

Anticancer Activity of Selected *Colocasia gigantea* Fractions

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The objective of this study is to investigate the anticancer potential of the extract of *Colocasia gigantea* C. gigantea), a plant member of the Araceae family. In the present study, we investigated the cytotoxic activity of C. gigantea extract on cervical cancer (Hela) and human white blood cells (WBC) in vitro. The authors then identified the bioactive ingredients that demonstrated cytotoxicity on tested cells and evaluated those bioactive ingredients using the bioassay-guided fractionation method. The results showed that not all parts of C. gigantea promote cytotoxic activity. The dichloromethane leaf fraction showed significant cell proliferation effect on Hela cells, but not on WBCs. Only the n-hexane tuber fraction (Fr. 1T) exhibited significant cytotoxicity on Hela cells ($IC_{50} = 585 \mu\text{g/ml}$) and encouraged WBC cell proliferation. From GC-Mass spectrometry, 4,22-Stigmastadiene-3-one, Diazoprogestosterone, 9-Octadecenoic acid (Z)-, hexyl ester, and Oleic Acid were the components of Fr. 1T that demonstrated cytotoxic potential. In conclusion, C. gigantea's Fr. 1T shows potential for cervical cancer treatment.

Keywords: *Colocasia gigantea* (Araceae), Cervical cancer, Cytotoxicity, Bioactive ingredients

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Cancer is a disease of uncontrollable cell fission in malignant tumors that invade normal human body systems^(1,2). Many factors are known to increase the risk of contracting cancer, such as environmental pollutants, radiation, stress, obesity, and diet⁽²⁾. Cancer is a primary health crisis problem for people in many parts of the world^(1,3). Thailand is one of the many countries that has been affected by cancer. National Cancer Institute Thailand, a cancer research and care center under the Thailand Ministry of Public Health reported 3,314 new cancer patients in Thailand in 2009⁽⁴⁾. Among all cancers, cervical cancer is the most common carcinoma found in Thailand⁽⁴⁾.

Many studies and articles from all over the world have reported the anticancer activity of phytochemical ingredients^(5,6). Plants are a source of

natural bioactive compounds. Various compounds are cytotoxic and therefore have the potential for anticancer activity⁽⁷⁻¹¹⁾. When contained in nutrients and foodstuffs, the cytotoxicity of phytochemicals must target specific cells, such as cancer cells, and must not be harmful to consumers⁽¹⁾.

Colocasia gigantea Hook. f. was selected for this study. *C. gigantea* belongs to the Araceae family, similar to *Colocasia esculenta* (L.) Schott (Taro). *C. gigantea* grows commonly in Thailand and other Southeast Asian countries⁽¹²⁾. In the Pacific islands, the tubers are cooked and eaten as a starch⁽¹²⁾. In India and Bangladesh, the tubers are used as a main ingredient in curries and stews⁽¹³⁾. In Thailand, *C. gigantea* is considered as a minor food crop and is mainly utilized as a stem vegetable. *C. gigantea*'s stem is often used for making homemade Thai food called "Bon curry". In Thai traditional medicine, *C. gigantea* tuber is heated over a fire. It is used to reduce "internal heat" (fever) and also for the treatment of drowsiness. Fresh tuber has been shown to ameliorate stomach problems, combat infection, and accelerate the healing

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of wounds. In the northern region of Thailand, fresh or dried tuber is used for the treatment of phlegm by mixing it with honey⁽¹⁴⁾.

The objective of the present study was to explore *C. gigantea* as an anticancer agent against cervical cancer cells. Our method of investigation centered on the use of *Hela in vitro* and bioassay-guided fractionation techniques to identify bioactive ingredients in the active fractions of *C. gigantea*.

Material and Method

Extraction

Tuber and leaf components of *C. gigantea* were obtained from the Department of Anatomy, Faculty of Medicine, Chulalongkorn University. The tuber and leaf samples were washed with water, cut, and dried by dehydrator at 40°C for 5 days. They were then ground into small pieces with a grinder. *C. gigantea* extract samples were obtained by macerating the *C. gigantea* in n-hexane (n-C₆H₁₄) for 3 days at room temperature; this step was performed 3 times. The mixture was then filtered using a vacuum filter. The filtrate was concentrated to remove n-C₆H₁₄ under reduced pressure by rotary vacuum evaporator to obtain a crude n-C₆H₁₄ fraction. Marc was then extracted using dichloromethane (CH₂Cl₂) and then again using methanol (CH₃OH), with increasing polarity. For both dichloromethane and methanol, the same extraction process used for n-hexane (n-C₆H₁₄) was used. These fractions were evaluated for cytotoxic activity, with the most active fractions identified for further fractionation. The *C. gigantea* extraction process is illustrated in Fig. 1.

