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ORIGINAL ARTICLE

Evaluation of the Anti-proliferation and Anti-migration Effects of *Leucaena leucocephala* and *Dolichandrone serrulata* Ethanolic Extracts against Human Cervical Cancer Cell Line

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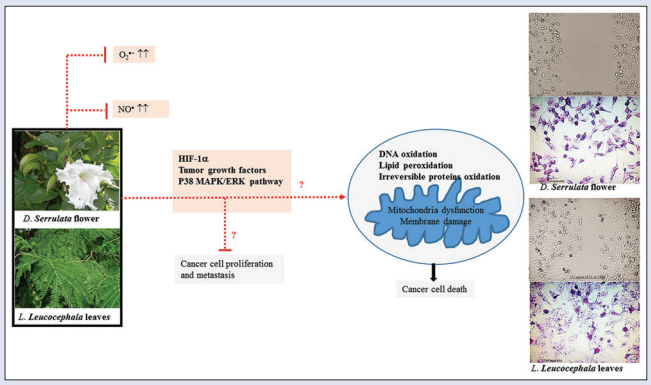
ABSTRACT

Background: Cervical cancer is a leading cause of death affecting women in developing country. Several medicinal plants have recently increasing in the involvement of chemotherapeutic drugs development, including *Leucaena leucocephala* (LL) and *Dolichandrone serrulata* (DS). **Objectives:** The aim of this study is to investigate the anti-proliferative and anti-migration effects of LL leaves and *D. serrulata* flowers ethanolic extracts against human cervical cancer cell line. **Materials and Methods:** The content of total phenolic and flavonoid and antioxidant activity of extracts collected from different fractions were determined. MTT assay, cell morphology, and wound healing assay were established to observe anti-proliferative and anti-migration effects in HeLa cell line. **Results:** Total phenolic contents of both medicinal plant extracts and flavonoids content of LL extract were observed highest in ethyl acetate fraction, while flavonoid content of *D. serrulata* extract was greatly observed in hexane fraction. LL extract in ethyl acetate fraction showed the most promising result in antioxidant activity assay using both 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid with inhibition concentration for fifty percent of 4.917 ± 0.955 and 11.005 ± 1.491, respectively. In cytotoxicity test, both herbal extract at concentration of 250 µg/ml were significantly reduced HeLa cell viability comparing with the control. The assay for wound healing showed that both LL and *D. serrulata* extract at concentration of 125 µg/ml could significantly decrease the migration rate of HeLa cell comparing with the control and Trolox-treated groups. **Conclusion:** LL and *D. serrulata* ethanolic extracts (125–500 µg/ml) have been proposed in this study of possessing antioxidant and anti-migration activity, as well as the ability in induce cancer cell death.

Key words: Anti-migration, anti-proliferation, cervical cancer, *Dolichandrone serrulata*, *Leucaena leucocephala*

SUMMARY

- *Leucaena leucocephala* (LL) and *Dolichandrone serrulata* provide the antioxidant activity in ethyl acetate fraction
- LL and *D. serrulata* can induce cervical cancer cell cytotoxicity in dose-dependent manner
- LL and *D. serrulata* efficiently reduced wound width, implying that both medicinal plants might be able to reduce the migration rate of cervical cancer cells without inducing cell death (125 µg/ml).



Abbreviations used: TPC: Total phenolic compound; GAE: Gallic acid equivalent; QE: Quercetin equivalent; FRAP: Ferric reducing radicals power; LL: *Leucaena leucocephala*; DS: *Dolichandrone serrulata*.

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INTRODUCTION

Cervical cancer is one of leading causes of death worldwide found in women in both developed and developing countries. Smoking, dietary, obesity, air pollution, chemical exposure, infection with certain microbial organisms and heredity have been previously reported as a crucial risk factors influencing cancer development.[1] The most common cause of disease is human papillomavirus (HPV). It can induce inflammation and progression of cervical cancer. Natural compound such as resveratrol and pterostilbene contain polyphenols that provide antioxidant activity have been proposed of anticancer activity by inducing cancer cell death.[2] The previous study suggested that antioxidant can reduce oxidative stress that exhibited anti-cancer and anti-proliferation properties.[3,4] With the incoming trend of plant-derived chemotherapeutic agent, *Leucaena leucocephala (*Lam.*)* (LL) *de wit* and *Dolichandrone serrulata* (DS) were introduced in the current study to investigate the anti-proliferative effects in cervical cancer cell line.

