

Antioxidant and cytotoxic activities of Thai medicinal plants named Hua-Khao-Yen

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Abstract

Two species of Thai medicinal plants named Hua-Khao-Yen were investigated for their antioxidant and cytotoxic activities against two types of human lung cancer cells (A549 and COR-L23) and human normal lung cells (MRC5). The extract procedures used were similar to those practiced by Thai traditional doctors (water and ethanolic extract). The results found that the ethanolic extract of *Dioscorea membranacea* Pierre (DME) exhibited the highest cytotoxic activities against A549 and COR-L23 cancer cells (IC_{50} values were 15.25 ± 1.36 and 12.63 ± 0.43 $\mu\text{g/ml}$, respectively), whereas the water extract of *Dioscorea membranacea* (DMW), the water and ethanolic extract of *Smilax corbularia* (SCW and SCE) showed no cytotoxic activity against all cancer cells. SCW, SCE and DME exhibited antioxidant activities by DPPH assay ($EC_{50} = 6.40 \pm 0.40$, 4.20 ± 0.12 and 10.34 ± 1.4 $\mu\text{g/ml}$, respectively). These results supported using *Dioscorea membranacea* for cancer treatment by Thai traditional doctors.

Keywords: Hua-Khao-Yen, antioxidant, cytotoxic.

Introduction

Thai medicinal plants named Hua-Khao-Yen have mostly been used in Thai traditional medicines preparation for treatment of leprosy, venereal diseases, inflammations, bacterial infections and cancers. Two species of Hua-Khao-Yen including *Dioscorea membranacea* Pierre (Dioscoreaceae) have been possessed potent cytotoxicity against breast cancers with IC_{50} of 11.8 ± 0.75 $\mu\text{g/ml}$, whereas they were nontoxic to normal cell line (SVK 14). Previous study showed *Dioscorea membranacea* have specific activity against the human large lung carcinoma cell (COR-L23) (Itharat *et al.*, 2003 and 2004). *Dioscorea membranacea* also showed high antioxidant activities by DPPH assay ($EC_{50} = 12.53$ $\mu\text{g/ml}$). However, no report about antioxidant and cytotoxic activities of *Smilax corbularia* Kunth (Smilacaceae).

The aim of this study was to compare antioxidant and cytotoxic activities of *Dioscorea membranacea* and *Smilax corbularia* extracts against three types of human lung cell i.e. A549, COR-L23 and MRC5.

Methods

Plant material and Preparation of extract

The fresh rhizome of *Dioscorea membranacea* Pierre (Dioscoreaceae) was collected from Chumphorn province and the rhizome of *Smilax corbularia* Kunth was collected from Chiangmai province and identified by comparison of authentic herbarium in Department of forestry Bangkok, Thailand where the herbarium vouchers kept.

The plants material were dried at 50°C , powdered and divided into two portions. The first portion (100 g of each plant) was boiled for 30 min in water and the filtrated was freeze dried to obtain water extract of each plant. The second portion (100 g of each plant) was macerated with 95% ethanol and the filtrated was evaporated to dryness under reduced pressure to obtain the ethanolic extract of each plants.

DPPH radical scavenging assay

The following assay procedure was modified from those described by Blois, 1958 and Yamasaki *et al.*, 1994. Samples for testing were dissolved in absolute ethanol or distilled water to obtain the highest concentration of 200 µg/ml. Each sample was further diluted for at least 4 concentrations (two-fold dilutions). Each concentration was tested in triplicate. A portion of sample solution (100 µl) was mixed with an equal volume of 6×10^{-5} M DPPH (in absolute ethanol) and allowed to stand at room temperature for 30 min. The absorbance (A) was then measured at 520 nm. BHT (butylated hydroxytoluene), a well known synthetic antioxidant, was tested in the same system as a positive standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The result was expressed as percentage inhibition in the formular below:

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

The EC₅₀ value (effective concentration of sample required to scavenge DPPH radical by 50%) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations by Prism program.

In vitro assay for cytotoxic activity

The cytotoxicity assay was carried out using sulphorhodamine B (SRB) assay (Skehan *et al.*, 1990). Three different types of human cell lines were used i.e. lung adenocarcinoma (A549), large cell lung carcinoma (COR-L23) and normal lung cell (MRC5) (Keawpradub *et al.*, 1996). The monolayered culture of each cell line were seeded in 96-well microtiter plate and incubated to allow for cell attachment (18-24 hours). Then treated cell with 4 serial dilution and 6 replications. The plates were incubated for the exposure time at 72 hours, then the medium was removed and added the new medium. The plates were incubated for recovery period of 72 hours. The survival percentage was measured colorimetrically using SRB assay and the IC₅₀ values (effective concentration of sample required to inhibit cell growth by 50%) was calculated from dose-response curves plotting between %inhibition and concentrations by Prism program. According to American National Cancer Institute (NCI) guidelines (Suffness and Pezzuto, 1990) crude extract with an IC₅₀ values < 30 µg/ml were considered active.

Results and discussion

Water and ethanolic extracts from two species of Thai medicinal plants named Hua-Khao-Yen were investigated antioxidant and cytotoxic activities (Table 1, Fig.1 and Fig. 2). The results indicated that the ethanolic extract of *Smilax corbularia* possessed the highest antioxidant activity by DPPH assay with an EC₅₀ value of 4.2 ± 0.12 µg/ml, followed by the water extract of *Smilax corbularia* and the ethanolic extract of *Dioscorea membranacea* (EC₅₀ = 6.4 ± 0.4 , 10.34 ± 1.4 µg/ml respectively). The water extract of *Dioscorea membranacea* were apparently inactive (EC₅₀ > 50µg/ml). Among four crude extracts were tested, only the ethanolic extract of *Dioscorea membranacea* showed appreciable activity against A549 and COR-L23 with IC₅₀ = 15.25 ± 1.36 and 12.63 ± 0.43 µg/ml, respectively).

