# Cytotoxicity of 114 Mongolian plant extracts on liver, colon, breast and cervix cancer cell lines

Sarangerel Oidovsambuu<sup>1</sup>\*, Tuul Tsagaantsooj<sup>2</sup>, Davaapurev Bekh-Ochir<sup>2</sup>, Nomin Myagmar<sup>2</sup>, Indra Batjikh<sup>2</sup>, Saruul Erdenebileg<sup>2</sup>, Orgilkhatan Munkhuu<sup>2</sup>, Odgerel Oidovsambuu<sup>2</sup>, and Batkhuu Javzan<sup>2</sup>

<sup>1</sup>Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Ulaanbaatar 13330, Mongolia

<sup>2</sup>School of Engineering and Technology, National University of Mongolia, Ulaanbaatar 14201, Mongolia

\*Author to whom correspondence should be addressed: Sarangerel Oidovsambuu

Institute of Chemistry and Chemical Technology Mongolian Academy of Sciences Ulaanbaatar 13330, Mongolia

Email: sarangerel\_o@mas.ac.mn

ORCID: https://orcid.org/0000-0001-5062-7440

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, and proofreading process, which may lead to differences between this version and the official version of record.

Please cite this article as: *Oidovsambuu S, Tsagaantsooj T., Bekh-Ochir D., Myagmar N., Batjikh I., Erdenebileg S., Munkhuu O., Oidovsambuu O., and Javzan B.* Cytotoxicity of 114 Mongolian plant extracts on liver, colon, breast and cervix cancer cell lines. *Mongolian Journal of Chemistry*, **25**(51), 2024, xx-xx

https://doi.org/10.5564/mjc.v25i51.2934

## Cytotoxicity of 114 Mongolian plant extracts on liver, colon, breast and cervix cancer cell lines

- 3 Sarangerel Oidovsambuu<sup>1\*</sup>, <a href="https://orcid.org/0000-0001-5062-7440">https://orcid.org/0000-0001-5062-7440</a>
  Tuul Tsagaantsooj<sup>2</sup>, <a href="https://orcid.org/0000-0003-2064-9205">https://orcid.org/0000-0003-2064-9205</a>
  Davaapurev Bekh-Ochir<sup>2</sup>, <a href="https://orcid.org/0000-0002-7623-2532">https://orcid.org/0000-0002-7623-2532</a>
- 6 Nomin Myagmar², <a href="https://orcid.org/0000-0002-3448-9137">https://orcid.org/0000-0002-3448-9137</a>
  Indra Batjikh², <a href="https://orcid.org/0000-0002-7292-8487">https://orcid.org/0000-0003-4474-7352</a>
  Saruul Erdenebileg², <a href="https://orcid.org/0000-0003-4474-7352">https://orcid.org/0000-0003-4474-7352</a>
- 9 Orgilkhatan Munkhuu², <a href="https://orcid.org/0009-0001-1594-654X">https://orcid.org/0009-0001-1594-654X</a>
  Odgerel Oidovsambuu², <a href="https://orcid.org/0000-0001-6872-3385">https://orcid.org/0000-0001-6872-3385</a>
  Batkhuu Javzan², <a href="https://orcid.org/0000-0002-8139-2311">https://orcid.org/0000-0002-8139-2311</a>
- <sup>1</sup>Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Ulaanbaatar 13330, Mongolia
  <sup>2</sup>School of Engineering and Technology, National University of Mongolia, Ulaanbaatar 14201,

#### 15 Mongolia

#### **ABSTRACT**

A total of 114 Mongolian plant species were subjected to cytotoxicity screening against liver (HepG2), colon (HCT116), breast (MCF7), and cervical (HeLa) cancer cell lines. Among 18 them, ethanolic extracts of Androsace incana, Artemisia rutifolia, Saussurea amara, and Inula salsoloides exhibited remarkable cytotoxicity, with IC<sub>50</sub> values below 1.5 µg/mL against at least 2 tested cell lines when treated for 48 hours. Erysimum flavum, Juniperus sibirica, 21 and Stellaria dichotoma demonstrated selective cytotoxicity against specific cancer cell lines. Extracts from 23 plant species, such as Artemisia xerophytica, Ajania trifida, Melandrium brachypetalum, Brachanthemum mongolicum, and Rhinanthus songaricus, 24 showed moderate toxicity. Further research on the phytochemicals and biological activities of these species is crucial for a deeper understanding and potential applications. This screening results of the cytotoxic effects of numerous Mongolian plants could establish a 27 foundational dataset for subsequent comprehensive studies on the screened plants.