LL and DS have been previously reported for providing antioxidation and anti-inflammatory activities, as well as the anti-cancer and anti-metastasis effects.[5-8] In oral cancer research, it has been suggested the anti-cancer properties of LL was introduced through ERK and p38 pathways, acting as signal transduction inhibitor.[9] Although no

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study has proposed the anti-cancer activity of DS, it was found that one of significant components found in this medicinal plant extract is protocatechuic acid (PCA) that has been previously proposed of having inhibitory effect in melanoma cell metastasis through downregulation of matrix metalloproteinase-2 MMP-2.[10] However, currently, there are no available data on cervical cancer as no studies have been conducted under this model. The current study aimed to investigate the antioxidant activity, the toxicity, anti-proliferative, and anti-migration effects of LL and DS in HeLa cell line.

MATERIALS AND METHODS

Plants extraction

LL leaves and DS flowers were collected from Mahasarakham province, Thailand. Briefly, the specific parts of both plants were dried using hot air oven at 50°C and blended into powder to exhibit the extraction process using 95% ethanol. The collection was processed after 7 days of fermentation. The solution was filtered, evaporated, and lyophilizing in freezer dryer. Ethyl acetate, chloroform, and hexane were further extracted using separatory funnel. All fractions were dried and kept at 50°C and 4°C, respectively.

Determination of total phenolic content

Total phenolic compound (TPC) was performed according to Folin-Ciocalteu standard protocol with some modifications. Briefly, 10% of Folin-Ciocalteu reagent was mixed with the ethanolic extracts, followed by 10.75% sodium carbonate and the mixture was incubated in room temperature for 30 min. Measurement of TPC was monitored at 765 nm using ultraviolet-visible (UV-VIS) spectrophotometer. The results were represented in microgram of gallic acid equivalent per milligram extracts (μg of GAE/mg extracts).

Determination of flavonoids content

The aluminum chloride ($AlCl_3$) colorimetric method was performed for evaluating flavonoids content compared with quercetin.[11] Briefly, 100 microliter of sample was incubated with 2% $AlCl_3$ for 45 min. The formation of flavonoids-aluminum complex was measured absorbance at 420 nm using UV-VIS spectrophotometer. Flavonoids content expressed as microgram quercetin equivalent per milligram of extracts (μg of QE equivalent/mg extract).

Measurement of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radicals scavenging assay

DPPH radical scavenging activity was performed according to Masuda method.[12] Briefly, the extract solution was incubated with 0.05 mM DPPH at room temperature for 30 min and observed at 517 nm using UV-spectrophotometer. The inhibition concentration for 50% (IC_{50}) determined 50% inhibition of DPPH radicals and capacity of DPPH radical scavenging activity was determined with the comparison of Vitamin C and Trolox as standard antioxidant.

Equation: Percent inhibition = $([A_{control} - A_{sample}] \times 100)/A_{control}$

Where $A_{control}$ and A_{sample} were absorbance data of control and sample, respectively.

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical scavenging assay

Capacity of extracts for radical scavenging assay was performed using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) as shown in previous method,[13] with some modification. Briefly, ABTS radical was induced by 7 mM ABTS solution added in ethanol with 2.45 mM potassium persulfate. The ABTS reagent was stored in dark at room temperature for 16 h and incubated with 0.1 ml of extracts for 30 s in dark at room temperature. IC_{50} of ABTS radicals was calculated comparing with Vitamin C and Trolox as standard antioxidant.

Equation: Percent inhibition = $([A_{control} - A_{sample}] \times 100)/A_{control}$

Where $A_{control}$ and A_{sample} were the absorbance of control and sample, respectively.

Ferric reducing radicals power assay

The ferric reducing power was determined according to Benzie method[14] with some modification. Briefly, extract solution was mixed with ferric reducing radicals power (FRAP) reagent containing 300 mM acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride and incubated at 37°C for 30 min in the darkroom. Ferrous tripyridyltriazine product was measured using spectrophotometer at 593 nm. FRAP value was expressed as mM of ferrous sulfate (II) equivalent/mg of extracts.

Cell culture

HeLa, human cervical cancer cell line was cultured in EMEM modification with L-glutamine supplement with 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin in an incubator at 37°C with 5% CO_2 and 95% humidity. The cells were cultured until it reaches 80%–90% confluent of a well plate before starting the experiment.

Cell cytotoxicity and proliferation assay

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay

MTT assay is a colorimetric assay for assessing cell viability and proliferation. Briefly, HeLa cell lines were placed in 96 wells plate. Different concentrations of LL leave and DS flower extracts (62.5, 125, 250, and 500 μg/ml) was incubated at 37°C with 5% CO_2 for 24 h before the experiment in parallel with the control group, doxorubicin (0.625, 1.25, 6.25, 12.5, and 25 μg/ml) and Trolox (31.5, 62.5, 125, 250, and 500 μM). The media without supplement was then replaced and 100 μl of 5 mg/ml MTT in PBS was added. The cells were incubated 37°C with 5% CO_2 for 4 h. The formazan product was dissolved with DMSO and quantified using microplate reader at 570 nm. Cytotoxic value of cells was expressed as percent of cell viability.