Table 1 Percentage of yield, antioxidant activity (DPPH assay) ($\mu\text{g/ml}$) and cytotoxic activity (SRB assay) ($\mu\text{g/ml}$) against two type of lung cancer cells (A549 and COR-L23) and normal lung cell (MRC5).

Plant extracts	Code	%yield	Antioxidant activity (n=3)	Cytotoxic activity (n=6)		
				IC ₅₀ ($\mu\text{g/ml}$)		
				(%inhibiton at concentration 50 $\mu\text{g/ml}$)		
			A549	COR-L23	MRC5	
<i>Dioscorea membranacea</i> (EtOH)	DME	4.25	10.34 \pm 1.40	15.25 \pm 1.36 (84.95)	12.63 \pm 0.43 (90.58)	>50 (52.31)
<i>Dioscorea Membranacea</i> (water)	DMW	24.90	>50	>50 (13.44)	>50 (6.09)	>50 (5.74)
<i>Smilax Corbularia</i> (EtOH)	SCE	12.38	4.20 \pm 0.12	>50 (20.14)	>50 (23.22)	>50 (7.52)
<i>Smilax Corbularia</i> (water)	SCW	8.25	6.40 \pm 0.40	>50 (10.12)	>50 (9.31)	>50 (5.68)
Butylate hydroxytoluene	BHT	-	12.10 \pm 1.20	-	-	-

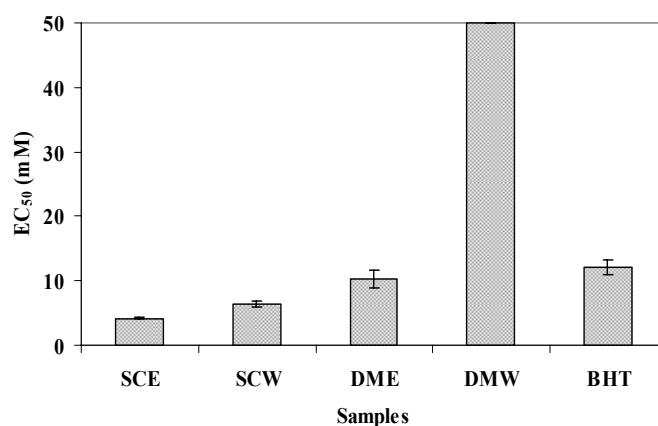


Fig. 1 Antioxidant activity by DPPH assay of Hua-Khao-Yen (EC₅₀ ($\mu\text{g/ml}$) \pm SEM). SCE = *Smilax corbularia* (EtOH extract), SCW= *Smilax corbularia* (water extract), DME = *Dioscorea membranacea* (EtOH extract), DMW= *Dioscorea membranacea* (water extract), BHT = butylated hydroxytoluene.

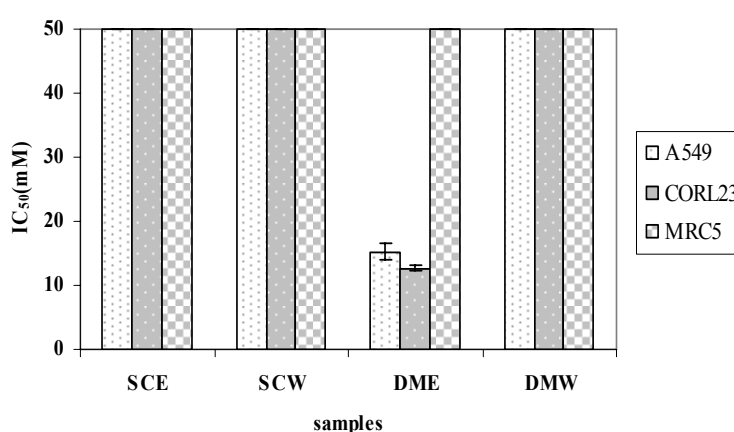


Fig. 2 IC₅₀ ($\mu\text{g/ml}$) of Hua-Khao-Yen against cell lines (n=6) between normal cell (MRC5) and each cancer cell (A549 and COR-L23). SCE = *Smilax corbularia* (EtOH extract), SCW= *Smilax corbularia* (water extract), DME = *Dioscorea membranacea* (EtOH extract), DMW= *Dioscorea membranacea* (water extract).

Conclusion

Cytotoxic activity screening of both species extracts by using the SRB assay was carried out against three human cell lines i.e. A549, COR-L23 and MRC5. The results found that the ethanolic extract of *Dioscorea membranacea* exhibited the highest cytotoxic activity against both types of lung cancer cells but no cytotoxic against lung normal cells and also showed high antioxidant activity whereas the water extract of *Dioscorea membranacea* and both extracts of *Smilax corbularia* showed no cytotoxic activity against all cells. However, both extract of *Smilax corbularia* exhibited high antioxidant activity by DPPH assay. This result conclude that *Dioscorea membranacea* showed specific against only cancer and safety for normal cell. *Smilax corbularia* also used in cancer preparation because it showed high antioxidant activity.

Acknowledgements

We would like to thanks National Research Council of Thailand (NRCT) for financial support.

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