Keywords: Medicinal plant, cytotoxicity, cancer cell line, ethanolic extract

#### INTRODUCTION

30

33

36

39

42

45

48

51

63

Cancer is one of the leading causes of morbidity and mortality globally. Among men, lung, prostate, colorectal, stomach, and liver cancer are the most prevalent types, whereas breast, colorectal, lung, cervical, and thyroid cancer are frequently diagnosed among women [1]. In Mongolia, liver cancer has the highest mortality rate, and the country ranks second for stomach cancer mortality [2]. Among Mongolian men, the most commonly diagnosed cancers include liver, stomach, lung, esophageal, and colorectal cancers, whereas liver, cervical, stomach, esophageal, and breast cancers are common among Mongolian women [3]. These statistics highlight the urgent need for comprehensive measures to address the burden of cancer in Mongolia and prioritize preventive and treatment strategies for the identified cancer types.

Throughout history, people have developed indigenous drug prescriptions based on the unique flora found in their native habitats [4]. Mongolia is divided into sixteen phytogeographical regions with various vegetation types, namely, alpine steppe, forest, meadow steppe, typical steppe, desert steppe, and desert [5]. Plant species growing in Mongolia synthesize protective compounds to survive the harsh climate, including UV radiation, aridity, winter coldness, and summer heat. In Mongolian ethnomedicine a diverse selection of native plant species was utilized for the treatment of cancer [6]. The identification of powerful anti-cancer drugs like paclitaxel, colchicine, camptothecin derivatives, podophyllotoxin, vincristine, and vinblastine through screening of natural products has inspired scientists to explore the potential anti-proliferative effects of natural herbs against human cancer cells [7]. Despite the discovery of numerous plant-derived compounds as anti-cancer agents and pharmacophores, a vast number of molecules still await discovery or thorough investigation for their anti-tumor activity.

In present study, the anti-proliferative effects of ethanolic extracts from 114 plant species growing in Mongolia were examined employing human liver, stomach, breast, and cervix cancer cell lines. The selection of plant species was based on their traditional use and unexplored biological effects. Liver, stomach, breast, and cervix cancer cell lines were chosen due to their prevalence among the Mongolian population.

#### **EXPERIMENTAL**

60 **Chemicals:** Analytical grade ethanol was purchased from Xilong Scientific and etoposide and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

*Plant material*: A total of 114 wild plant species were collected from various locations in Mongolia (Table S1). Voucher specimens of these species were deposited in the Herbarium

66

75

78

81

84

87

90

93

96

of the Laboratory of Bioorganic Chemistry and Pharmacognosy, School of Engineering and Technology, National University of Mongolia. The identification of plant species was carried out by Dr. Chinbat Sanchir, a former taxonomist at the Institute of Botany, Mongolian Academy of Sciences. Prior to extraction, the collected plant materials were dried in the shadow, chopped, and ground.

69 **Plant sample extraction:** Plant samples weighing between 50 g and 200 g were extracted three times with 96% ethanol at a ratio of 1:10 (plant sample to ethanol) at room temperature for at least 3 days. The resulting extracts were then filtered and dried under vacuum at 40°C.

72 The dried extracts were subsequently stored at 4°C for future use.

Cell culture: HepG2 human hepatocellular carcinoma cell line, HCT116 human colorectal carcinoma cell line, MCF7 human breast adenocarcinoma cell line and HeLa human cervix adenocarcinoma cell line were obtained from American Type Culture Collection (ATCC, Manassas, VA). HepG2 cell line was maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% (w/v) fetal bovine serum (FBS, HyClone), 100 U/ml penicillin (HyClone), and 100 μg/ml streptomycin (HyClone). MCF7 cells were cultured in DMEM supplemented with 0.01 mg/mL insulin (Sigma-Aldrich), 10% (w/v) FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. HCT116 cells were maintained in Minimum Essential Media (MEM, Gibco) supplemented with 10% (w/v) FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. HeLa cell line was cultured in RPMI 1640 media (Gibco) with 10% (w/v) heat inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. All cell lines were maintained at subconfluence in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> at 37°C.

**Cell viability assay:** The cytotoxicity of the extracts was evaluated by a cell viability assay as previously described [8, 9]. All cells (1×10<sup>4</sup> cells per well) were plated in 96-well plates and incubated for 24 hrs at 37°C. Cells were treated with the plant extracts, which was previously stocked in DMSO at 20 mg/mL, at the final dose of 100 μg/mL, then the cells were incubated for additional 24 hrs. The cell viability was measured using the EZ-Cytox cell viability assay kit (Daeil Lab Service, Seoul, Republic of Korea) employing BioTek Microplate Reader (Agilent). Etoposide (100 μM) was included as a positive control in this study due to its known toxicity against the employed cell lines [10].

**Calculation of results:** The cytotoxicity of the extracts was calculated as a percentage of the negative control value treated with the vehicle (DMSO), which was set as 100%. The cell viability assay was conducted in triplicate for all the extracts, and IC<sub>50</sub> values were determined for the selected extracts. The data were presented as the mean ± standard deviation (SD).

