



Research Article

Anticancer Activities of *Phlogacanthus pulcherrimus* Leaf Extracts on HeLa Cancer Cells: *In vitro* study

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Abstract

Background and Objective: Medicinal plants have gained considerable attention and are widely used as alternative traditional medicines for the treatment of cancer. *Phlogacanthus pulcherrimus* (T. Anderson) is a plant that grows in various regions of Thailand. Therefore, the goal of the present study was to investigate the mechanism by which the ethanolic PP leaf extract affected HeLa cancer cell proliferation. Fast dissolving tablet (FDTs) formulations containing the extract were developed. **Materials and Methods:** The anticancer activities of the PP leaf extract were determined using specific assays assessing cell cytotoxicity, colony formation, cell migration, reactive oxygen species (ROS) production and cell apoptosis. The experiments were performed using HeLa cervical cancer cells. The FDTs were formulated using a direct compression method. **Results:** The PP extract was cytotoxic on HeLa cells, with half-maximal inhibitory concentration (IC₅₀) values of 20.9±3.6 and 7.3±1.7 µg mL⁻¹ following 48 and 72 hrs of incubation, respectively. Moreover, the extract inhibited the ability of HeLa cells to form cell colonies with an IC₅₀ value of 26.0±3.5 µg mL⁻¹. The extract inhibited the migratory ability of HeLa cells, with a significant effect at 25 µg mL⁻¹. It also induces the production of ROS and cell death in a dose-dependent manner at a concentration of 50-250 µg mL⁻¹. The FDTs containing the PP extract were successfully developed and indicated optimal physical properties. **Conclusion:** These findings suggest that the PP extract exhibits anticancer properties on HeLa cells, which may be used to prevent and treat cervical cancer.

Key words: *Phlogacanthus pulcherrimus*, apoptosis, colony formation, migration suppression, ROS formation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cervical cancer is the fourth most common cancer found in women worldwide. In 2018, >50% of women who were diagnosed with cervical cancer, succumbed to the disease¹. The failure to respond to cancer treatment due to side effects and drug resistance of chemotherapy has caused the development of plant-derived chemotherapeutic agents as an alternative treatment for cancer². *Phlogacanthus pulcherrimus* (T. Anderson) is a member of the *Acanthaceae* family³. In the northern and northeastern regions of Thailand, the leaves of *P. pulcherrimus* (PP) are mostly consumed fresh. In traditional medicine, an aerial part of PP has been used as a diuretic, while the leaves of PP have been used as nutritional supplements and as a tonic^{3,4}. The dichloromethane extract of the PP leaves has been investigated for its cytotoxicity against breast cancer (MCF-7), cervical cancer (HeLa), colon cancer (HT29), liver cancer (HepG2) and oral cancer (KB) cell lines⁴. The 50% inhibitory concentration (IC_{50}) values reported were 45.3, 81.5, 271.7, 124.9 and 105.1 $\mu\text{g mL}^{-1}$, respectively⁴. Based on these findings, the extract could be used as an anticancer agent. However, limited data are available on the anticancer activities of PP leaves. Compared with the dichloromethane extract, an ethanolic extract may be safer to be used in patients with cancer. Previous study examined the anticancer activities of the ethanolic PP leaf extract against MCF-7 cells⁵. The extract induced the cell death via apoptosis with an IC_{50} value of $51.3 \pm 4.7 \mu\text{g mL}^{-1}$. It also inhibited colony formation and migration of MCF-7 cancer cells. In addition, the extract could induce the production of reactive oxygen species (ROS). The ethanolic leaf extract of PP has not been previously examined for its ability to limit the survival of cervical HeLa cells. In addition, cell cytotoxicity, induction of cell apoptosis and ROS production should be investigated as possible modes of action of this extract⁶. Colony formation and cell migration of HeLa cells were also suppressed by this plant extract. Excessive ROS production has been confirmed to be the cause of cell apoptosis. Therefore, chemotherapeutic agents can be used to produce ROS in cancer cells⁷. Fast dissolving tablets (FDTs) or dispersible tablets are designed to dissolve the active substance in saliva within a few seconds. Therefore, a faster rate of tablet dissolution may lead to a faster rate of drug absorption, resulting in rapid onset of action. The FDTs usually consist of an active ingredient having high solubility or porous structure resulting in rapid disintegration in the mouth following oral administration^{8,9}. The present investigation aimed to assess the cytotoxicity of the PP ethanolic leaf extract on HeLa cervical cancer cell lines. Inhibition of colony formation and cell migration, as well as the induction of ROS generation and cell death, were also studied to determine the

underlying mechanism of action of this medicinal herb. The FDTs containing the extract was subsequently formulated and characterized.

MATERIALS AND METHODS

Study area: The study was carried out at the Laboratory of Pharmaceutical Technology, School of Pharmaceutical Sciences, University of Phayao, Phayao and the Laboratory of Pharmacology, Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand, from December, 2020 to June, 2021.

Materials: Corn starch, croscarmellose sodium (Ac-Di-Sol®), sodium starch glycolate (Explotab®), lactose, magnesium stearate, microcrystalline cellulose and talcum were obtained from Union Science Co. Ltd. (Chiang Mai, Thailand). Crospovidone (Kollidol CL®) was purchased from Chanjao Longevity Co. Ltd. (Bangkok, Thailand).

Preparation and phytochemical screening of the extracts:

The PP specimen (voucher No. MSUT_7390) was retained in the Faculty of Science, Mahasarakham University. An ethanolic PP extract was prepared using the maceration method. Phytochemical screening of extracts was evaluated using specific chemical reactions for secondary metabolites, including flavonoids, alkaloids, tannins, cyanogenic glycosides, saponins, anthraquinones, terpenes and steroids. The positive results of the screening tests were detected with the intensity of the colour or the formation of the precipitate.