Fractionation

Column chromatography was used for isolating compounds which stationary phase was silica gel 60 (230-400 mesh ASTM). Thin layer chromatography (TLC, aluminum sheet silica gel 60F₂₅₄) was used for the detection of compound fractions. UV light at wavelengths of 254 and 365 nm and 5% anisaldehyde in sulfuric acid heated to 110°C were used for the detection of compound fractions on TLC sheets.

Identification of fractions

Modification of Mitova et al 2003⁽¹⁵⁾, GC-Mass spectroscopy (Leco Corporation, St. Joseph, MI, USA) was used for fraction identification. The column HP5-MS (30 m x 0.25 mm, 0.25 μm film thickness) was used. The injector port was set at 300°C. The injection volume was 0.2 μl and injections were carried out under split/

splitless: split/split ratio: 1:20. Helium was used as a carrier gas with a constant flow of 1 ml/min. The temperature was programmed from 40°C (2 min) to 300°C at a rate of 6°C min⁻¹ with a 10 min hold. The fractions in the test solution were identified by comparing the spectra with known compounds stored in the internal library.

Cell lines and culture

Cervical cancer cells (Hela) were obtained from the Department of Anatomy, Faculty of Medicine, Chulalongkorn University. Human white blood cells (WBC) were collected from the blood of one of the research authors and this sample was used as the primary cell line in the experiment. Hela cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM). WBCs were maintained in Roswell Park Memorial Institute Medium (RPMI) at 37°C in an incubator containing 5% CO₂. Both DMEM and RPMI were supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin/streptomycin, and 3.7 g/L sodium bicarbonate (Na₂CO₃).

In vitro assay for cytotoxicity

Hela cells were washed two times with phosphate buffered saline (PBS) and were then

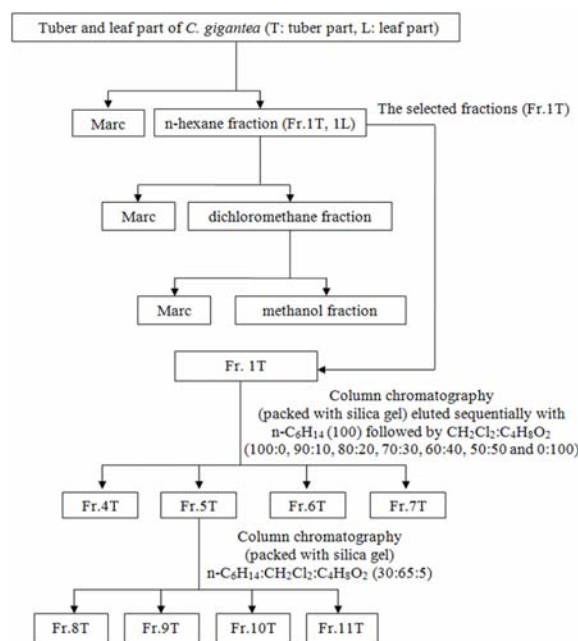


Fig. 1 Bioactive compound extraction and fractionation process for the evaluation of cytotoxic activity in *C. gigantea*.

collected by trypsinization. HeLa cells and WBC cells were stained with trypan blue. Cell counts were adjusted to 1×10^3 cell/well and seeded in 96-well plates containing DMEM medium for HeLa and RPMI medium for WBC cells and then incubated for 24 hours in an incubator. After 24 hours of incubation, the HeLa and WBC cells were treated with various concentrations of *C. gigantea* fractions and then incubated for an additional 48 hours. *C. gigantea* fraction-free plates containing DMEM for HeLa and RPMI for WBC were used as a negative control. Doxorubicin (Adriamycin), an effective anticancer drug, was used as a positive control. PrestoBlue™ Cell Viability Reagent was used as a cell viability indicator after treatment (48-hour incubation). 10 µl of PrestoBlue™ Cell Viability Reagent was added to each well and then incubated for 30 minutes. Cytotoxicity was determined by measurement of the fluorescence of the converted PrestoBlue™ Cell Viability Reagent at a wavelength of 590 nm in a microplate reader (BioTeK Synergy HT, Winooski, VT, USA). Percent (%) of cell viability was calculated from:

$$\text{Cell viability (\%)} = \frac{\text{Treated cells}}{\text{Negative control}} \times 100$$

Statistical analysis

All experiments were conducted as three independent replicates. The experimental data are shown in terms of both mean and standard deviation. The data were analyzed using Microsoft Excel software (Microsoft Software Inc., Redmond WA, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA).