#### 99 **RESULTS AND DISCUSSION**

102

105

108

111

114

117

120

123

Numerous libraries containing natural product extracts and fractions have been established to gather information on the cytotoxic effects of plant extracts and fractions on cancer cells worldwide [11]. However, there is currently a lack of screening data regarding the antiproliferative potential of Mongolian plants on cancer cells. Interestingly, Mongolian nomads have developed their own traditional prescriptions utilizing Mongolian herbs for treating various tumors. Despite this, no comprehensive collection of data regarding the cytotoxicity effects of these plants on cancer cells has been conducted thus far. In this study, we assessed the anti-proliferative effect of extracts derived from 114 Mongolian plant species belonging to 39 families. To evaluate this effect, we employed a cell viability assay conducted on four human cancer cell lines: liver cancer HepG2, colon cancer HCT116, breast cancer MCF7, and cervical cancer HeLa. We utilized the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which is widely employed as one of the most common methods for screening the anti-proliferative effects of both natural products and synthesized compounds [8]. As a positive control, we selected etoposide, a well-known anticancer drug widely prescribed for the treatment of various human cancers. Etoposide exerts its therapeutic effects by targeting topoisomerase II, an enzyme critical for DNA replication, transcription, and repair [10]. This mechanism leads to G2/M cell cycle arrest, nuclear enlargement, and induction of apoptosis [12].

The cytotoxicity of the extracts was categorized as follows: potent (cell viability below 5.0% for at least 2 cell lines), selective (cell viability below 6.0% for at least 1 cell line), moderate (cell viability below 70.0% for at least 2 cell lines), weak (cell viability below 90.0% for at least 2 cell lines), and without cytotoxicity (cell viability higher than 90.0% for 2 cell lines).

The plant extracts with potent, selective, and moderate cytotoxicity were presented in Table 1, while extracts with weak and no cytotoxic effects were demonstrated in Table S2.

Table 1. The plant extracts with potent, selective and moderate cytotoxicity against four cancer cell lines at 100  $\mu$ g/mL (n=3)

	ser sen intes at 100 μg/m² (n=0)	Cell viability (%)									
No	Name	HCT116	MCF7	HepG2	HeLa						
L	Potent cytotoxicity										
1	Androsace incana Lam.	3.04±0.95	1.36±0.76	1.34±1.40	16.75±5.41						
2	Artemisia rutifolia Steph. Ex Spreng.	3.87±0.26	19.04±5.23	2.06±3.03	10.67±1.39						
3	Inula salsoloides (Turcz.) Ostenf.	1.29±0.76	1.57±4.63	3.87±5.55	1.31±2.26						
4	Saussurea amara DC.	2.73±1.58	5.81±1.06	1.84±2.62	9.73±3.13						
	Selective cytotoxicity										
5	Erysimum flavum (Georgi) Bobr.	45.62±1.15	5.38±0.83	38.66±5.91	44.56±7.34						
6	Juniperus sibirica Burgsd.	1.45±0.29	8.98±1.58	41.41±17.99	18.67±1.22						
7	Stellaria dichotoma L.	5.66±2.09	18.76±0.63	81.79±5.82	41.01±2.11						
	Moderate cytotoxicity										
8	Allium bidentatum Fisch. ex Prokh.	75.18±3.25	65.18±3.53	70.43±9.42	62.94±0.26						
9	Ajania trifida (Turcz.) Tzvel.	24.80±0.59	43.54±0.33	26.48±13.67	39.00±3.87						
10	Artemisia annua L.	85.92±5.74	67.93±9.55	52.00±2.13	69.51±1.69						
11	Artemisia demissa Krasch.	100<	86.61±0.77	65.16±8.67	57.67±3.07						
12	Artemisia xerophytica Krasch.	23.31±1.74	38.91±0.92	35.98±7.40	32.33±2.76						
13	Brachanthemum mongolicum Krasch.	55.85±2.64	50.35±2.10	46.12±1.09	59.60±0.97						
14	Brachanthemum gobicum Krasch.	100<	68.02±4.00	55.03±8.62	61.58±4.88						
15	Bupleurum bicaule Helm.	77.42±5.64	64.78±1.72	29.00±2.40	52.36±1.24						
16	Bupleurum multinerve DC.	74.86±5.64	62.81±2.72	46.24±12.42	42.13±2.54						
17	Caragana pygmaea (L.) DC.	77.89±2.99	66.05±2.15	66.49±7.60	50.51±1.61						
18	Cirsium arvense (L.) Scop.	98.91±1.45	64.78±7.49	68.85±3.89	71.85±1.82						
19	Delphinium pumilum W.T.Wang	76.49±3.96	67.73±4.59	54.44±1.44	64.77±3.88						
20	Geranium pratense L.	81.20±3.71	65.57±1.83	84.64±7.54	59.35±2.20						
21	Goniolimon speciosum (L.) Boiss.	93.44±4.69	93.93±2.57	58.43±13.82	57.44±1.87						
22	Haplophyllum dauricum (L.) G. Don	77.62±7.34	78.65±2.26	44.29±8.14	55.51±7.27						
23	Heracleum dissectum Ldb.	92.58±3.56	68.63±4.93	71.51±4.91	48.86±0.99						
24	Melandrium brachypetalum (Hornem.) Fenzl	16.79±2.04	80.20±1.41	23.27±0.19	69.38±7.59						
25	Polygonum sericeum Pall. ex Georgi	66.80±1.77	77.61±3.42	49.14±6.28	63.62±4.51						
26	Potentilla strigosa Pall. ex Pursh.	91.34±2.95	84.00±2.37	65.54±6.36	60.15±2.65						
27	Potentilla viscosa G. Don	78.02±1.49	82.19±1.20	61.47±1.48	55.69±3.74						
28	Rhinanthus songaricus (Sterneck) B. Fedtsch	40.80±2.00	49.17±5.14	58.43±4.05	32.07±0.39						
29	Sphallerocarpus gracilis (Bess. ex Trev.) KPol.	73.59±3.54	60.66±0.98	57.41±4.63	69.79±1.55						
30	Urtica angustifolia Fisch. ex Hornem	67.84±1.59	66.94±3.81	70.89±11.51	61.87±1.04						
	Positive control										
31	Etoposide	71.05±2.95	72.72±4.80	37.36±2.33	33.62±2.14						