Cell viability assay: The effects of PP on HeLa cell viability was assessed using the sulforhodamine B (SRB) assay¹⁰. Briefly, the cellular protein content was measured to represent the cellular density. HeLa cells were exposed to the extract ($0-500 \mu\text{g mL}^{-1}$) for 24-72 hrs. The percentage of HeLa cell viability was determined following comparison with the non-treated control groups and the dose-response curve was used to calculate IC_{50} .

Colony formation assay: To determine the colony formation inhibition of the extract, viable cells (500 cells/well) were seeded in a 6-well plate, incubated for 24 hrs and finally incubated with the extract (100 μL) at a concentration range of $0-500 \mu\text{g mL}^{-1}$. Subsequently, the cells were incubated for 24 hrs washed and the new culture medium was added. The cells were subsequently grown for an additional 10 days. The culture medium was changed every 2 days. Finally, crystal violet was dissolved in methanol (0.5%) and used to stain the cells. The number of colonies was determined by a direct counting method.

Cell migration assay: The cells (2.5×10^5 cells) were plated in a 24-well plate and incubated for 24 hrs at 37°C . Subsequently, the cells in each well were scratched with a sterile pipette tip to make a straight wound. The extract solution ($100 \mu\text{L}$) was used at a concentration range of $0\text{-}100 \mu\text{g mL}^{-1}$ and was incubated with the cells for 48 hrs. Finally, an inverted microscope was used to capture the area of the wound that was uncovered (TS100, Nikon, Japan). The area data were used to compute the percentage of the relative scratch closure, which represented the influence of PP on the suppression of cell migration.

ROS production assay: The effects of PP on ROS production were evaluated using the fluorescent probe 2',7' dichlorodihydrofluorescein diacetate (DCF-DA)¹¹. Briefly, the cells (2.5×10^5 cells) were treated with the extract at doses ranging from $50\text{-}250 \mu\text{g mL}^{-1}$, as well as with 25 mM DCF-DA . The treated cells were incubated for 30 min in the dark and subsequently washed with PBS. Flow cytometry was used to measure the ROS levels (BD Biosciences, CA, USA). Increased ROS levels were determined by a shift of the signal to the right.

Apoptosis assay: The effect of PP on cell apoptosis was determined using flow cytometry. In brief, 2.5×10^5 cells/well were seeded on 6-well plates and subsequently treated with the extracts at a concentration range of $50\text{-}250 \mu\text{g mL}^{-1}$. Following incubation for 24 hrs, the cells were washed twice in PBS and subsequently collected in 0.25% trypsin-EDTA. The cells were further incubated with $100 \mu\text{L}$ binding buffer and mixed with $5 \mu\text{L}$ Annexin V-FITC and $1.5 \mu\text{L}$ propidium iodide solution (Cat No. 558547, BD Biosciences, CA, USA). An additional incubation was performed for 15 min at room temperature in the dark. Finally, viable, early apoptotic, late apoptotic and necrotic cells were measured using a flow cytometer. The data were analyzed by BD Accuri C6 Plus software.

Preparation and characterization of the FDTs: In the present study, the FDTs were prepared using a direct compression method. Before tablet compression, the powder mixtures were formulated. The compositions of the formulations are illustrated in Table 1. The powder formulation consisted of four types of disintegrates including corn starch, Explotab®, Ac-Di-Sol® and Kollidol CL®. D-mannitol and microcrystalline cellulose acted as diluents, while magnesium stearate and talcum served as lubricants. The eight formulations were denoted as follows: FT-1, FT-2, FT-3, FT-4, FT-5, FT-6, FT-7 and

Table 1: Composition of the formulated fast dissolving tablet

Compositions	Composition weight (mg/tablet)							
	FT-1	FT-2	FT-3	FT-4	FT-5	FT-6	FT-7	FT-8
Extract	-	-	-	-	0.50	0.50	0.50	0.50
D-mannitol	125.25							
Corn starch	41.75	-	-	-	41.75	-	-	-
Explotab®	-	41.75	-	-	-	41.75	-	-
Ac-Di-Sol®	-	-	41.75	-	-	-	41.75	-
Kollidol CL®	-	-	-	41.75	-	-	-	41.75
Mg. stearate				0.0040				
Talcum				0.0012				
MCC ad to				500.0				

The extract: *P. pulcherrimus* extract, MCC: Microcrystalline cellulose, Mg.: Magnesium

FT-8. Subsequently, the properties of the powders were studied as follows: Bulk density, tapped density, compressibility index and Hausner's ratio using the following equation:

$$\text{Bulk density } (\rho_B) = \frac{\text{Weight of the powders}}{\text{Bulk volume of powders}} \quad (1)$$

$$\text{Tapped density } (\rho_T) = \frac{\text{Weight of the powders}}{\text{Tapped volume of powders}} \quad (2)$$

$$\text{Compressibility index } (\%) = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \quad (3)$$

$$\text{Hausner's ratio} = \frac{\rho_T}{\rho_B} \quad (4)$$

The angle of repose was determined by allowing the powders to flow through the funnel freely and enter its bottom. The height and radius of the powder pile were measured. The angle of repose of the powder was calculated using the following equation:

$$\tan \theta = \frac{h}{r} \quad (5)$$

Where:

θ = Angle of repose

h = Height of the powder pile (cm)

r = Radius of the powder pile (cm)

Tablet compression: A hydraulic press (PerkinElmer, IL, USA) was used to compress eight powder formulations used in the FDT formulation. A round, flat-faced punch with a diameter of 12.70 mm was used at a compaction force of 1.5 kN . The obtained tablets were further evaluated for their properties

concerning thickness and hardness (Erweka TBH 220 TD, Erweka, Heusenstamm, Germany), weight variation (ME204E, Mettler Toledo, Pune, India) and disintegration time (DT) (Erweka ZT322, Erweka, Heusenstamm, Germany). All measurements were performed according to the USP guidelines¹². In addition, the wetting time (WT) of the formulated FDTs was evaluated as follows: A tablet was placed in a Petri dish with a diameter of 12 cm containing distilled water (10 mL). The time required for complete wetting of the tablet was measured.