Results

Screening of crude n-hexane, dichloromethane, and methanol fractions for cytotoxicity

The objective of the initial experiment was to screen the crude n-C₆H₁₄, CH₂Cl₂, and CH₃OH fractions of tuber and leaf parts of *C. gigantea* for cytotoxicity. The concentrations of each fraction were 62.5, 125, 250, 500, and 1,000 µg/ml. As shown in Table 1, only fraction 1T from the tuber part of *C. gigantea* exhibited cytotoxicity on HeLa cells. The half maximal inhibitory concentration value (IC₅₀) was 585 µg/ml for HeLa cells. Fig. 2 describes how increasing concentrations of fraction 1T resulted in a dramatic decrease in HeLa cell viability. The IC₅₀ value of doxorubicin on HeLa cells was 7.03 µM, which is equivalent to 3.82 µg/ml. Fraction 1T from the tuber part not only expressed cytotoxicity on HeLa cells, but also significantly stimulated WBC

Table 1. Cytotoxicity of the crude fractions on HeLa and WBC cells *in vitro*

Fractions	IC ₅₀ (µg/ml)	
	HeLa	WBC
1T	585	– ^a
2T	–	–
3T	–	–
1L	–	–
2L	–	–
3L	–	–
Doxorubicin	3.82	–

Fraction cytotoxicity is considered statistically significant if >1,000 µg/ml and doxorubin cytotoxicity is considered statistically significant if >5.43 µg/ml.

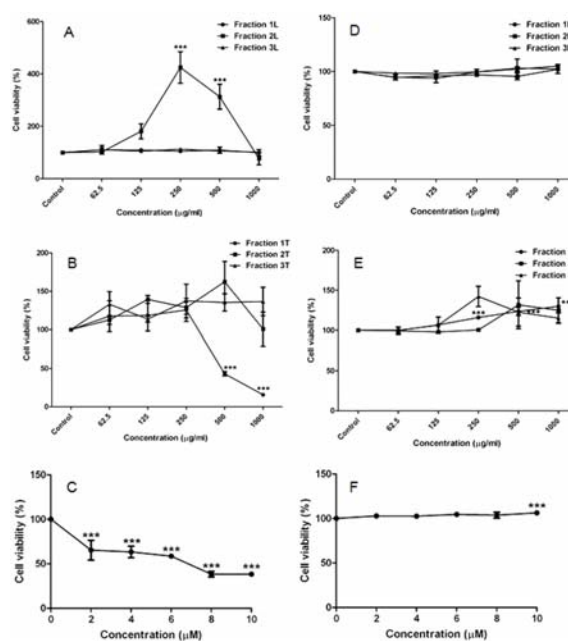


Fig. 2 Screening of crude n-C₆H₁₄, CH₂Cl₂, and CH₃OH fractions for cytotoxicity *in vitro*: (A) Growth inhibition of leaf part fractions on HeLa cells, (B) growth inhibition of tuber part fractions on HeLa cells, (C) growth inhibition of doxorubicin on HeLa cells, (D) growth inhibition of leaf part fractions on WBC cells, (E) growth inhibition of tuber part fractions on WBC cells, (F) growth inhibition of doxorubicin on WBC cells (the triple asterisk (***) indicates a significant difference from the control $p < 0.001$, one-way ANOVA, Dunett's test) Results are mean values \pm SD of independent experiments performed in triplicate.

cell proliferation. Inexplicably, fraction 2L from the leaf part stimulated Hela cell proliferation very significantly (Fig. 2). Hela cell proliferation varied according to fraction 2L concentration, with the highest level of cell proliferation occurring at 250 µg/ml. The IC₅₀ values of all fractions and doxorubicin on WBC cells were more than 1,000 µg/ml and 10 µM (equivalent to 5.43 µg/ml), respectively. From the experimental results, fraction 1T was selected for further investigation.

Bioassay-guided fractionation of the fraction 1T

Fraction 1T was loaded into column chromatography, which was packed with silica gel and eluted sequentially with n-C₆H₁₄ (100), followed by CH₂Cl₂: C₄H₈O₂ (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, and 0:100), to yield four fractions (4T, 5T, 6T and 7T). Each fraction was once again subjected to cytotoxicity testing to observe the most active fraction. As described in Table 2, fraction 5T (CH₂Cl₂: C₄H₈O₂; 80:20) was associated with the lowest IC₅₀ value (IC₅₀ = 93.0 µg/ml). Fig. 3 further illustrates that IC₅₀ values for all fractions exceeded 500 µg/ml with regard to WBC cells. Moreover, fraction 5T not only delivered the lowest IC₅₀ value with regard to Hela cells, but also stimulated significant WBC cell proliferation. Increasing concentrations of fraction 5T dramatically decreased Hela cell viability. Therefore, fraction 5T was chosen for further fractionation. Fraction 5T was then subjected to column chromatography on silica gel eluted with n-C₆H₁₄: CH₂Cl₂: C₄H₈O₂ (30:65:5) to yield four fractions (8T, 9T, 10T and 11T). Once again, a descriptive outline of the fractionation process is illustrated in Fig. 1. Additionally, Fig. 4 pictorially describes Hela cell morphology resulting from treatment with fraction 5T.