Equation: Percent of cell viability = $([OD_{sample}\text{-}OD_{blank}]/[OD_{control} - OD_{blank}]) \times 100$

Cell morphology

Medicinal plant extracts with different concentrations (62.5, 125, and 250 μg/ml), 6.25 μg/ml doxorubicin and 50 μM Trolox were treated in HeLa cell lines. The *in vitro* cell density and cell morphology were stained with carmine and observed under cell imager using 20X objective lens and light microscope at 24 h.

Wound healing assay

The study of cell migration was evaluated in wound healing assay. The cell line was cultured in 24 wells plate until it reached 90%–100% confluence. The wound was generated using scratcher to the size of 0.5 mm. The scratcher was pressed against the top of the tissue culture plate and generated a vertical wound down through the cell monolayer according to the protocol reported in previous study.[15] The cells were then treated with the normal media used as a control group, Trolox (50 or 200 μM), or either of herbal extracts in the concentration of 125 and 250 μg/ml.

Migration of the cells was observed at 0, 3, 6, 12, and 24 h under cell imager. The width of the wound was measured using Image J program and the length of control group and treatment groups were compared according to their corresponding times.

Statistical analysis

All variables were summarized using means and standard deviations. Statistical analysis was carried out to compare between groups by Brown-Forsythe Test and followed by Bonferroni correction for multiple comparison. Statistical significant was considered when value of $P < 0.05$ ($R = 3.4.0$).

RESULTS

Total phenolic compound and flavonoids content

LL leaves and DS flowers ethanolic extracts were measured for TPC and flavonoids content using different solvents (hexane, chloroform, and ethyl acetate) in separatory funnel. TPC and flavonoids content of LL leaves were mostly found in ethyl acetate fraction were 319.290 ± 10.934 µg of GAE/mg extract and 399.572 ± 10.905 µg of QE/mg extract, respectively [Table 1]. Most flavonoid content of DS flower extract was monitored at 152.908 ± 23.372 µg of QE equivalent/mg of hexane fraction.

Antioxidant activity

LL and DS ethanolic extracts in the fraction of ethyl acetate were observed for antioxidant activity using DPPH, ABTS, and FRAP method, as shown in Table 2. The radicals scavenging activities of DPPH and ABTS radicals have shown in IC_{50} in equivalent to Vitamin C and Trolox. FRAP value was represented in the equivalent to ferrous (Fe[II]) in the comparison with the extracts. The result represented IC_{50}. The scavenging properties observed under DPPH method showed the IC_{50} of 4.917 ± 0.955 and 36.331 ± 1.683 µg/ml, for LL and DS, respectively. On the other hand, the Trolox equivalent of both herbs is 27.823 ± 0.361 and 1.472 ± 0.314 mM of TAE/mg, respectively. The result was shown in the same way when compared with Vitamin C with 1.472 ± 0.314 and 0.143 ± 0.032 mg of Vitamin C equivalence/mg extract, for LL and DS, respectively. The result of scavenging activity observed using ABTS method was found most effective in ethyl acetate fraction of LL (IC_{50}: 11.005 ± 1.491 µg/ml), as well as the activity to reduce Fe^{3+} that the result showed the 3.981 ± 0.262 mM Fe (II)/mg extracts.

Table 1: Total phenolic content and flavonoid content of *Leucaena leucocephala* and *Dracaena serrulata*

Extracts	TPC (µg of GAE/mg extract)	Flavonoids (µg of QE equivalent/mg extract)
1. LL leaves in ethanol	172.940±1.895	146.220±2.788
LL hexane	39.623±0.255	206.343±0.895
LL chloroform	69.799±0.175	203.460±1.710
LL ethyl acetate	319.289±10.934	399.572±10.905
2. DS flower in ethanol	34.796±1.994	8.038±2.642
DS hexane	8.086±3.562	152.908±23.372
DS chloroform	41.510±6.293	94.012±1.776
DS ethyl acetate	71.089±1.475	6.9207±2.794

Data are expressed as mean±SD. *L. leucocephala: Leucaena leucocephala; D. serrulata: Dracaena serrulata*; TPC: Total phenolic content; LL: *L. leucocephala*; DS: *D. serrulata*; QE: Quercetin equivalent; GAE: Gallic acid equivalent; SD: Standard deviation

HeLa cell cytotoxicity test by MTT assay

HeLa cell lines were treated in 96 wells cultured plate for 24 h and further incubated with 100 µl of 5 mg/ml MTT for 4 h in CO_2 incubator. Percent of cell viability were observed by the measurement of formazan production at absorbance 570 nm using microplate reader. The result showed that the viability of HeLa cell line were significantly reduced in dose at 250–500 µg/ml of both LL and DS extracts when compared with control [Figure 1]; while Trolox, an antioxidant standard, was showed no statistical significant in cell viability comparing with control.