Statistical analysis: All analyses were carried out using Sigma Stat software version 3.5. The data were analyzed using one-way ANOVA with a *post hoc* least significant difference test. The $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Phytochemical profiles of the extracts: The phytochemical constituent analysis indicated that flavonoids, alkaloids,

tannins, cyanogenic glycosides and saponins were present in the PP, while anthraquinones, terpenes and steroids were absent.

Effect of PP extract on cell cytotoxicity: The effect of the extracts on the viability of HeLa cancer cells was examined using the SRB test. The results indicated that the PP extract inhibited HeLa cell viability at 24, 48 and 72 hrs (Fig. 1a-c). After 24 hrs of incubation, HeLa cell death was increased following the increase in the treatment dose of the PP extract (0-500 $\mu\text{g mL}^{-1}$) as shown in Fig. 1a. Also, the cell death was increased with the extension of the incubation time as shown in Fig. 1b for 48 hrs and Fig. 1c for 72 hrs. Compared to the control group, the PP extract significantly reduced the number of HeLa cells at a concentration value of 25, 10 and 10 $\mu\text{g mL}^{-1}$ after 24, 48 and 72 hrs of incubation, respectively. As shown in Fig. 1d, the IC_{50} values of the extract were 50.3 ± 6.8 , 20.9 ± 3.6 and 7.3 ± 1.7 $\mu\text{g mL}^{-1}$ following 24, 48 and 72 hrs of incubation, respectively. The results were expressed as a maximum effect on HeLa cell viability following treatment with the maximal extract concentration (E_{max})

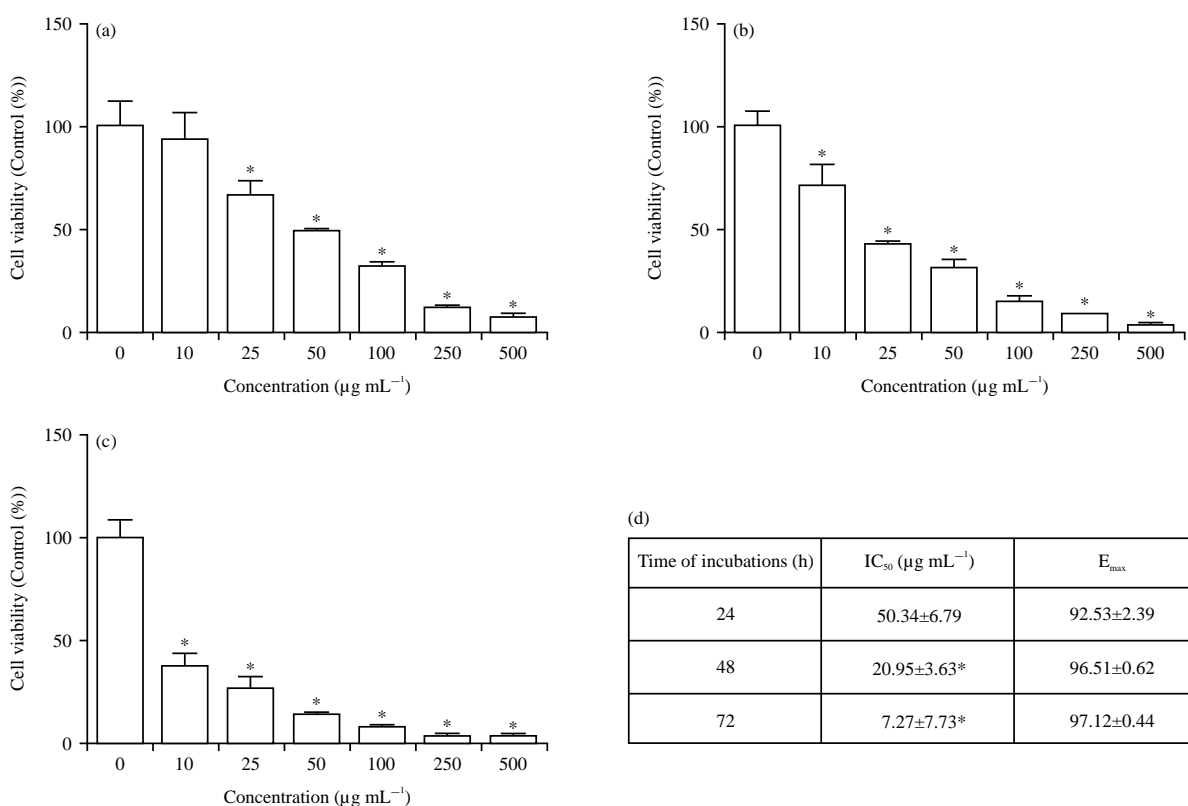


Fig. 1(a-d): Cytotoxicity of *Phlogacanthus pulcherrimus* extract on HeLa cell viability, (a) Cells were treated with the extract (0–500 $\mu\text{g mL}^{-1}$) for 24 hrs, (b) Cells were treated with the extract (0–500 $\mu\text{g mL}^{-1}$) for 48 hrs, (c) Cells were treated with the extract (0–500 $\mu\text{g mL}^{-1}$) for 72 hrs and (d) Results are expressed as a half-maximal inhibitory concentration (IC_{50}) and maximum effect on HeLa cell viability at maximal extract concentration (E_{max})