Furthermore, 12 extracts exhibiting potent, selective and moderate cytotoxicity against the cancer cell lines were selected and evaluated for their IC<sub>50</sub> values (Table 2).

Table 2. IC<sub>50</sub> values of some selected plant extracts (µg/mL)

ıar	ole 2. IC <sub>50</sub> values	of some sele	ected plant e	extracts (µg.	/mL)					
	Species	Treatment time: 24 hrs			Treatment time: 48 hrs					
		MCF7	HepG2	HCT116	MCF7	HepG2	HCT116			
Potent toxicity										
1	Androsace incana Lam.	3.96±5.29	7.13±4.97	9.27±5.90	1.5>	1.5>	3.62±4.41			
2	Artemisia rutifolia Steph. Ex Spreng.	35.85±5.24	7.53±4.27	10.21±6.3	12.54±4.47	1.5>	1.5>			
3	Inula salsoloides (Turcz.) Ostenf.	9.67±8.63	6.04±4.52	6.25±3.68	1.5>	1.5>	1.5>			
4	Saussurea amara DC.	17.88±6.06	7.34±9.55	7.26±8.10	1.5>	1.5>	1.5>			
Selective toxicity										
5	Erysimum flavum (Georgi) Bobr.	9.22±4.52	47.61±8.70	200<	1.5>	20.77±6.10	200<			
6	<i>Juniperus</i> sibirica Burgsd.	32.07±8.40	65.88±8.41	16.65±08	9.09±6.56	41.50±6.25	1.5>			
7	Stellaria dichotoma L.	42.60±6.30	96.97±8.25	27±8.1	29.3±5.29	74.84±8.63	18.0±6.9			
Moderate toxicity										
8	<i>Ajania trifida</i> (Turcz.) Tzvel.	121.30±9.53	46.51±13.70	200<	74.6±8.6	19.6±6.4	200<			
9	<i>Artemisia</i> <i>xerophytica</i> Krasch.	68.15±8.92	64.56±7.74	200<	31.21±9.65	21.10±6.52	200<			
10	Brachanthemu m mongolicum Krasch.	104.09±10.32	72.68±8.47	200<	48.32±9.20	25.84±12.19	200<			
11	<i>Melandrium</i> <i>brachypetalum</i> (Hornem.) Fenzl	143.71±14.44	33.09±9.31	25.53±6.68	96.94±12.23	13.7±6.1	8.1±6.4			
12	Rhinanthus songaricus (Sterneck) B. Fedtsch	69.28±8.35	82.71±6.41	200<	45.06±9.91	65.30±6.82	200<			
	Positive control									
13	Etoposide*	72.79±4.80	37.36±2.33	200<	47.11±5.25	12.68±3.55	200<			

<sup>\*</sup>IC<sub>50</sub> values of etoposide were expressed as µM.

129

132

135

The extract of *Androsace incana* (*AI*) exhibited strong cytotoxicity against all cell lines, except for HeLa. In Mongolian traditional medicine, *AI* is commonly used for its anti-swelling, wound healing, detoxifying, body strengthening, and dehydrating properties [6]. In previous studies, other members of the Androsace genus, namely *Androsace umbellate* and *Androsace integra*, were investigated for their cytotoxic effects [13-15]. Triterpenoid saponins, including saxifragifolin A, saxifragifolin B, saxifragifolin C, and saxifragifolin D,

isolated from *Androsace umbellate*, demonstrated apoptotic effects on RAW 264.7 cells and exhibited cytotoxicity against various multidrug resistance and non-multidrug resistance human tumor cell lines [13]. In HepG2 cells, saxifragifolin B induced apoptosis through the accumulation of sub-G1 population, mitochondrial membrane depolarization, cytochrome c leakage, and activation of poly (ADP-ribose) polymerase (PARP) and caspase cascades [14]. Additionally, another triterpenoid saponin called ardisiacrispin A from *Androsace integra* exhibited cytotoxicity against HepG2 cells [15]. Based on these previous reports, it is plausible to hypothesize that *AI* may also contain cytotoxic triterpenoid saponins similar to those found in other Androsace species. However, further research is needed to explore the chemical constituents and biological activities of *AI*.

