Antioxidant and Anticancer Activities of the Extract from *Tiliacora triandra* Diels. in Nakhonsawan Province

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ABSTRACT

In this research, antioxidant and anticancer activities including quantity of the extract from *Tiliacora triandra* Diels. In Nakhonsawan province from Mueang District, Ta Khli District, Banphot Phisai District, Kao Liao District and Lat Yao District were studied. The aerial parts of *T. triandra* were macerated with hexane, chloroform and methanol, sequentially. The antioxidant and anticancer activities of the crude extract of this plant were determined. The percentage yields of the crude extract were calculated based on a dry weight. The methanol extract of this plant from Kao Liao District gave the highest percentage yields. The antioxidant activity of the crude extract of this plant were determined using ABTS and DPPH methods. The hexane extract of the plant from Banphot Phisai District exhibited the highest antioxidant activity (ABTS) with an IC_{50} of 2.94 mg/mL. The methanol extract of the plant from Ta Khli District exhibited the highest antioxidant activity (DPPH) with an IC_{50} of 0.36 mg/mL. The anticancer activity of the plant extracts were performed using the resazurin microplate assay (REMA). Only hexane extract of this plant from Mueang District showed anticancer activity against of two cancer cell lines: KB-Oral cavity cancer and MCF7-Breast cancer with the IC_{50} values of 41.14 and 38.26 µg/mL, respectively.

Keywords: Antioxidant Activity, Anticancer Activity, *Tiliacora triandra* Diels.

1. INTRODUCTION

Natural products are well known as sources for drugs in several human ailments including cancers for example, vincristine and innotecan etc. [1]. *Tiliacora triandra* Diels, one of the medicinal plants is known in Thai as Ya Nang. Its root has been widely used as antipyretic agent for a fever and also prescribed in the preparation of antimalarial in folk medicine [2]. *T. triandra* showed antimalarial activity against *Plasmodium falciparum* in vitro [3]. There are no reports on antioxidant and anticancer activities including quantity of the extract from *T. triandra*. This is the first report on antioxidant and anticancer activities including quantity of the extract from *T. triandra*. In Nakhonsawan Province.

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2. MATERIAL AND METHODS

Plant Materials :

Aerial parts of *Tiliacora triandra* Diels. in Nakhonsawan province

Preparation of Plant Extracts :

The aerial parts of *Tiliacora triandra* Diels. were cleaned and dried in a hot air oven at 40°C for 24 h. The dried plant was finely ground. Then, the dried plant powders were extracted successively with hexane, chloroform, and methanol, respectively for three days. For each extraction step, the solvent was removed in vacuo to give crude extract.

Determination of Antioxidant Activity

DPPH radical scavenging activity :

The antioxidant activity of the extracts was determined by the DPPH radical scavenging assay [4] with some modifications. The DPPH 6.6 mg/mL (in ethanol) was prepared and stored in the dark before use. Various concentrations of Trolox standard solutions and the sample solutions were prepared using ethanol as solvent. For the DPPH assay, added 100 µL of the sample (concentration of 5 mg/mL), 0.3 mL of absolute ethanol and 3.0 mL of DPPH solution were mixed. The absorbance was determined at 540 nm after incubation for 5 min. at room temperature. All determinations were carried out at least three times, and in triplicate. The percentage inhibition was calculated by the following formula (Equation 1) :

$$\% \text{ inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

A linear regression ($R^2 = 0.9995$) of standard Trolox (Figure. 1) was also used to calculate the radical scavenging capacity.

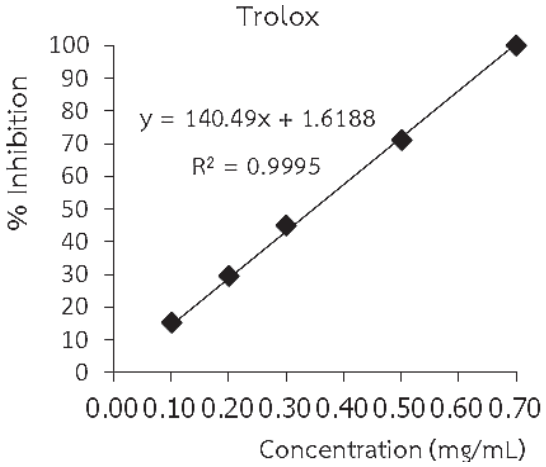

Figure 1. Calibration plot for DPPH assay

ABTS•+ radical scavenging activity :

The antioxidant activity of the extract was performed using the ABTS radical cation scavenging assay and [5] compared with the Trolox standard. For the ABTS assay, 20 µL of the extract (concentration of 5 mg/mL) was mixed with 2.0 mL of diluted ABTS solution. The absorbance (0.025±0.700) was determined at 734 nm after incubation for 5 min. at room temperature. All determinations were carried out at least three times, and in triplicate. The percentage inhibition was calculated by the following formula (Equation 1) A linear regression ($R^2 = 0.9992$) of standard Trolox (Figure. 2) was also used to calculate the radical scavenging capacity.