* $p < 0.05$ when compared with control

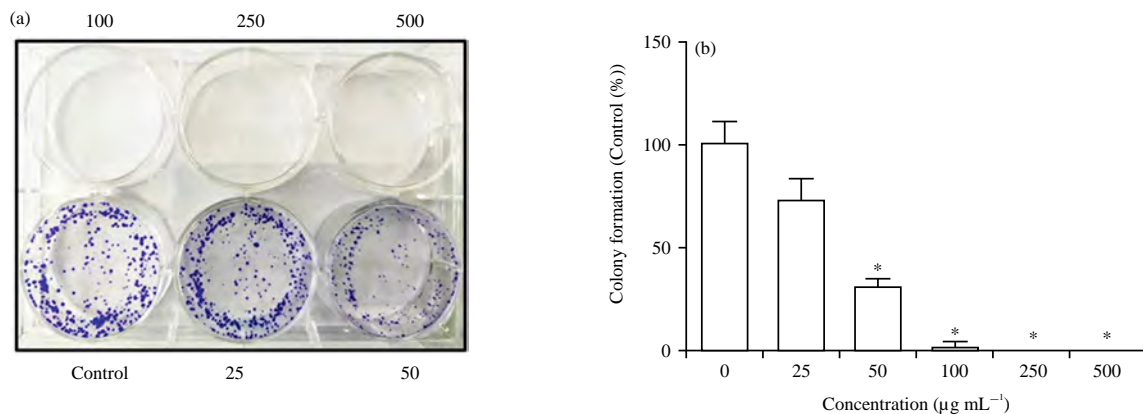


Fig.2(a-b): Effect of *Phlogacanthus pulcherrimus* extract on colony formation of HeLa cell, (a) Cells were then stained with crystal violet and photographed and (b) Colony formation percentage relative to the control
The cells were exposed to extracts of *Phlogacanthus pulcherrimus* with the concentration of 0-500 µg mL⁻¹ for 24 hrs, cultured for 10 days and the *p<0.05 when compared with the control

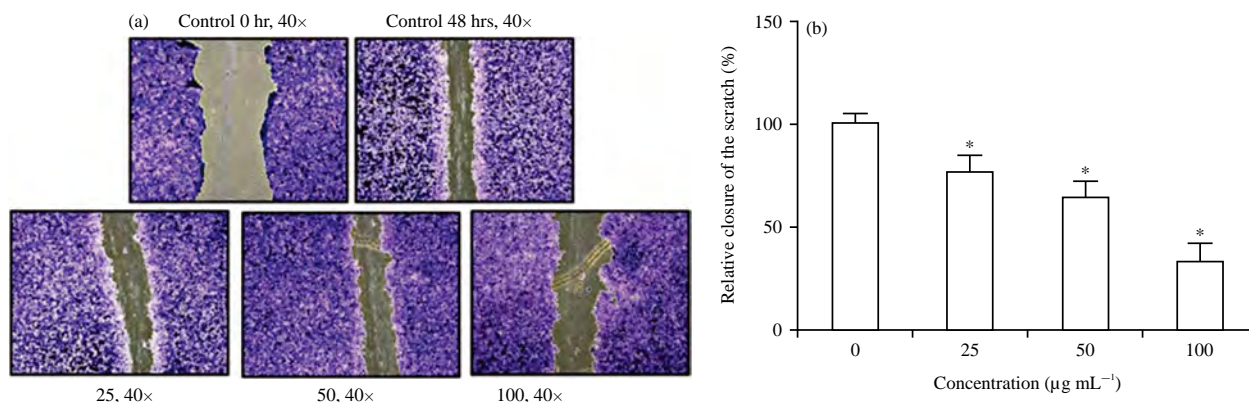


Fig. 3(a-b): Effect of *Phlogacanthus pulcherrimus* extract on the migration of HeLa cell, (a) Inverted microscopy (40×) and (b) Relative closure of scratch
The cells were exposed to the extract (0-100 µg mL⁻¹) for 48 hrs and migration was captured using inverted microscopy (40×). The results are expressed as a percentage of control groups with three independent experiments and represent Mean ± SEM values and *p<0.05 when compared with control

as shown in Fig. 1d. Following 24, 48 and 72 h of incubation, the E_{max} values of the extract were 92.5 ± 2.4, 96.5 ± 0.6 and 97.1 ± 0.4 µg mL⁻¹, respectively. The PP was considered cytotoxic on HeLa cells based on the National Institute guidelines, which define the criteria of crude extracts with IC₅₀ values <20 µg mL⁻¹. At that concentration, the extracts were considered active for decreasing cancer cell viability¹³.

Effect of PP extract on inhibition of colony formation: The inhibition of colony development of HeLa cancer cells caused by the extracts was carried out by the colony formation assay. The extract could inhibit colony formation at low concentrations. The present study indicated that treatment of HeLa cells by the extract suppressed cell colony development in a concentration-dependent manner in Fig. 2a. Treatment of the cells with 50 µg mL⁻¹ PP caused inhibition of HeLa cell

colony formation. The IC₅₀ value of the PP for the colony formation was 26.0 ± 3.5 µg mL⁻¹ in Fig. 2b.