The ethanol extract of *Artemisia rutifolia* (*AR*) exhibited potent inhibition of cell growth in both the HepG2 and HCT116 cell lines, with IC<sub>50</sub> values of less than 1.5 μg/mL after 48 hours of treatment. However, the extract demonstrated moderate toxicity in other cell lines. *AR* has limited data available regarding its use in traditional folk medicine. Few reports have focused on its specific sesquiterpene lactone content, revealing the presence of several guaianolides, seco-guaianolides, germacranolide, and eudesmane derivatives in the aerial parts of *AR* along with its *in vitro* cytotoxic properties [16,17]. Artemisia (Asteraceae) is a diverse genus comprising 200-400 species, known for its rich reservoir of active biological compounds. Within the phytochemicals isolated from Artemisia species, terpenoids, particularly sesquiterpenoids, as well as flavonoids, coumarins, and lignans, have exhibited noteworthy anti-proliferative activity against cancer cells. Notably, sesquiterpenoids like artemisinin, artesunate, artemether, dihydroartemisinin, and arteether derived from *Artemisia annua*, along with flavonoids such as eupatilin, jaceosidin, cirsilineol, and 6-methoxytricin from *Artemisia asiatica*, have been reported for their cytotoxic effects against cancer cells [18].

Inula salsoloides (IS) exhibited significant cytotoxicity against all cell lines tested. Inula species are famous for their anti-tumor effects in the folk medicine of China, Mongolia, and Korea [6]. Sesquiterpene lactones are considered the active components in Inula plants, displaying anti-cancer activity against various human cancer cell lines. Inula sesquiterpene lactones exert their anti-tumor effects through cell cycle arrest induction, inhibition of neoangiogenesis, and stimulation of apoptosis signaling pathways [19]. However, there are limited reports regarding the chemical constituents with cytotoxic activity of IS, except for the cytotoxicity demonstrated by sesquiterpene lactones inulasalsolin and eupatolide, against human cancer P-388 and KB3 cell lines [20].

The extract of Saussurea amara (SA) demonstrated remarkable toxicity against all cell lines utilized in this study. In Mongolian traditional medicine, SA is commonly employed for 171 treating bacterial, protozoal, and viral infections, intoxication, jaundice, and tumors [6]. SA contains several flavonoids, including apigenin, luteolin, genkwanin, quercitrin, and apigenin-7-O-glucoside, as well as terpenoids such as taraxasterol, taraxasterol-acetate, 174 cynaropicrin, and desacylcynaropicrin [21]. Although the biological activity of SA has not been extensively studied, it has shown choleretic effects in isolated perfused rat liver [22]. 177 Other Saussurea species have exhibited cytotoxic potential against various human cancer cells. Sesquiterpene lactones, particularly guaiane-type sesquiterpene lactones from Saussurea deltoidea and Saussurea calcicola have demonstrated strong toxicity against cancer cell lines [23, 24]. Cynaropicrin, a guaiane sesquiterpene lactone identified in 180 Saussurea calcicola, Saussurea pulchella, and Saussurea salicifolia, exhibited cytotoxic potential against skin melanoma SK-MEL-2, ovary malignant ascites SK-OV-3 [25], nonsmall cell lung adenocarcinoma A549, skin melanoma SK-MEL-2, human CNS solid tumor 183 XF498, colon adenocarcinoma HCT15 [24], gastric adenocarcinoma AGS cells, and murine hepatoma Hepa1c1c7 cells [26]. Dehydrocostus lactone and costunolide, which are sesquiterpene lactones isolated from Saussurea lappa, exhibited potent cytotoxicity against 186 ovarian carcinoma OVCAR-3, hepatoma HepG2, and cervical adenocarcinoma HeLa cell lines [27] and induced apoptosis in neuroblastoma IMR-32, NB-39, SK-N-SH, and LA-N-1 cell lines by activating caspase-7 and cleaving PARP [28]. Therefore, the cytotoxicity 189 observed in the extract from SA may be attributed to its constituents of sesquiterpene lactones.

Several plant extracts demonstrated selective toxicity towards specific cell lines in this study. *Erysimum flavum, Juniperus sibirica*, and *Stellaria dichotoma* exhibited cytotoxic effects on certain cancer cell lines.