Cells morphology

After HeLa cell line were treated with media (control), doxorubicin, Trolox or the extracts in different concentrations for 24 h, morphology of cells were observed directly under cell imager and the microscope [Figure 2]. The result showed the cell death characteristics in doxorubicin treated group and both LL and DS extracts at concentration of 125 and 250 µg/ml. Followed by staining with carmine, the cell line treated with either 6.25 µg/ml of doxorubicin, 125 µg/ml of LL, and 125 µg/ml of DS were re-observed under cell imager with 20X objective lens [Figure 3]. The result showed the almost identical pattern of cell death between doxorubicin and LL treated groups [Figure 3a and b].

Wound healing assay

HeLa cell migration was observed using wound healing assay *in vitro* by introducing the "wound" using sterile scratcher with tip size of 0.5 mm as described in method previously. Figures 4 and 5 show the migration rate of HeLa cell in control, Trolox, LL or DS treated groups in different point of time under cell imager. According to the progress of time, the width collected from media treated group (control) and Trolox treated

Table 2: Antioxidant activities of extracts and standard using 2,2-diphenyl-1-picrylhydrazyl radical, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid and ferric reducing radicals power method

Group	DPPH			ABTS			FRAP
	IC_{50}	mM of TAE/mg extracts	mg of Vitamin C/mg extracts	IC_{50}	mM of TAE/mg extracts	mg of Vitamin C/mg extracts	mM Fe (II)/mg extracts
Vitamin C (µg/ml)	5.853±0.254	-	-	0.606±0.031	-	-	5.695±0.119
Trolox (µM)	111.923±2.329	-	-	13.940±0.335	-	-	-
1. LL ethanol (µg/ml)	14.693±0.122	6.365±0.137	0.834±0.180	25.552±0.572	0.716±0.043	0.016±0.005	1.802±0.099
Hexane	67.729±7.269	1.634±0.488	0.080±0.024	122.121±3.530	0.158±0.093	0.004±0.001	0.550±0.016
Chloroform	126.889±12.016	1.043±0.062	0.040±0.013	21.535±1.359	0.626±0.015	0.014±0.001	0.182±0.005
Ethyl acetate	4.917±0.955	27.823±0.361	9.589±0.118	11.005±1.491	1.331±0.186	0.051±0.001	3.981±0.262
2. DS ethanol (µg/ml)	44.807±5.087	1.272±0.345	0.126±0.036	343.825±31.141	0.042±0.001	0.001±0.002	0.215±0.003
Hexane	554.072±23.836	0.182±0.015	0.008±0.001	413.599±35.867	0.062±0.001	0.001±0.001	0.437±0.001
Chloroform	636.050±4.753	0.179±0.012	0.009±0.001	85.598±4.730	0.014±0.003	0.004±0.004	0.245±0.001
Ethyl acetate	36.331±1.684	1.472±0.314	0.143±0.032	41.733±2.740	0.340±0.067	0.008±0.003	0.720±0.033

Data are expressed as mean±SD. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; ABTS: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; FRAP: Ferric reducing radicals power; *L. leucocephala: Leucaena leucocephala; D. serrulata: Dracaena serrulata*; TAE: Trolox equivalent; LL: *Leucaena leucocephala*; DS: *Dolichandrone serrulata*; SD: Standard deviation; IC_{50}: Inhibition concentration for fifty percent