Effects of the PP extract on inhibition of cell migration: The inhibitory effect of the PP extract on HeLa cell migration was determined by the scratch wound assay. The extract indicated significant effects on the suppression of HeLa cell migration at the concentration of 25 µg mL⁻¹. The IC₅₀ value of the PP extract for the suppression of HeLa cell migration was 73.0 ± 14.4 µg mL⁻¹ in Fig 3a and b.

Effect of PP extract on ROS production: An excess ROS production leads to cell cycle arrest or cell death. In cancer cells, the majority of the anticancer agents produce ROS⁷. The flow cytometry histograms of ROS were monitored and an increase in ROS levels was shown by the right shift in the

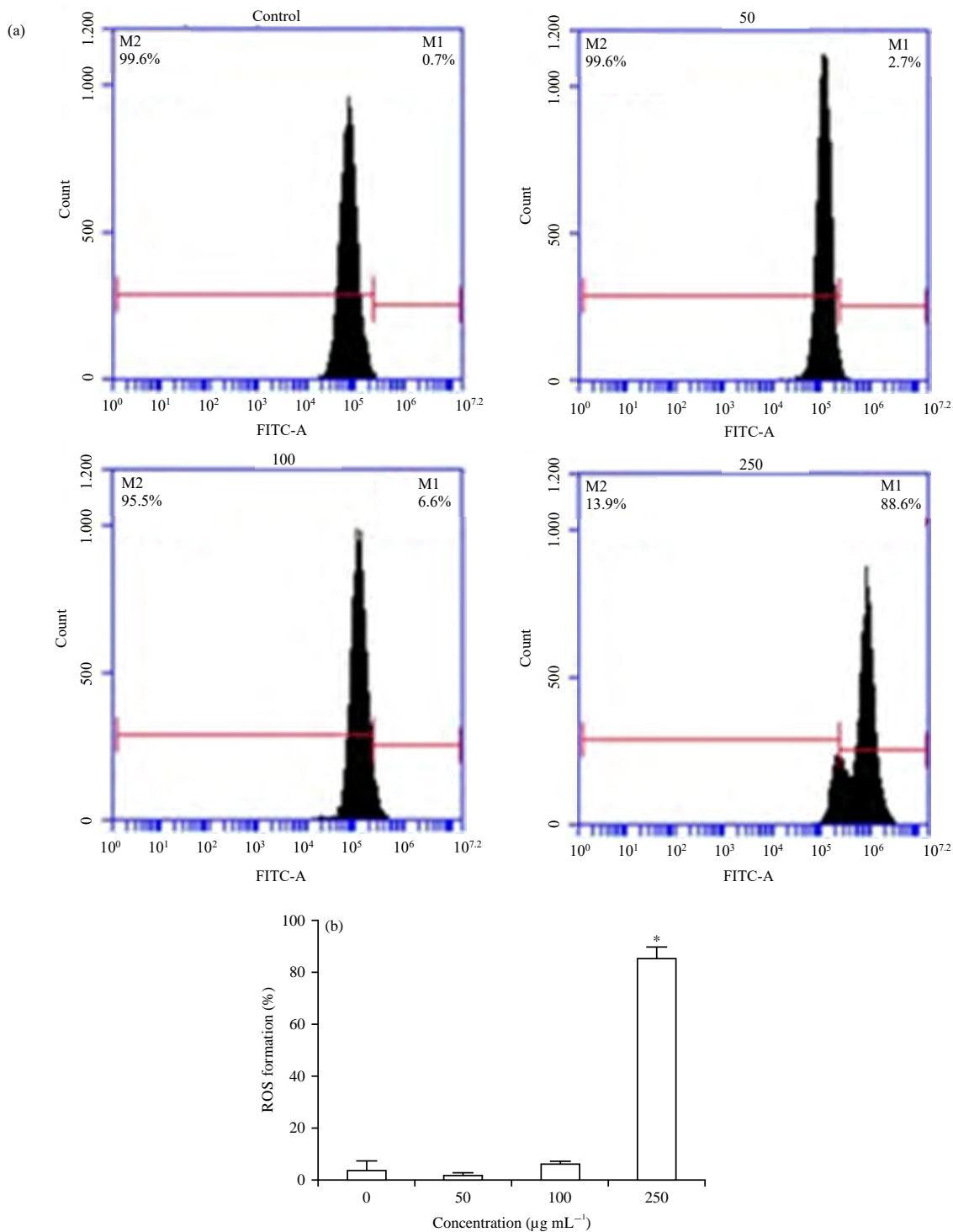


Fig. 4(a-b): Flow cytometry histograms of reactive oxygen species (ROS) of HeLa cells treated with *Phlogacanthus pulcherrimus* extract at concentrations of 0-250 $\mu\text{g mL}^{-1}$ (a) Flow cytometry histograms of ROS and (b) Percentage of ROS formation * $p < 0.05$ when compared with control

fluorescence signal of the DCF-DA¹¹. As shown in Fig. 4a, the results indicated that the PP induced ROS production at concentrations of 50, 100 and 250 $\mu\text{g mL}^{-1}$. The ROS values were 2.7 ± 0.5 , 7.2 ± 1.9 and $1.6 \pm 0.1\%$, respectively. The ROS

production was monitored following treatment of the cells with PP (concentration range of 50-250 $\mu\text{g mL}^{-1}$). Significant induction of ROS was shown following treatment of the cells with 250 $\mu\text{g mL}^{-1}$ PP extract in Fig. 4b.