The bioactive ingredients in fractions 8T, 9T, 10T and 11T, which were fractionated from fraction 5T, were analyzed by GC-Mass spectroscopy and the analyzed results are shown in Table 3. Analysis of the

chromatograms showed that the most commonly occurring and the highest peak height of expected bioactive compounds found in fractions 8T, 9T, 10T and 11T belonged to 4,22-Stigmastadiene-3-one,

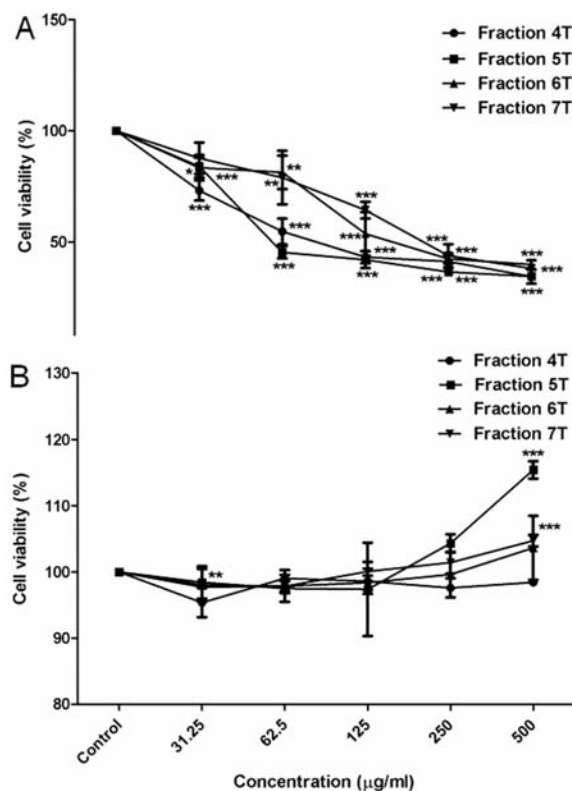


Fig. 3 Cytotoxicity of 4T, 5T, 6T and 7T fractions *in vitro*: (A) The cytotoxicity of each fraction on Hela cells; (B) The cytotoxicity of each fraction on WBC cells (the triple asterisk (***) indicates a significant difference from the control $p < 0.001$, one-way ANOVA, Dunett's test). Results are mean values \pm SD of independent experiments performed in triplicate.

Table 2. Cytotoxicity of 4 fractions fractionated from fraction 1T on Hela and WBC cells *in vitro*

Cell	IC ₅₀ (µg/ml)				
	Fraction 1T				Doxorubicin
	4T	5T	6T	7T	
Hela	104	93.0	166	210	3.82
WBC	- ^a	-	-	-	-

Fraction cytotoxicity is considered statistically significant if > 500 µg/ml and Doxorubin cytotoxicity is considered statistically significant if > 5.43 µg/ml

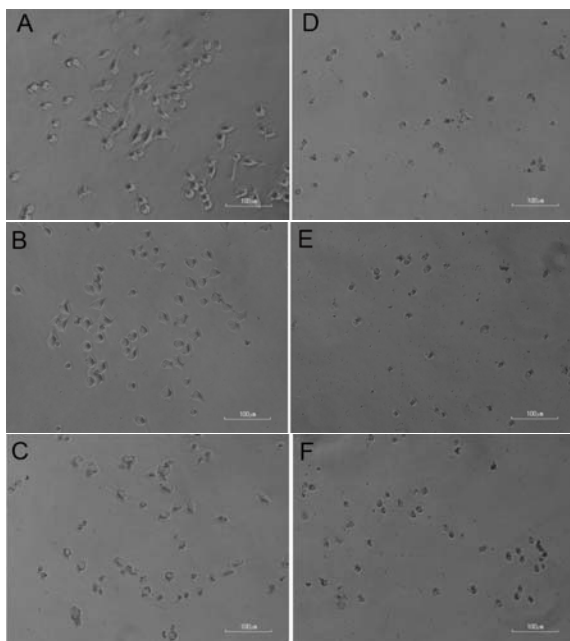


Fig. 4 HeLa cell morphology after being treated fraction 5T: (A) control; (B-F) HeLa cells treated with fraction 5T concentrations of 32.25, 61.5, 125, 250, and 500 µg/ml, respectively.

Diazoprogestosterone, 9-Octadecenoic acid (Z)-, hexyl ester, and Oleic Acid, respectively.