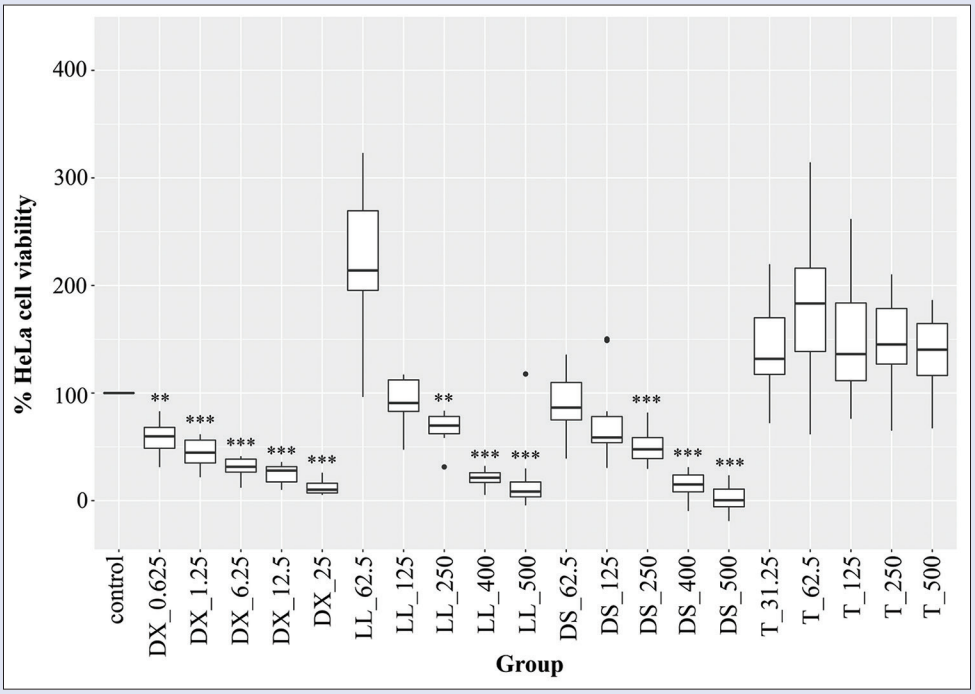

Figure 1: Percent viability of HeLa cell in the control group, together with doxorubicin, *Leucaena leucocephala*, *Dolichandrone serrulata* and trolox. **$P < 0.01$ and ***$P < 0.001$ compared to control. DX: doxorubicin; LL: *Leucaena leucocephala*; DS: *Dolichandrone serrulata*; T: Trolox

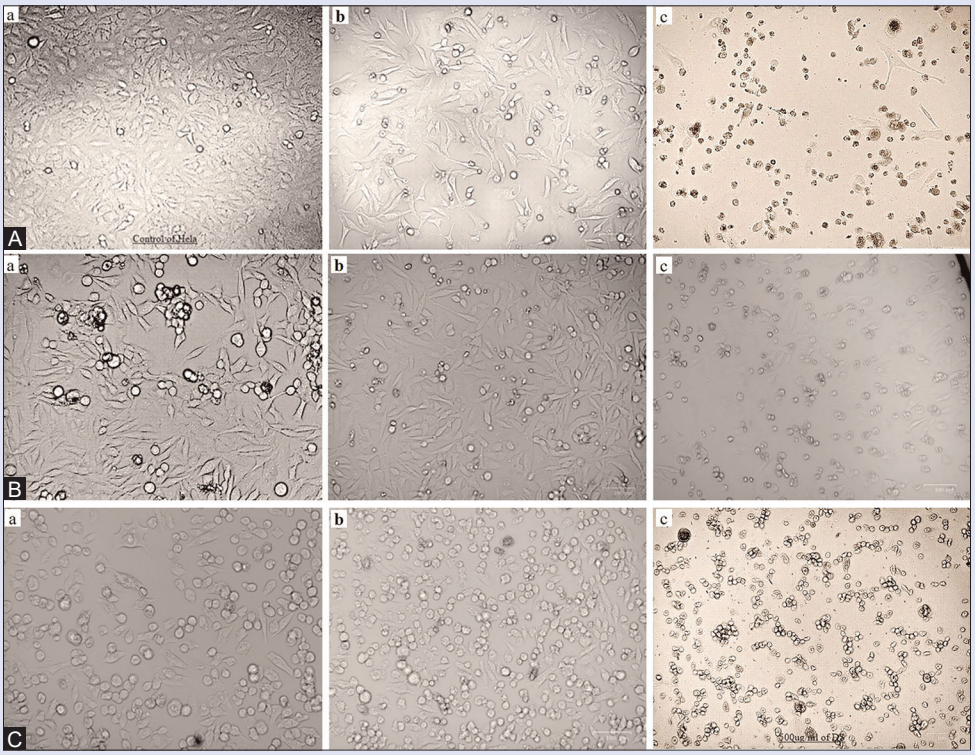

Figure 2: Morphology of HeLa cells observed under fluorescent cell imager using brightfield mode with ×20 objective len (ZOE™ fluorescent cell imager) after 24 h treatment. Row A represents the incubation of HeLa cell in the control group, with the treatment of (Aa) supplement free media (negative control), (Ab) 62.5 μg/ml of doxorubicin and (Ac) 50 μM of trolox. Row B represents the incubation of HeLa cell with *Leucaena leucocephala* extracted at different concentrations of (Ba) 62.5, (Bb) 125 and (Bc) 250 μg/ml. Row C represents the incubation of HeLa cell with *Dracaena serrulata* extracted at different concentrations of (Ca) 62.5, (Cb) 125 and (Cc) 250 μg/ml (left to right)

group were gradually reduced [Figures 4 and 5]. While the results observed from LL and DS treatment groups showed the significantly difference comparing with their corresponding time in control group [Figures 4 and 5].