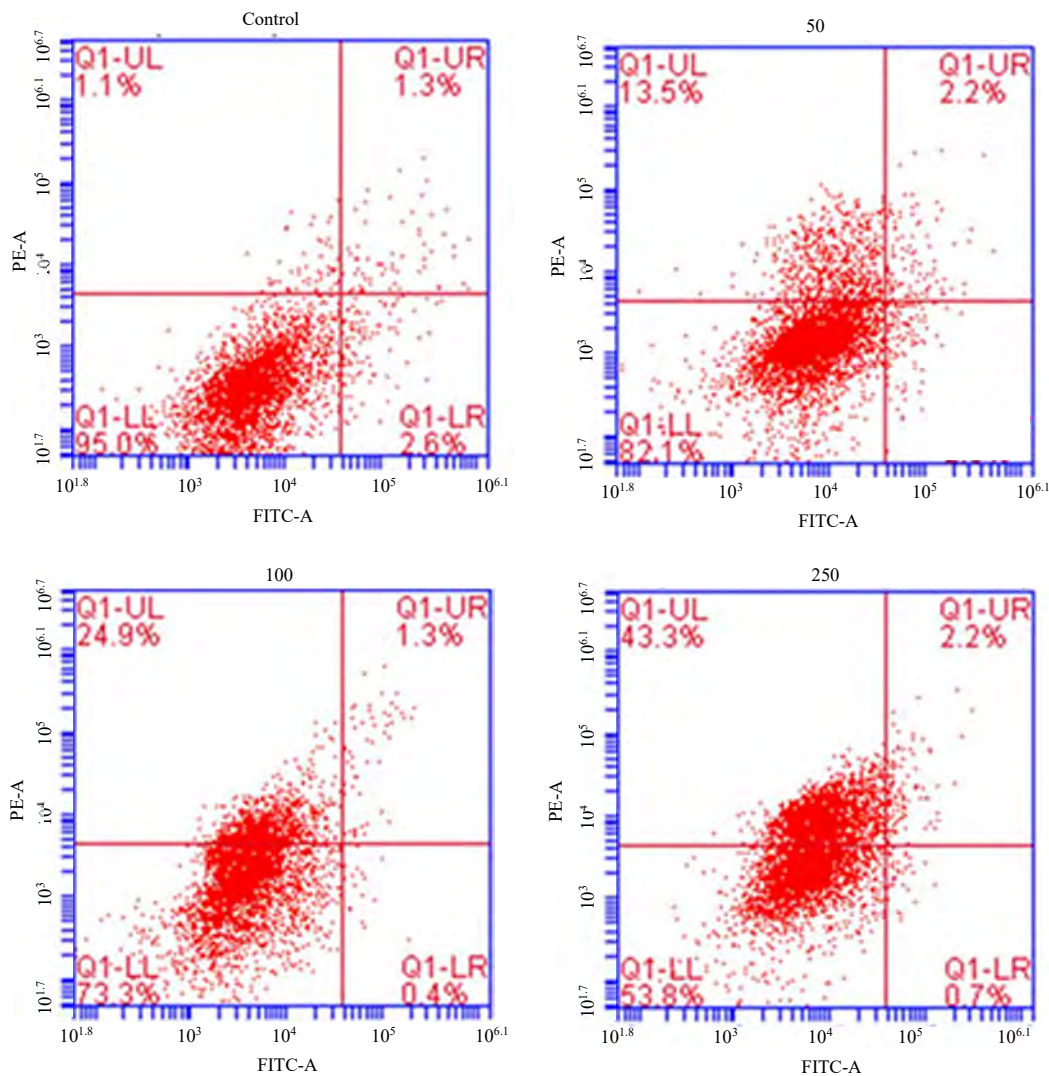


Fig.5: Flow cytometry histograms of cell apoptosis of HeLa cells treated with *Phlogacanthus pulcherrimus* extract at concentrations of 0-250 µg mL⁻¹

Effects of the PP extract on the induction of cell apoptosis:

The effects of PP on the induction of HeLa cell apoptosis are shown in Fig. 5. The data at the left lower, right lower, right upper and left upper quadrants to represent the percentage of viable, early apoptotic, late apoptotic and non-viable cells, respectively. As shown in Fig. 5, PP induced HeLa cell apoptosis in a dose-dependent manner. The percentages of late apoptotic HeLa cells treated with 0, 50, 100 and 250 µg mL⁻¹ extract were 1.3, 2.2, 1.3 and 2.2%, respectively.

Properties of the mixture containing the ingredient powders:

The evaluation parameters of the mixture powders are shown in Table 2. The mixture of the ingredient powders indicated poor flowability as determined by Hausner's ratio. The value was >1.25 and the Car's index was in the range of

18-29% for all the powder formulations except for FT-7, which demonstrated Hausner's ratio of 1.23. In addition, poor flowability of all blend powders was demonstrated with the angle of repose values, which were more than 30¹². According to the characteristics of the mixture of the ingredients, the formulation could improve its flow abilities by using the granulation method before tablet compression.

Physical parameters of the formulated FDT tablets:

The physical parameters of the formulated FDT tablets are shown in Table 3. The thickness of the flat surface FDT tablets was in the range of 2.76-3.05 mm, while the hardness of the FDTs was in the range of 7.47-13.67 KP. The results of the FDT hardness assessment demonstrated that this tablet was sufficiently strong to avoid friability during package and transport