Discussion

C. esculenta has been reported to contain several biologically active ingredients and has been used as a food and a traditional medicinal plant⁽¹⁴⁾. Although, there are many reports that highlight the biological activities of the phytochemicals in *C. esculenta*, there are few reports on *C. gigantea*'s biological activities and its bioactive ingredients. This study is the first to report the cytotoxic activity and the suspected bioactive ingredients of some *C. gigantea* fractions on HeLa cells. The results showed that not all parts of *C. gigantea* demonstrated cytotoxicity on HeLa cells. In fact and paradoxically, some *C. gigantea* leaf part fractions promoted significant HeLa cell proliferation. Similarly, Wei et al (2011)⁽¹⁶⁾ reported that extract from the leaf part of *C. esculenta* encouraged human breast adenocarcinoma (MCF-7) proliferation. Accordingly, cancer patients should perhaps avoid reasonable precautions against the consumption of *C. gigantea* leaf parts as a food ingredient. *C. gigantea* fraction 1T, which comes from the tuber portion of the plant, demonstrates two different pathways for HeLa cell inhibition. First, it directly restrains HeLa cell

proliferation by the effects of its bioactive ingredients. Second, fraction 1T encourages the immune system by acting as a mitogen. Ohno et al (1994)⁽¹⁷⁾ reported that mitogen activated lymphocytes can kill numerous types of colon cancer cells in humans and in rodents. To demonstrate that the cytotoxicity of the bioactive ingredients in this case is specific to HeLa cells, but is safe for human white blood cells, fraction 1T from the tuber part of *C. gigantea* stimulated WBC cell proliferation significantly. The experimental results on WBC cells in the present study were similar to the results reported by Brown et al (2005)⁽⁵⁾, in which normal mouse splenocyte cells were treated with *C. esculenta* extract. Brown et al suggested that *C. esculenta* contains an endogenous mitogen and a mannose-binding lectin, similar to *Phaseolus vulgaris*, that activates lymphocytes^(18,19). Some lectins can induce lymphocyte proliferation through interleukin-2 production. Lymphocytes incubated 1-2 days with a high dose of interleukin-2 induced normal lymphocytes to become lymphokine-activated killer cells (LAK). LAK cells have a non-specific tumoricidal activity that kills various types of cancer cells⁽¹⁷⁾.

The main idea of bioactive ingredients in anti-cancer property *in vitro* is that it acts on non-nutritive plant compounds⁽⁵⁾. Bioassay-guided fractionation was the method used for the identification of active ingredients. Column chromatography was used for the fractionation of bioactive ingredients. TLC and GC-Mass spectroscopy were used to observe various components contained within each fraction.

4,22-Stigmastadiene-3-one and Diazoprogestosterone can be classified as a phytosterol. There have been many reports regarding the anticancer activities of phytosterol^(20,21). Phytosterol can act against carcinogenesis by various mechanisms. The authors expected that phytosterol in *C. gigantea* might be a bioactive ingredient that plays an important role as an anticancer compound. In 2004, Awad et al⁽²²⁾ reported on mechanisms of phytosterol acting on cancer cells. Phytosterol suppressed cancer development by reducing the production of carcinogens. In 2005, Vivancos and Moreno⁽²³⁾ reported that phytosterol increased the activities of antioxidant enzymes, superoxide dismutase, and glutathione peroxidase in cultured macrophages. They suggested that phytosterol prevented cells from damage by reactive oxygen species. Phytosterol also induces apoptosis in cancer cells, an important mechanism in the inhibition of carcinogenesis⁽²⁰⁾. Park et al (2007)⁽²⁴⁾, Moon et al (2007)⁽²⁵⁾, and Rubis et al (2008)⁽²⁶⁾ published the

Table 3. The chemical ingredients of fractions 8T and 9T that were isolated from fraction 5T and analyzed by GC-Mass spectroscopy

No.	Rate (sec)	Molecular Formula	%	Name
Fraction 8T				
1	150.9	C ₆ H ₁₂ O	1.34	Furan, tetrahydro-2,5-dimethyl-
2	183.75	C ₃ H ₈ O ₂	39.08	(S)-(+)-1,2-Propanediol
3	226.95	C ₅ H ₈ O ₂	1.23	1-Propen-2-ol, acetate
4	240.75	C ₆ H ₁₂ O	3.49	Hexanal
5	1160.75	C ₁₀ H ₁₈ O ₃	1.46	Nonanoic acid, 9-oxo-, methyl ester
6	1627.7	C ₆ H ₁₂ O	1.31	Pentanal, 2-methyl-
7	1868.8	C ₇ H ₁₇ NO	0.74	Dimethylamine, N-(neopentyloxy)-
8	2022.7	C ₁₈ H ₃₄ O ₂	3.90	Oleic Acid
9	2037.1	C ₁₉ H ₃₇ MoNO ₃ P ₂	0.95	Molybdenum, tricarbonyl-[N-butyl-bis [2-(butylphosphino)ethyl] amine]
10	2099.8	C ₁₁ H ₁₉ ClO	4.74	10-Undecenoyl chloride
11	2240.1	C ₂₈ H ₄₆ O ₄	0.98	Phthalic acid, dodecyl 2-ethylhexyl ester
12	2276.3	C ₁₂ H ₂₂ N ₂ O ₄	6.63	N,N'-Bis(2-methyl-2-nitrosopentan-4-one)
13	2464.75	C ₁₀ H ₁₆ O ₂	3.04	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane,
14	2804.05	C ₂₆ H ₃₆ O ₈	2.41	1-methyl-5-(1-methylethyl)-, (1a,3a,5a,7a)-1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-9a-triacetate, [1aR-(1aa,1ba,4aa,5a,7aa,7ba,8a,9a,9aa)]-tetramethyl-, 5,9
15	2824.4	C ₂₉ H ₄₆ O	10.66	4,22-Stigmastadiene-3-one
16	2874.1	C ₂₁ H ₃₀ N ₄	5.15	Diazoprogesterone
Fraction 9T				
1	239.55	C ₇ H ₁₆	4.18	Hexane, 3-methyl-
2	241.05	C ₄ H ₁₀ N ₂	4.12	(2-Aziridinylethyl)amine
3	2022.45	C ₁₈ H ₃₄ O ₂	13.65	Oleic Acid
4	2718.2	C ₂₇ H ₃₈ O ₄ S ₂	18.17	2a,4a-Epoxymethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene)methyl-acetate 1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-
5	2736.7	C ₂₁ H ₃₀ N ₄	48.42	Diazoprogesterone