DISCUSSION AND CONCLUSION

Cervical cancer is one of the major threats of cancer to women in developing country. Many risk factors can induce the disease development, including HPV. Several studies have been introduced the novel therapeutic methods for treating this cancer and investigated on how they work. The current study is to introduce and investigate the two Thai traditional medicines, LL and DS, in the activity of antioxidant, the toxicity, anti-proliferative, and anti-migration effects of LL and DS in cervical cell line.

In this study, ethyl acetate fraction of LL ethanolic leave extract provided highest content of total phenolic and flavonoid content, 319.289 ± 10.934 µg of GAE/mg extract and 399.572 ± 10.905 µg of QE/mg extract, respectively. In contrast, the flavonoid content of DS flower extract showed the highest content in hexane fraction (152.908 ± 23.372 µg of QE equivalent/mg), while total phenolic content found the highest amount in ethyl acetate fraction (71.089 ± 1.475 µg of GAE/mg extract). It could be suggested that most of components from each fraction contain polar, as it could be likely collected from ethyl acetate fraction. While flavonoid content of DS extract was found the highest in hexane fraction, this may be due to the hydrocarbon group containing in flavonoid collected in this fraction. The findings of LL were in line with the previous study of Hassan *et al.*[16] who found that ethyl acetate fraction of LL contains the highest flavonoid content. The previous study of Sharma and Chaurasia[17] explored the total phenolic and flavonoid contents LL methanolic crude extract with the quantity of 1.550 ± 0.008 mg GAE/g extract and 2.933 ± 0.19 mg QE equivalent/g extract, respectively. While the study of Sreeprasert *et al.* (unpublished data) reported the highest total phenolic content found in its leave with 63.38 ± 2.00 mg GAE/g extract, while in flowers, it was found 9.27 ± 1.95 mg GAE/g extract. The study also investigated the amount of flavonoid contents in leave and flower part and the result were 33.06 ± 2.28 and 2.28 ± 0.14 mg QE equivalent/g, respectively. It is suggested the source of this plant may influence the number of total phenolic and flavonoid contents. In addition, it is suggested that the extra step in separatory funnel combined with different solvents, chloroform, hexane or ethyl acetate, could increase the purity of the extracts.

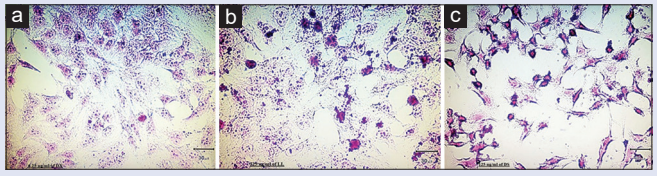

Figure 3: Morphology and density of *in vitro* culture of HeLa cell lines stained with carmine observed under cell imager with 20X objective lens after 24 h treated with (a) 6.25 µg/ml of doxorubicin, (b): 125 µg/ml of *Leucaena leucocephala*, or (c) 125 µg/ml of *Dolichandrone serrulata*