Table 2: Evaluation parameters of the formulated mixture powders

Product code	Bulk density (g mL ⁻¹)	Tapped density (g mL ⁻¹)	Hausner's ratio	Carr's index (%)	Angle of repose (°)
FT-1	0.63±0.02	0.83±0.00	1.31±0.05	23.70±3.03	51.59±1.43
FT-2	0.60±0.02	0.81±0.02	1.35±0.02	25.53±1.24	49.90±1.85
FT-3	0.61±0.03	0.68±0.10	1.29±0.08	22.41±4.80	50.10±0.96
FT-4	0.55±0.03	0.78±0.01	1.43±0.08	29.57±4.07	48.04±1.59
FT-5	0.63±0.01	0.83±0.01	1.31±0.01	23.79±0.80	45.57±3.94
FT-6	0.63±0.02	0.81±0.01	1.28±0.04	22.14±2.29	49.23±1.80
FT-7	0.64±0.03	0.78±0.01	1.23±0.07	18.68±4.98	48.23±0.97
FT-8	0.53±0.02	0.77±0.01	1.39±0.07	28.12±3.71	49.64±0.00

Table 3: Physical parameters of the formulated FDTs tablet

Product code	Thickness (mm)	Hardness (KP)	Weight variation (mg)	Weight variation (%)	Wetting time (sec)	Disintegration time (sec)
FT-1	2.95±0.04	7.47±0.50	501.13±3.97	1.60	24.18±1.94	28.98±1.20
FT-2	2.76±0.04	11.27±0.85	499.00±1.00	4.70	31.78±0.37	37.58±1.00
FT-3	2.95±0.04	9.57±0.99	500.00±3.00	1.46	163.70±16.16	58.91±1.02
FT-4	2.99±0.03	13.67±1.30	502.62±3.90	2.51	13.51±0.45	30.80±1.26
FT-5	2.95±0.04	7.73±1.26	502.00±3.00	1.40	13.51±2.01	38.18±0.88
FT-6	2.93±0.05	11.08±1.03	506.00±1.00	2.37	33.84±1.96	48.16±4.02
FT-7	2.93±0.03	11.84±0.84	505.00±1.00	1.78	108.80±7.58	107.00±1.69
FT-8	3.05±0.04	9.44±0.78	503.00±3.00	1.19	25.28±0.69	38.00±0.00

processing. The FDT tablet weight was estimated to be 502.34 ± 2.37 mg. The tablet weight variation was <5%, which met the acceptance criteria of the target weight. All FDT formulations were completely wet within 3 min and completely disintegrated within 3 min. Therefore, the formulated FDTs met an acceptable range under the criteria of the European pharmacopoeia (EU) standard¹⁴. By using the different disintegrates, the FDTs were formulated using Ac-Di-Sol®. The FT-3 and FT-7 indicated the longest time required for wetting and disintegration (Table 3).

DISCUSSION

The use of plant-based chemotherapeutic agents, containing phenolic and flavonoid compounds, has been reported in cancer therapy. In the present study, the results indicated that PP contained flavonoids. A recent study reported that the PP extract contained flavonoids and phenolic contents corresponding⁵ to 161.8 ± 8.8 mg RE g⁻¹ and 103.4 ± 13.6 mg GAE g⁻¹, respectively. The PP extract was considered to be cytotoxic on HeLa cells and its IC₅₀ values were 20.9 ± 3.6 and 7.3 ± 1.7 µg mL⁻¹ following 48 and 72 hrs incubation with the cells. This conclusion was based on the National Institute guidelines, which define the criteria of the crude extracts with IC₅₀ values¹³ <20 µg mL⁻¹. This finding was confirmed by the cytotoxicity of the PP extract against HeLa cells as previously reported³. Moreover, the ethanolic PP extract indicated higher cytotoxicity on HeLa cells than the dichloromethane PP extract (IC₅₀ values of 81.5 µg mL⁻¹). This is the first study that examined the activity of the PP extract concerning the inhibition of colony formation and cell

migration, as well as the induction of ROS production and cell apoptosis in HeLa cervical cancer cells. The colony formation and cell migration assays are representative of cancer cell survival recurrence and cancer cell metastasis. These two processes significantly affect cancer prognosis. Therefore, the extract which possesses significant inhibitory activities against colony formation and migration of cancer cells can be potentially used for cancer treatment. The present study indicated that the PP extract (25 µg mL⁻¹) exhibited significant inhibitory activity concerning colony formation and migration of HeLa cells. Furthermore, the extract induced ROS formation and apoptosis, which are major causes of cell death. In the present study, the extract induced ROS formation and cell apoptosis at a concentration range of 50 - 250 µg mL⁻¹. According to its *in vitro* anticancer activities, the present study indicated that 50 µg mL⁻¹ of the PP extract could induce HeLa cell death and produce ROS, resulting in the induction of cell apoptosis. In addition, PP could suppress colony formation and migration of HeLa cells. Nevertheless, the extract exhibited significant cytotoxicity on normal cells, such as fibroblast cells, which was confirmed by the MTT assay. The results indicated that 50 µg mL⁻¹ PP extracts decreased 35% of cell viability following 48 hrs incubation with the cells. Therefore, the cytotoxicity of the PP extract on more than one normal cell line may be possible and should be further assessed. FDT was selected for the development of the extracted base since it is preferable for patients with rapid onset of the disease and more convenient for oral administration. Disintegrate play a key role in the disintegration time. Therefore, four disintegrating agents were used to formulate FDTs in the present study. The mixture of

the ingredient formulations indicated poor flow properties. Therefore, the formulation improved its flow properties by using a granulation method before tablet compaction. Eight FDT formulations were formulated and subsequently characterized. All FDT formulations were completely wet and could disintegrate within 3 min. Therefore, the formulated FDTs demonstrated an acceptable efficacy as determined by the criteria of the EU standards¹⁴. The FDTs which contained Ac-Di-Sol® (i.e., FT-3 and FT-7) indicated the longest periods compared with WT and DT. The other formulations exhibited lower periods and are shown in Table 3. This may be due to the lower swelling and hydration capacities of Ac-Di-Sol® compared with those of corn starch and Explotab®. Moreover, the FDTs using Ac-Di-Sol® have been reported to exhibit the longest disintegration compared with the FDTs using starch and Kollidol CL® due to the lowest wet ability time¹⁵. The resulting FDT formulations may be used to develop the PP extract as a dietary supplement formulated for the prevention of cervical cancer.