apoptosis mechanism of β -sitosterol and showed that it increased the activity of caspase-3 and, at the same time, deactivated the Bcl-2 pathway. Furthermore, Moon et al (2007)⁽²⁵⁾ explained the activation of caspase-3 showing that phytosterol could be mediated by extracellular signals that were complemented by mitochondrial pathways. Moreover, phytosterol also inhibited angiogenesis and metastasis in cancer cell proliferation⁽²⁷⁾. Choi et al (2007)⁽²⁸⁾ found basic fibroblast growth factor (bFGF)-induced angiogenesis in endothelial cells isolated from human umbilical veins and successfully reduced proliferation of the cells with campesterol treatment. The anticancer property of Oleic Acid was proposed by Menendez et al (2005)⁽²⁹⁾. They observed that Oleic Acid supported trastuzumab (Herceptin, an anticancer drug) when used in cancer

cell cultures. They suggested that Oleic Acid up-regulates polyomavirus enhancer activator 3 (PEA3), which suppressed the expression of human epidermal growth factor receptor 2 (HER2/neu), a cause of breast cancers. Therefore, high levels of PEA3 would result in lower levels of HER2/neu, thereby lowering the risk of cancer onset as a result of HER2/neu over-expression. Valko et al (2007)⁽³⁰⁾ and Sakthivel et al (2010)⁽³¹⁾ reported that Diazoprogesterone, 9-Octadecenoic acid (Z)-, hexyl ester, and Oleic Acid not only displayed anticancer activity, but also had antimicrobial, hypercholesterolaemic, and anti-ulcerogenic effects.

Conclusion

The potential anticancer properties of *C. gigantea* tuber parts had the combined effect of

Table 4. The chemical ingredients of fractions 10T and 11T that were isolated from fraction 5T and analyzed by GC-Mass spectroscopy

No.	Rate (sec)	Molecular Formula	%	Name
Fraction 10T				
1	150.7	C ₆ H ₁₂ O	2.66	Furan, tetrahydro-2,5-dimethyl-
2	226.45	C ₅ H ₈ O ₂	4.44	1-Propen-2-ol, acetate
3	239.55	C ₃₂ H ₄₅ NO ₃	10.36	3'H-Cycloprop(1,2) cholesta-1,4,6-trien-3- one, 1'-carboethoxy-1' -cyano-1a,2a-dihydro-
4	240.45	C ₆ H ₁₂ O	16.27	Hexanal
5	573.45	C ₆ H ₁₂ O ₂	0.65	Pentanoic acid, 2-methyl-
6	700.65	C ₁₂ H ₂₀ O ₄	0.76	Oxalic acid, allyl heptyl ester
7	728.75	C ₉ H ₁₈ O ₂	1.69	Octanoic acid, methyl ester
8	932.95	C ₇ H ₁₂ O	0.94	4-Pental, 2-ethyl-
9	1026	C ₉ H ₁₆ O ₃	1.42	Methyl 8-oxooctanoate
10	1040.35	C ₁₁ H ₂₀ O	1.01	2-n-Hexylcyclopentanone
11	1116	C ₉ H ₁₆ O ₂	4.78	4-Octenoic acid, methyl ester, (Z)-
12	1160.9	C ₁₀ H ₁₈ O ₃	9.82	Nonanoic acid, 9-oxo-, methyl ester
13	1627.7	C ₈ H ₁₆ O	1.71	2-Heptanone, 5-methyl-
14	1712.2	C ₈ H ₁₆ O ₂	0.96	2-Methylheptanoic acid
15	1869.95	C ₁₃ H ₂₆ O ₂	2.69	Decanoic acid, propyl ester
16	2023.7	C ₂₄ H ₄₆ O ₂	18.08	9-Octadecenoic acid (Z)-, hexyl ester
17	2047	C ₇ H ₁₇ NO	0.77	Dimethylamine, N-(neopentyl)-
18	2051.7	C ₉ H ₁₇ ClO	2.92	Nonanoyl chloride
19	2095.35	C ₁₀ H ₁₆ O ₂	1.44	Tetrahydrofuran-2-one, 3-[2-pentenyl]-4-methyl-
Fraction 11T				
1	151.15	C ₆ H ₁₂ O	1.40	Furan, tetrahydro-2,5-dimethyl-
2	536.55	C ₅ H ₁₀ O ₂	13.02	Pentanoic acid
3	1115.75	C ₇ H ₁₄ O ₂	2.69	Hexanoic acid, methyl ester
4	2022.7	C ₁₈ H ₃₄ O ₂	23.58	Oleic Acid
5	2050.4	C ₁₁ H ₂₀ O ₃	2.43	Decanoic acid, 2-oxo-, methyl ester
6	2240.15	C ₂₇ H ₃₈ O ₄ S ₂	17.86	2a,4a-Epoxymethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene) methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate