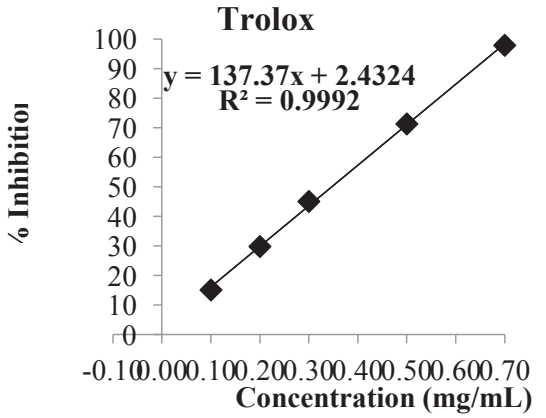

Figure 2. Calibration plot for ABTS assay

Determination of Anticancer Activity

The anticancer activity of the extracts was assayed using three cancerous human cell lines: KB cell line (epidermoid carcinoma of oral cavity, ATCC CCL-17), MCF 7 cell line (breast adenocarcinoma, ATCC HTB-22), and NCI-H 187 cell line (small cell lung carcinoma, ATCC CRL-5804). This assay was performed using the method described by Brien *et al*., [6] with some modifications. In brief, cells at a logarithmic growth phase were harvested and diluted in fresh medium to 7×10^4 cells/mL for KB and 9×10^4 cells/mL for MCF-7 and NCI-H 187. Successively, 5 µL of each test sample (the hexane, chloroform, and methanol extracts) was diluted in 5% DMSO. Then, 45 µL of cell suspension were added to 384-well plates and incubated at 37°C in 5% CO_2 incubator. After the incubation period (3 days for KB and MCF-7; 5 days for NCI-H187), 12.5 µL of 62.5 µg/mL Resazurin solution was added to each well, and the plates were then incubated at 37°C for 4 hours. Fluorescence signal was measured using a SpectraMax M5 multidetection microplate reader at the excitation and emission wavelengths of 530 nm and 590 nm, respectively. The percentage inhibition of the cell growth was calculated by the following equation (Equation 2) :

$$\% \text{ Inhibition} = [1 - (FUT / FUC)] \times 100$$

where FUT and FUC are the mean fluorescent unit from treated and untreated conditions, respectively. IC_{50} values were derived from dose-response curves using six concentrations of twofold serially diluted samples, by the SOFTMax Pro software (Molecular device). Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

3. RESULT AND DISCUSSION

The percentage yields of the crude extracts were calculated based on a dry weight. The hexane, chloroform and methanol extract of the plant from Mueang District gave the percentage yields (w/w) at 35%, 58% and 67% , respectively. The extracts of this plant from Ta Khli District gave the percentage yields are as follow: the hexane extract gave the percentage yields (w/w) 28 %, the chloroform extract gave the percentage yields (w/w) 51% and the methanol extract gave the percentage yields (w/w) 72 %, respectively. The extracts of this plant from Banphot Phisai District gave the percentage yields are as follow: the hexane extract gave the percentage yields (w/w) 31 %, the chloroform extract gave the percentage yields (w/w) 44% and the methanol extract gave the percentage yields 72% (w/w), respectively. The extracts of this plant from Kao Liao District gave the percentage yields are as follow: the hexane extract gave the percentage yields (w/w) 29 %, the chloroform extract gave the percentage yields (w/w) 57% and the methanol extract gave the percentage yields (w/w) 77 %, respectively. The extracts of this plant from Lat Yao District gave the percentage yields are as follow: the hexane extract gave the percentage yields (w/w) 27 %, the chloroform extract gave the percentage yields (w/w) 35 % and the methanol extract gave the percentage yields (w/w) 59 %, respectively. The results are shown in Table 1.

Antioxidant Activity:

In the ABTS assay, all three plant extract (hexane, chloroform and methanol) from Mueang District, Ta Khli District and Kao Liao District were not exhibited the antioxidant activity (ABTS) with an IC_{50}.

Only the hexane extracts of the plant from Banphot Phisai District and Lat Yao District exhibited the antioxidant activity (ABTS) with an IC_{50} 2.94 and 2.97 mg/mL, respectively. For DPPH assay, the hexane and methanol extract of the plant from Mueang District exhibited the antioxidant activity (DPPH) with an IC_{50} 4.41 and 0.39 mg/mL, respectively. Only the methanol extract of the plant from Ta Khli District exhibited the antioxidant activity (DPPH) with an IC_{50} 0.36 mg/mL. The hexane and chloroform extract of the plant from Banphot Phisai District exhibited the antioxidant activity (DPPH) with an IC_{50} 4.28 and 3.33 mg/mL, respectively. Only the chloroform extract of the plant from Kao Liao District exhibited the antioxidant activity (DPPH) with an IC_{50} 3.87 mg/mL. Only the hexane extract of the plant from Lat Yao District exhibited the antioxidant activity (DPPH) with an IC_{50} 4.27 mg/mL. The results are shown in Table 1.