The ethanol extract of *Erysimum flavum* (*EF*) exhibited selective cytotoxicity against the breast cancer cell line MCF7, while the extract demonstrated only a moderate cytotoxic effect on other cell lines (Table 1). In Mongolian traditional medicine, this plant is utilized for the treatment of heart disorders, indigestion, and swellings [29]. However, the phytochemical composition and biological activities of *EF* have not been investigated to date. Similarly, limited research has been conducted on the chemical composition and biological activities of other Erysimum species. For example, a study focused on assessing the *in vitro* cytotoxicity of *Erysimum corinthium* seeds against colorectal, hepatic, and Hela cell lines [30]. Therefore, there is a need for future studies aimed at identifying and evaluating phytochemicals of *EF* that possess potential anti-proliferative effects.

207

210

213

216

219

Juniperus sibirica (JS) exhibited selective cytotoxicity against colorectal carcinoma HCT116 cells. In Mongolian culture, JS is commonly utilized as an air fragrance by smoking and fuming the dried needles. JS has been traditionally employed in Mongolian oriental medicine for the treatment of kidney diseases and bladder inflammation through oral administration and for wound healing and rheumatism treatment through immersion methods [29]. While the phytochemical constituents with cytotoxic effect of JS have not been investigated yet, it is worth noting that the Juniperus family is known for its cytotoxic lignan constituents, such as podophyllotoxin and deoxypodophyllotoxin, which hold significant pharmaceutical potential for cancer chemotherapy [31]. In addition, chemical composition and cytoxicity of essential oil from JS against MCF7 human breast cancer cell line was reported previously [32]. Therefore, it can be suggested that the presence of lignans and volatile components in the extract of JS may be responsible for its toxic effects on cancer cell lines.

The extract of *Stellaria dichotoma* (*SD*) demonstrated cytotoxic effects on HCT116 and MCF7 cells (Table 1, 2). Two alkaloids dichotomine B and glucodichotomine B isolated from this plant displayed moderate toxicity towards colon carcinoma HCT116 and hepatocarcinoma SMMC7721 cells [33]. Despite this finding, the cytotoxic effect of *SD* has not been extensively documented up until now.

Moderate cytotoxicity was exhibited in the extracts of 23 plant species including previously unexplored ones such as *Artemisia xerophytica, Melandrium brachypetalum, Brachanthemum mongolicum*, and *Rhinanthus songaricus* (Table 1). Conversely, weak cytotoxicity was observed in the extracts of 47 plant species, while no cytotoxicity was detected in the extracts of 36 plant species. (Table S2).

#### CONCLUSION

The cytotoxic effects of ethanolic extracts from a total of 114 Mongolian plant species were evaluated against human liver (HepG2), colon (HCT116), breast (MCF7), and cervix (HeLa) cancer cell lines. Notably, *Androsace incana*, *Artemisia rutifolia*, *Saussurea amara*, and *Inula salsoloides* exhibited the most potent cytotoxic effects against all tested cell lines with IC<sub>50</sub> values below 1.5 μg/mL against at least 2 tested cell lines when treated for 48 hours. *Juniperus sibirica*, *Stellaria dichotoma*, and *Erysimum flavum* demonstrated cytotoxic effects against specific cancer cell lines. On the other hand, moderate cytotoxicity was observed in the extracts of 23 plant species, while weak cytotoxicity was observed in the extracts of 47 plant species. It is essential to further investigate the chemical constituents and biological activities of these plants exhibiting cytotoxic effects. This first comprehensive screening

results of the cytotoxic effects of a great number of Mongolian plants could provide the basis for subsequent in-depth studies on the screened plants.

### 240 **ACKNOWLEDGEMENT**

This work was supported by the basic research project (ShuSs-2020/54) sponsored by the Foundation of Science and Technology, Ministry of Education and Science of Mongolia. In addiditon, this study was partially supported by the JICA M-JEED Project (J12A15), JST/JICA SATREPS (JPMJSA1906). We thank Dr. Nho Chu Won from KIST Gangneung Natural Product Institute for generously sharing the cancer cell lines.

#### **REFERENCES**

243

- 1. Mattiuzzi C., Lippi G. (2019) Current cancer epidemiology. *Journal of Epidemiology* and Global Health, **9**(4), 217–222. <a href="https://doi.org/10.2991/jegh.k.191008.001">https://doi.org/10.2991/jegh.k.191008.001</a>
- Cancer Mongolia 2020 country profile. Technical document, World Health Organization. <a href="https://www.who.int/publications/m/item/cancer-mng-2020">https://www.who.int/publications/m/item/cancer-mng-2020</a> (accessed 22 June 2023)
- 3. World Cancer Research Fund International. <a href="https://www.wcrf.org/cancer-trends/global-cancer-data-by-country/">https://www.wcrf.org/cancer-trends/global-cancer-data-by-country/</a> (accessed 22 June 2023)
- Süntar I. (2020) Importance of ethnopharmacological studies in drug discovery: Role of medicinal plants. *Phytochem. Rev.*, 19, 1199-1209. <a href="https://doi.org/10.1007/s11101-019-09629-9">https://doi.org/10.1007/s11101-019-09629-9</a>
- 5. Shukherdorj B., Magsar U., Batlai O., Sukhorukov A.P., Zagarjav T., *et al.* (2022) Flora of Mongolia: Annotated checklist of native vascular plants. *PhytoKeys*, **192**, 63-169. <a href="https://doi:10.3897/phytokeys.192.79702">https://doi:10.3897/phytokeys.192.79702</a>
- 6. Ligaa U. (1996) Methods of uses of medicinal plants in Mongolian traditional medicine and prescriptions. 1st volume, Ulaanbaatar.
- 7. Mann J. (2002) Natural products in cancer chemotherapy: Past, present and future.