inhibiting cervical cancer Hela cell proliferation and stimulating human white blood cell growth. These experimental results may stimulate future research and *in vivo* mechanism studies on cell death. The results demonstrate that a fraction extracted from *C. gigantea* tuber parts (using n-hexane (n-C₆H₁₄) as a solvent) contains powerful bioactive ingredients with anticancer properties. With further study, these findings may contribute to the development of new-targeted cancer chemotherapy agents.

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Potential conflicts of interest

None.

References

- Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; 275: 218-20.
- Anand P, Kunnumakkara AB, Sundaram C,

- Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008; 25: 2097-116.
3. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; 61: 212-36.
 4. Attasara P, Buasom R. Hospital-Based Cancer Registry. Bangkok: Rumthai press; 2010.
 5. Brown AC, Reitzenstein JE, Liu J, Jadus MR. The anti-cancer effects of poi (*Colocasia esculenta*) on colonic adenocarcinoma cells In vitro. *Phytother Res* 2005; 19: 767-71.
 6. Reynertson KA, Charlson ME, Gudas LJ. Induction of murine embryonic stem cell differentiation by medicinal plant extracts. *Exp Cell Res* 2011; 317: 82-93.
 7. Bonham M, Arnold H, Montgomery B, Nelson PS. Molecular effects of the herbal compound PC-SPES: identification of activity pathways in prostate carcinoma. *Cancer Res* 2002; 62: 3920-4.
 8. Hu H, Ahn NS, Yang X, Lee YS, Kang KS. Ganoderma lucidum extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. *Int J Cancer* 2002; 102: 250-3.
 9. Lee SM, Li ML, Tse YC, Leung SC, Lee MM, Tsui SK, et al. Paeoniae Radix, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. *Life Sci* 2002; 71: 2267-77.
 10. El Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita K. Willow leaves' extracts contain anti-tumor agents effective against three cell types. *PLoS One* 2007; 2: e178.
 11. Nassr-Allah A, Aboul-Enein A, Aboul-Enein K, Lightfoot D, Cocchetto A, El-Shemy H. Anti-cancer and anti-oxidant activity of some Egyptian medicinal plants. *Journal of Medicinal Plants Research* 2009; 3: 799-808.
 12. Manner HI. Farm and forestry production and marketing profile for giant taro (*Alocasia macrorrhiza*). In: Elevitch CR, editor. Specialty crops for pacific island agroforestry [Internet]. Honolulu, Hawaii: Permanent Agriculture Resources (PAR); 2011 [cited 2011 Apr 12]. Available from: <http://agroforestry.net/scps>
 13. Kay DE. (revised by Gooding EGB). Crop and product digest No. 2-root crops. 2nd ed. London: Tropical Development and Research Institute; 1987.
 14. Essence of the Agriculture, Faculty of Natural Resources, Prince of Songkla University.
 15. Mitova M, Taskova R, Popov S, Berger RG, Krings U, Handjieva N. GC/MS analysis of some bioactive constituents from *Carthamus lanatus* L. *Z Naturforsch C* 2003; 58: 697-703.
 16. Wei LS, Wee W, Siong JY, Syamsumir DF. Antimicrobial, antioxidant, anticancer property and chemical composition of different parts (corm, stem and leave) of *Colocasia esculenta* extract. *Annales Universitatis Mariae Curie-Sklodowska Lublin-Polonia* 2011; XXIV: 1-8.
 17. Ohno S, Tanaka N, Hizuta A, Miyagi K, Orita K. Lectin-activated killer cells rapidly induced by pokeweed mitogen conjugated beads and their in vivo antitumor effects. *Int J Immunopharmacol* 1994; 16: 761-8.
 18. Bezerra IC, Castro LA, Neshich G, de Almeida ER, de Sa MF, Mello LV, et al. A corm-specific gene encodes tarin, a major globulin of taro (*Colocasia esculenta* L. Schott). *Plant Mol Biol* 1995; 28: 137-44.
 19. Shewry PR. Tuber storage proteins. *Ann Bot* 2003; 91: 755-69.
 20. Woyengo TA, Ramprasath VR, Jones PJ. Anticancer effects of phytosterols. *Eur J Clin Nutr* 2009; 63: 813-20.
 21. Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. *J Nutr* 2000; 130: 2127-30.
 22. Awad AB, Toczek J, Fink CS. Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. *Prostaglandins Leukot Essent Fatty Acids* 2004; 70: 511-20.
 23. Vivancos M, Moreno JJ. beta-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. *Free Radic Biol Med* 2005; 39: 91-7.
 24. Park C, Moon DO, Rhu CH, Choi BT, Lee WH, Kim GY, et al. Beta-sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. *Biol Pharm Bull* 2007; 30: 1317-23.
 25. Moon DO, Lee KJ, Choi YH, Kim GY. Beta-sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. *Int Immunopharmacol* 2007; 7: 1044-53.
 26. Rubis B, Paszel A, Kaczmarek M, Rudzinska M, Jelen H, Rybczynska M. Beneficial or harmful *Colocasia gigantea* used in folk medicine [Internet]. 2006 [cited 2012 Apr 11]. Available from: http://natres.psu.ac.th/radio/radio_article/radio48-49/48-490043.htm

