the proliferation of the A549 cancer cell line *via* autophagy and the modulation of the AMPK/mTOR/AKT and MAPK signaling pathways [33]. The compound platycodin D is the main component in the roots of *P. grandiflorum*, which has been reported to exhibit time- and concentration-dependent cytotoxic activities [34], on cell lines such as HepG2 (IC_{50} value of 30 mM at 48 h) [35], BEL-7402 (IC_{50} values of 37.70±3.99, 24.30±2.30, and 19.70±2.36 μmol/L at 24, 48, and 72 h, respectively.) [36]. The extract containing saponin (platycodinD is the main component) was cytotoxic against four cell lines, RWPE-1, RC-59T/h/SA#4, LNCap.FGC, and PC-3,in a dose-dependent manner with IC_{50} values at 72 h ranging from 28.84 to 45.25 μg/mL. The compounds platycodonoids A and B, platycodin D, deapioplatycodin D, glucopyranosyl platycodigenin, and polygalacin D demonstrated antiproliferative activity against HSC-T6 cells with IC_{50} values of 5.27, 69.63, 1.77, 8.24, 13.36, and 1.04 μM, respectively [37]. The compound lycopodine isolated from the ethanolic extract of *L. clavatumca* used a decrease in the survival of HeLa cells from 50% to 40% in regard to exposure from 24 h to 48 h at the dose (200 μg/mL)38. The lycopodine inhibited proliferation of PC3 and LnCaP cells with IC_{50} values of 57.62 and 51.46 μg/mL, respectively [39]. The highly-diluted, dynamized homeopathic remedies LC-5C and LC-15C demonstrated their capabilities to induce apoptosis in HeLa cancer cells [40]. In this present study, the ethyl acetate extract demonstrated the most potent cytotoxic activities compared to the n-hexane and n-butanol extracts, while the aqueous extract did not show any activity. Polar extracts of *O. japonicus* (75% ethanol extract, methanol extract) have reported proliferative inhibitory activities against many cancer cell lines. Sterol and steroidal saponin components such as ruscogenin, liriopesides B, ophiopogonin B, ophiopogonin D and ruscogenin-1-O-[β-D-glucopyranosyl(1→2)][β-D-xylopyranosyl (1→3)]-β – D - fucopyranoside (DT-13) have been reported to be responsible for the cytotoxic activities of O. japonicas [41]. In this present study, ethyl acetate and n-butanol extracts showed strong cytotoxic activities, while n-hexane extracts and aqueous extracts did not show activity.

CONCLUSION: This study evaluated the anti-proliferative activities of 24 Vietnamese traditional medicinal herbs against nine cancer cell lines including HepG2, A549, MCF7, HT-29, Hela, RD, LNCaP, HL-60, and NTERA-2.Eight of them include *Phyllanthus urinaria, Solanum trilobatum, Angelica sinensis, Xanthium strumarium*, *Scutellaria baicalensis*, *Platycodon grandiflorus*, *Lycopodium clavatum* and *Ophiopogon japonicushave* shown potential for further research for cancer treatment purposes with IC_{50} values below 100 μg/mL. There is a need to verify the phytochemical makeup of each of these herbal extracts, to isolate and screen the individual components for activity, and to evaluate the antiproliferative effects and therapeutic dosages *in-vivo*.

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