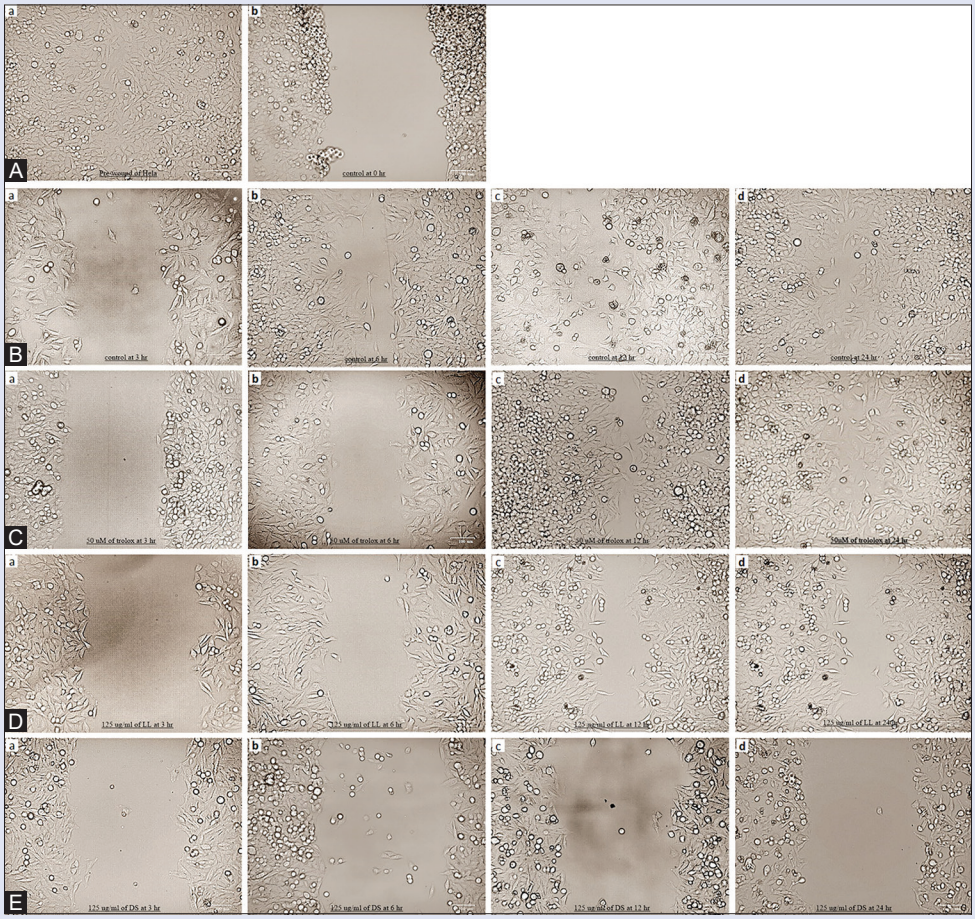

Figure 4: Migration of HeLa cell observed under fluorescent cell imager using brightfield mode with 20X objective (ZOE™ fluorescent cell imager). Row A represents before (Aa) and after (Ab) applying of scratcher and generating 0.5 mm wound. After the scratch was applied, the monolayer cell lines were treated with media (control group, row B), 50 µM trolox (row C), 125 µg/ml *Leucaena leucocephala* (row D) and 125 µg/ml *Dracaena serrulata* (row E). The width of the wound was measured at 3 (Ba, Ca, Da, Ea), 6 (Bb, Cb, Db, Eb), 12 (Bc, Cc, Dc, Ec) and 24 h (Bd, Cd, Dd, Ed) using image J program

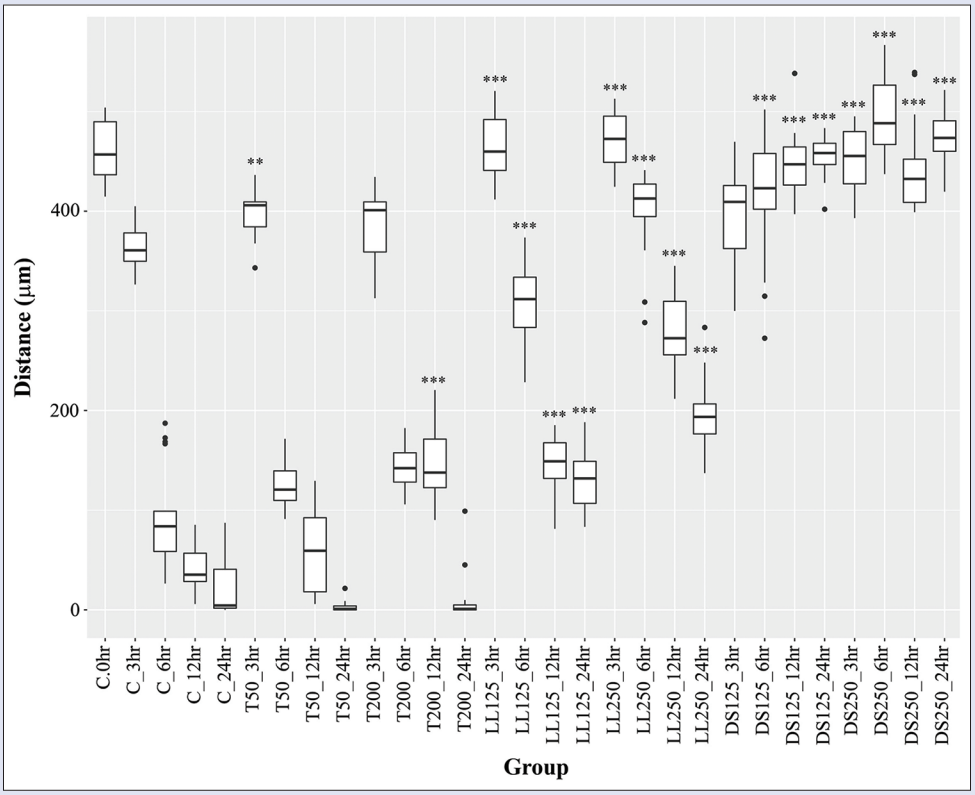

Figure 5: Distance of the wound (μm) generated by 0.5 mm scratcher at different time points measured by image J program. Statistical analysis was performed in R 3.4.0 using Brown-Forsythe Test/Bofferroni. ***P* < 0.01 and ****P* < 0.001 compared to control according to its corresponding time. C: Control, T: Trolox, LL: *Leucaena leucocephala*; DS: *Dolichandrone serrulata*