  Nature Reviews Cancer, 2(2), 143-148. <a href="https://doi.org/10.1038/nrc723">https://doi.org/10.1038/nrc723</a>
- 8. Ediriweera M.K., Tennekoon K.H., Samarakoon S.R. (2018) In vitro assays and techniques utilized in anticancer drug discovery. *Journal of Applied Toxicology*, **39**(1), 11-34. <a href="https://doi.org/10.1002/jat.3658">https://doi.org/10.1002/jat.3658</a>
- Oidovsambuu S., Kim C.Y., Kang K.S., Dulamjav B., Jigjidsuren T., Nho C.W. (2013)
   Protective effect of *Paeonia anomala* extracts and constituents against tert-butylhydroperoxide-induced oxidative stress in HepG2 Cells. *Planta Medica*, 79(02), 116-122. https://doi.org/10.1055/s-0032-1328062

- Baldwin E.L., Osheroff N. (2005) Etoposide, topoisomerase II and cancer. Curr. Med. Chem. Anticancer Agents, 5(4), 363-372. https://doi.org/10.2174/1568011054222364
- Wilson B.A.P, Thornburg C.C., Henrich C.J., Grkovic T., O'Keefe B.R. (2020)
   Creating and screening natural product libraries. *Natural Product Reports*, 37, 893-918. https://doi.org/10.1039/C9NP00068B
- 12. Kang K.S., Lee S.B., Yoo J.H., Nho C.W. (2010) Flow cytometric fluorescence pulse width analysis of etoposide-induced nuclear enlargement in HCT116 cells. *Biotechnology Letters*, **32**, 1045-1052. https://doi.org/10.1007/s10529-010-0277-x
- Park J.H., Kwak J.H., Khoo J.H., Park S.H., Kim D.U., et al. (2010) Cytotoxic effects of triterpenoid saponins from *Androsace umbellata* against multidrug resistance (MDR) and non-MDR Cells. *Archives of Pharmacal Research*, 33(8), 1175-1180. https://doi.org/10.1007/s12272-010-0807-z
- 14. Zhang D.M., Wang Y., Tang M.K., Chan Y.W., Lam H.M., *et al.* (2007) Saxifragifolin B from *Androsace umbellata* induced apoptosis on human hepatoma cells. *Biochem. Biophys. Res. Commun.*, **362**(3), 759-765. <a href="https://doi.org/10.1016/j.bbrc.2007.08.068">https://doi.org/10.1016/j.bbrc.2007.08.068</a>
- 15. Dong W., Liu X., Li X., Yang D., Ding L. (2011) A new triterpene saponin from *Androsace integra*. *Fitoterapia*, **82**(5), 782-785. <a href="https://doi.org/10.1016/j.fitote.2011.04.001">https://doi.org/10.1016/j.fitote.2011.04.001</a>
- 16. Tan R.X., Jia Z.J., Jakupovic J., Bohlmann F., Huneck S. (1991) Sesquiterpene lactones from *Artemisia rutifolia*. *Phytochemistry*, **30**(9), 3033-3035. https://doi.org/10.1016/S0031-9422(00)98246-3
- 17. Tan R.X., Jia Z.J. (1992) Sesquiterpenes from *Artemisia rutifolia*. *Phytochemistry*, **31**(7), 2534-2536. <a href="https://doi.org/10.1016/0031-9422(92)83319-T">https://doi.org/10.1016/0031-9422(92)83319-T</a>
- 18. Taleghani A., Emami S.A., Tayarani-Najaran Z. (2020) Artemisia a promising plant for the treatment of cancer. *Bioorg. Med. Chem.*, **28**(1), 115-180. <a href="https://doi.org/10.1016/j.bmc.2019.115180">https://doi.org/10.1016/j.bmc.2019.115180</a>
- 19. Wang G.W., Qin J.J., Cheng X.R., Shen Y.H., Shan L., *et al.* (2014) Inula sesquiterpenoids: Structural diversity, cytotoxicity and anti-tumor activity. *Expert Opinion on Investigational Drugs*, **23**(3), 317-345. <a href="https://doi.org/10.1517/13543784.2014.868882">https://doi.org/10.1517/13543784.2014.868882</a>
- 20. Zhou B.N., Bai N.S., Lin L.Z., Cordell G.A. (1994) Sesquiterpene lactones from *Inula salsoloides*. *Phytochemistry*, **36**(3), 721-724. <a href="https://doi.org/10.1016/S0031-9422(00)89804-0">https://doi.org/10.1016/S0031-9422(00)89804-0</a>