Anticancer Activity:

The anticancer activity of the extract of the plant were performed using the resazurin microplate assay (REMA). Only hexane extract of this plant from Mueang **District** showed anticancer activity against of two cancer cell lines: KB-Oral cavity cancer and MCF7-Breast cancer with the IC_{50} values of 41.14 and 38.26 µg/mL, respectively. The results are shown in Table 2.

Table 1. Antioxidant activity and % yield of the extracts

Sample	Antioxidant activities, ABTS	DPPH	% Yield (w/w)
1. Mueang District			
Hexane extract	-	4.41	35 %
Chloroform extract	-	-	58 %
Methanol extract	-	0.39	67 %
2. Ta Khli District			
Hexane extract	-	-	28 %
Chloroform extract	-	-	51 %
Methanol extract	-	0.36	72 %
3. Banphot Phisai District			
Hexane extract	2.94	4.28	31 %
Chloroform extract	-	3.33	44 %
Methanol extract	-	-	72 %
4. Kao Liao District			
Hexane extract	-	-	29 %
Chloroform extract	-	3.87	57 %
Methanol extract	-	-	77 %
5. Lat Yao District			
Hexane extract	2.97	4.27	27 %
Chloroform extract	-	-	35 %
Methanol extract	-	-	59 %
6. Trolox	0.35	0.34	

Trolox was used as a positive control.
IC_{50} is inhibition by 50%.
- is mean of the extracts exhibited antioxidant activity but can not calculate to IC_{50}
*Results from three determinations

Table 2. Anticancer activity of the extracts

Sample	KB-Oral cavity cancer		MCF7-Breast cancer		NCI-H187-Small cell lung cancer	
	Activity	IC_{50} * (µg/ml)	Activity	IC_{50} * (µg/ml)	Activity	IC_{50} * (µg/ml)
Hexane extract						
1. Mueang District	active	41.14	active	38.26	Inactive	-
2. Ta Khli District	Inactive	-	Inactive	-	Inactive	-
3. Banphot Phisai District	Inactive	-	Inactive	-	Inactive	-
4. Kao Liao District	Inactive	-	Inactive	-	Inactive	-
5. Lat Yao District	Inactive	-	Inactive	-	Inactive	-
Chloroform extract						
1. Mueang District	Inactive	-	Inactive	-	Inactive	-
2. Ta Khli District	Inactive	-	Inactive	-	Inactive	-
3. Banphot Phisai District	Inactive	-	Inactive	-	Inactive	-
4. Kao Liao District	Inactive	-	Inactive	-	Inactive	-
5. Lat Yao District	Inactive	-	Inactive	-	Inactive	-
Methanol extract						
1. Mueang District	Inactive	-	Inactive	-	Inactive	-
2. Ta Khli District	Inactive	-	Inactive	-	Inactive	-
3. Banphot Phisai District	Inactive	-	Inactive	-	Inactive	-
4. Kao Liao District	Inactive	-	Inactive	-	Inactive	-
5. Lat Yao District	Inactive	-	Inactive	-	Inactive	-
Ellipticine	active	1.20				
Doxorubicin	active	0.68	active	6.64	active	0.11
Tamoxifen			active	8.23		

Ellipticine, Doxorubicin and Tamoxifen were used as positive control.

IC_{50} is inhibition of cell growth by 50%.

- is mean of the extracts exhibited anticancer activity but

can not calculate to IC_{50}

***Results from three determinations**

For discussion, The hexane, chloroform and methanol extract of this plant exhibited antioxidant activity using the DPPH assay. Only hexane extract of this plant showed antioxidant activity using the ABTS assay. As the result was described by Phadungkit *et al.*, [7] exhibited the 95 % ethanol extract of *Tiliacora triandra* Diels also showed antioxidant activity using the DPPH assay It showed high antioxidant activity with the EC_{50} values at concentrations of 12.69 ± 1.02 µg/mL.

4. CONCLUSION

In conclusion, The methanol extracts of this plant from Kao Liao District gave the highest percentage yields. The hexane extract of the plant from Banphot Phisai District exhibited the highest antioxidant activity (ABTS) with an IC_{50} of 2.94 mg/mL. The methanol extract of the plant from Ta Khli District exhibited the highest antioxidant activity (DPPH) with an IC_{50} of 0.36 mg/mL. Only hexane extract of this plant from Mueang District showed anticancer activity against of two cancer

cell lines: KB-Oral cavity cancer and MCF7-Breast cancer with the IC_{50} values of 41.14 and 36.28 µg/mL, respectively.

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