The experiment observing antioxidant activity [Table 2] showed that ethyl acetate fraction of LL ethanolic extract provided the highest antioxidant activity with IC_{50} of 4.917 ± 0.955 and 11.005 ± 1.491 in DPPH and ABTS assay, respectively. While the highest antioxidant activity among different fractions of DS ethanolic extract was collected from ethyl acetate fraction with IC_{50} of 36.311 ± 1.684 and 41.733 ± 2.740 in DPPH and ABTS assay, respectively. It could be suggested that the flavonoid containing hydrocarbon group in hexane fraction may not contribute high antioxidant activity comparing to ethyl acetate fraction. The findings of the previous study of Hassan *et al.*[16] also found that ethyl acetate fraction of LL acquired the antioxidant activity in of Ehrlich-ascites carcinoma cell. Contradicted with another study of Sharma and Chaurasia,[17] the result showed the highest antioxidant activity in chloroform extract of LL leaves. The other study showed that although flavonoid is the major component in LL leaves, not all of the flavonoid provides scavenging activity[6] This may supported the current result showed in DS fractions that the flavonoid collected from hexane fraction provided less antioxidant activity comparing with those from ethyl acetate fraction. Another study from Heijnen *et al.*[18] showed that the scavenging activity containing in flavonoid also possess the activity against peroxynitrite by the AC-ring with hydroxyl groups (5-and 7-OH). The study of Phanthong *et al.*[8] found that methanolic extract DS flower-containing PCA, hallerone and rengyolone exhibited potent scavenging activity for reactive oxygen species with IC_{50} of 25–30 μM. Notably, the different in total phenolic and flavonoid content collected from different fraction of both medicinal plant may imply that the bioactive compounds for antioxidant activity and cytotoxic effect in cervical cancer cell could be non-identical.

The cytotoxic activities of LL leaves and DS flower extracts in HeLa cell line were observed under cell imager (×20) and MTT assay. Percent of cell viability were decreased significantly comparing with control and Trolox, which is a potent antioxidant control [Figure 1]. Cell death could be observed starting from LL and DS extracts at concentration 250 μg/ml treated groups. Although Trolox, one of the potent antioxidant, possessed the cytotoxic activity against colon cancer cell line[19] and it was previously suggested that antioxidant might have protective effect in hypoxia-induced apoptosis,[20] the result in this study showed no significant difference comparing with control in observing anti-proliferation activity [Figure 1]. It can be suggested that Trolox might only involve in some specific cancers. The observing cell death characteristic [Figures 2 and 3] showed the present of nuclear fragments in the dose-response manner. It is possible that the active ingredients of LL, tannin and mimosine, might induce the cell death by inducing apoptosis pathway.[21,22] Although no prior information about cytotoxic effect of DS, this study is the first to claim the cytotoxic effect against cervical cancer. The mechanism underlying cell death is still unclear but it may involve in Ras/Akt/NF-κB pathway and MMP-2 production.

To observe whether or not LL and DS extract can inhibit the cancer metastasis, we introduced the wound to the monolayer cell line by scratching HeLa cell line with 0.5 mm scratcher and observed the results in 3, 6, 12, and 24 h. Surprisingly, the result in Figure 5 showed significant difference of wound width comparing between 125 and 250 μg/ml of LL and DS ethanolic extracts and control in its corresponding time, suggested the reduction in migration rate of cancer cell. The previous study in oral cancer[9] suggested LL could inhibit metastasis oral cancer cells through expression MMP-2 and mitogen-activated protein kinase pathway. DS flower also contains PCA, a phenolic compound with strong free radical scavenging activity.[8] This medicinal plant extract was proposed of possessing anti-metastasis activity through the

down-regulation of Ras/Akt/NF-κB pathway and MMP-2 production by targeting RhoB activation.[10]

Although the mechanism underlying cytotoxicity and anti-proliferative effects are still unclear, the result clearly demonstrated that both LL and DS ethanolic extracts could induce cell death and reduced migration of cervical cancer cell lines. The further study needs to focus on the mechanism involving cell death and their major active components both *in vivo* and *in vitro* for developing a novel chemotherapeutic agent in the future.

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Conflicts of interest

There are no conflicts of interest.

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