CONCLUSION

The present study provides the first report on cell cytotoxicity, colony formation, cell migration, ROS production and cell apoptosis of the PP extract using the HeLa cervical cancer cell model. The results indicated that PP exhibited significant cytotoxicity on HeLa cells depending on the dose of treatment. The extract also inhibited colony formation and cell migration of HeLa cells. Moreover, the extract induced ROS formation leading to the induction of cell apoptosis. Current findings led to the conclusion that the PP leaf extract exhibited anticancer activities. The FDTs containing the extract was successfully formulated and characterized. All FDT formulations exhibited improved physical properties and met the criteria of the EU disintegration period of 3 min.

SIGNIFICANCE STATEMENT

The present study demonstrated that the PP ethanolic leaf extract exhibited cytotoxicity on HeLa cells. The HeLa cell death was caused by the PP extract by induction of apoptosis and ROS production. In the present study, the data indicated that PP may be a potential source of the chemopreventive substances used for the treatment of cervical cancer. The FDTs containing the PP leaf extract were successfully formulated and indicated optimal physical properties. The formulated FDTs may be developed as dietary supplement formulations for the prevention of cervical cancer.

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REFERENCES

1. Haron, N.H., Z.M. Toha, R. Abas, M.R. Hamdan, N. Azman, M. Khairuddean and H. Arsad, 2019. *In vitro* cytotoxic activity of clinacanthus nutans leaf extracts against HeLa cells. Asian Pac. J. Cancer Prev., 20: 601-609.
2. Mitra, S. and R. Dash, 2018. Natural products for the management and prevention of breast cancer. Evidence-Based Compl. Alt. Med., Vol. 2018. 10.1155/2018/8324696.
3. Jongrungruangchok, S., T. Songsak and S. Wongwiwatthanakit, 2014. Evaluation of nutrient and mineral content of the leaves of *Phlogacanthus pulcherrimus* cultivated in Thailand. Bull. Health. Sci. Technol., 12: 22-26.
4. Poeaim, S., P. Lordkhem, P. Charoenying and P. Laipasu, 2016. Evaluation of antioxidant, cytotoxic activities and total phenolic content from leaf extracts of *Phlogacanthus pulcherrimus*. Int. J. Agric. Technol., 12: 1659-1669.
5. Boontha, S., B. Buranrat, P. Temkitthawon and T. Pitaksuteepong, 2021. Anticancer activities of *Phlogacanthus pulcherrimus* T. anderson leaves extract on MCF-7 breast cancer cells. Key Eng. Mater., 901: 16-21.
6. Amith, S.R., J.M. Wilkinson and L. Fliegel, 2016. Assessing Na⁺/H⁺ exchange and cell effector functionality in metastatic breast cancer. Biochimie Open, 2: 16-23.
7. Shehat, M.G. and J. Tigno-Aranjuez, 2019. Flow cytometric measurement of ros production in macrophages in response to fcyr cross-linking. J. Visualized Experiments, Vol. 145. 10.3791/59167.
8. Olmez, S.S., I. Vural, S. Sahin, A. Ertugrul and Y. Capan, 2013. Formulation and evaluation of clozapine orally disintegrating tablets prepared by direct compression. Pharmazie-An Int. J. Pharm. Sci., 68: 110-116.
9. Pabari, R.M. and Z. Ramtoola, 2012. Effect of a disintegration mechanism on wetting, water absorption, and disintegration time of orodispersible tablets. J. Young Pharmacists, 4: 157-163.
10. Boontha, S., N. Kaewjaiboon, P. Rattanatanyapat, W. Nanto, S. Taolam, B. Buranrat and T. Pitaksuteepong, 2018. Cytotoxicity and cell migration suppression by noni fruit extract on michigan cancer foundation-7 human breast cancer cells and development of topical microemulsions. Pharmacogn. Mag., 14: 499-506.
11. Buranrat, B., S. Noiwetech, T. Suksar and A. Ta-Ut, 2020. Inhibition of cell proliferation and migration by *Oroxylum indicum* extracts on breast cancer cells via Rac1 modulation. J. Pharm. Anal., 10: 187-193.

12. Boontha, S., D. Jaichum, K. Kunseang, K. Wanjai, W. Boonyo and T. Pitaksuteepong, 2021. Effect of trimethyl chitosan with different degrees of quaternization on the properties of tablets prepared using charged model drugs. *Walailak J. Sci. Tech.*, Vol. 18. 10.48048/wjst.2021.9880.
13. Ruangnoo, S., A. Itharat, I. Sakpakdeejaroen, R. Rattarom, P. Tappayutpijam and K.K. Pawa, 2012. *In vitro* cytotoxic activity of Benjakul herbal preparation and its active compounds against human lung, cervical and liver cancer cells. *J. Med. Assoc. Thail.*, 95: S127-S134.
14. Council of Europe, 2013. *European Pharmacopoeia Version 8. 8th Edn.*, EDQM, Strasbourg, France, ISBN: ISBN: 978-92-871-7525-0, Pages: 1456.
15. Zhao, N. and L.L. Augsburger, 2005. The influence of swelling capacity of superdisintegrants in different pH media on the dissolution of hydrochlorothiazide from directly compressed tablets. *AAPS PharmSciTech*, 6: E120-E126.