- 21. Glasl S., Mayr K., Daariimaa K., Narantuya S., Kletter C. (2006) Phytochemical investigation of the Mongolian medicinal plant *Saussurea amara* (L.) DC (Asteraceae). *Planta Medica*, **72**, 56. <a href="https://doi.org/10.1055/s-2006-949856">https://doi.org/10.1055/s-2006-949856</a>
- 22. Glasl S., Tsendayush D., Batchimeg U., Holec H., Wurm E., *et al.* (2007) Choleretic effects of the Mongolian medicinal plant *Saussurea amara* in the isolated perfused rat liver. *Planta Medica*, **73**(1), 59-66. https://doi.org/10.1055/s-2006-957063
- 23. Xu J.J., Huang H.Q., Zeng G.Z., Tan N.H. (2012) Cytotoxic sesquiterpenes and lignans from *Saussurea deltoidea*. *Fitoterapia*, **83**(6), 1125-1130. <a href="https://doi.org/10.1016/j.fitote.2012.04.022">https://doi.org/10.1016/j.fitote.2012.04.022</a>
- 24. Choi S.Z., Cho S.U., Lee K.R. (2005) Cytotoxic sesquiterpene lactones from Saussurea calcicola. Archives of Pharmacal Research, 28(10), 1142-1146. <a href="https://doi.org/10.1007/BF02972976">https://doi.org/10.1007/BF02972976</a>
- 25. Yang M.C., Choi S.U., Choi W.S., Kim S.Y., Lee K.R. (2008) Guaiane sesquiterpene lactones and amino acid-sesquiterpene lactone conjugates from the aerial parts of Saussurea pulchella. Journal of Natural Products, 71, 678-683. <a href="https://doi.org/10.1021/np800005r">https://doi.org/10.1021/np800005r</a>
- 26. Kang K.S., Lee H.J., Kim C.Y., Lee S.B., Tunsag J., *et al.* (2007) The chemopreventive effects of *Saussurea salicifolia* through induction of apoptosis and phase II detoxification enzyme. *Biological and Pharmaceutical Bulletin*, **30**(12), 2352-2359. <a href="https://doi.org/10.1248/bpb.30.2352">https://doi.org/10.1248/bpb.30.2352</a>
- 27. Sun C.M., Syu W.J., Don M.J., Lu J.J., Lee G.H. (2003) Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa. Journal of Natural Products*, **66**(9), 1175-1180. https://doi.org/10.1021/np030147e
- 28. Tabata K., Nishimura Y., Takeda T., Kurita M., Uchiyama T., Suzuki T. (2015) Sesquiterpene lactones derived from *Saussurea lappa* induce apoptosis and inhibit invasion and migration in neuroblastoma cells. *Journal of Pharmacological Sciences*, **127**, 397-403. https://doi.org/10.1016/j.jphs.2015.01.002
- 29. Ligaa U. (1997) Methods of uses of medicinal plants in Mongolian traditional medicine and prescriptions. 2nd volume, Artsot Co. Ltd. Ulaanbaatar.
- Al-Gendy A.A., El-gindi O.D., Hafez Al.S., Ateya A.M. (2010) Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). Food Chemistry, 118(3), 519-524. <a href="https://doi.org/10.1016/j.foodchem.2009.05.009">https://doi.org/10.1016/j.foodchem.2009.05.009</a>
- 31. Renouard S., Lopez T., Hendrawati O., Dupre P., Doussot J., *et al.* (2011) Podophyllotoxin and deoxypodophyllotoxin in *Juniperus bermudiana* and 12 other Juniperus species: Optimization of extraction, method validation, and quantification.

- Journal of Agricultural and Food Chemistry, **59**, 8101-8107. https://doi.org/10.1021/jf201410p
- 32. Javzmaa N., Altantsetseg Sh., Sarantuya J., Tuul Ts., Shiretorova V.G. (2019) Study on chemical composition, anti-pathogenic micro-organisms activity and inhibition activity against human breast cancer cell (MCF 7) of *Juniperus sibirica* essential oil. *Bulletin of the Institute of Chemistry and Chemical Technology*, **7**, 41-48. https://doi.org/10.5564/bicct.v0i7.1272
- 33. Cao L.H., Zhang W., Luo J.G., Kong L.Y. (2012) Five new β-carboline type alkaloids from *Stellaria dichotoma* var. *lanceolate. Helvetica*, **95**(6), 1018-1025. <a href="https://doi.org/10.1002/hlca.201100485">https://doi.org/10.1002/hlca.201100485</a>