- influence of phytosterols on human cells? Br J Nutr 2008; 100: 1183-91.
27. Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest 2000; 105: 1511-3.
 28. Choi JM, Lee EO, Lee HJ, Kim KH, Ahn KS, Shim BS, et al. Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities. Phytother Res 2007; 21: 954-9.
 29. Menendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer cells with Her-2/neu oncogene amplification. Ann Oncol 2005; 16: 359-71.
 30. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.
 31. Sakthivel K, Palani S, Kalash RS, Devi K, Kumar BS. Phytoconstituents analysis by GC-MS, cardioprotective and antioxidant activity of *Buchanania axillaris* against Doxorubicin-Induced cardio toxicity in albino rats. Int J Pharmaceut Sci Res 2010; I: 34-48.

ฤทธิ์ต้านมะเร็งของส่วนสกัดที่ถูกคัดเลือกจาก *COLOCASIA GIGANTEA*

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งานวิจัยนี้มีวัตถุประสงค์ที่จะตรวจสอบศักยภาพในการต่อต้านมะเร็งของสารสกัดจากต้นบอนคนุน (*Colocasia gigantea*) ซึ่งอยู่ในวงศ์ Araceae ในการศึกษาได้มีการตรวจสอบฤทธิ์ความเป็นพิษต่อเซลล์ของสารสกัดจากต้นบอนคนุนโดยใช้เซลล์มะเร็งปากมดลูก (Hela) และเซลล์เม็ดเลือดขาวในหลอดทดลอง อีกทั้งตรวจสอบสารออกฤทธิ์ทางชีวภาพในสารสกัดจากต้นบอนคนุนซึ่งมีความเป็นพิษต่อเซลล์ด้วยวิธี bioassay-guided fractionation ผลการทดลองแสดงให้เห็นว่าไม่ใช้สารสกัดจากต้นบอนคนุนทุกส่วนที่ก่อให้เกิดความเป็นพิษต่อเซลล์มะเร็งปากมดลูก โดยสารสกัดที่ได้จากส่วนของใบซึ่งใช้ไดคลอโรมีเทนเป็นตัวทำละลายสามารถเร่งการเจริญเติบโตของเซลล์มะเร็งปากมดลูกแต่ไม่เร่งการเจริญเติบโตของเซลล์เม็ดเลือดขาว ในขณะที่สารสกัดซึ่งได้จากส่วนของลำต้นใต้ดินโดยใช้เฮกเซนเป็นตัวทำละลาย (Fr.1T) มีความเป็นพิษต่อเซลล์มะเร็งปากมดลูก ($IC_{50} = 585$ ไมโครกรัมต่อมิลลิกรัม) แต่เร่งการเจริญเติบโตของเซลล์เม็ดเลือดขาว ผลการวิเคราะห์ด้วยเทคนิค GC-MS พบว่าสารสกัด Fr.1T ประกอบไปด้วย 4,22-Stigmastadiene-3-one, Diazoprogerone, 9-Octadecenoic acid (Z)-, hexyl ester และ Oleic acid ซึ่งคาดว่าจะมีฤทธิ์ความเป็นพิษต่อเซลล์ดังนั้นจึงสรุปว่าสารสกัด Fr.1T จากต้นบอนคนุนมีศักยภาพที่จะใช้รักษามะเร็งปากมดลูกได้
