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Comparative biological activities of five Thai medicinal plants called Hua-Khao-Yen

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Abstract

Plants named 'Hua-Khao-Yen' are common ingredients in traditional cancer remedies in Thailand and are best-selling medicinal plants in traditional drugstores. From the selective interview found that Hua-Khao-Yen was found to comprise at least five species such as *Dioscorea membranacea* Pierre (Dioscoreaceae) (DM), *D. burmanica* Prain ex Burkill (Dioscoreaceae) (DB), *Smilax corbularia* Kunth (Smilacaceae) (SC), *S. glabra* Roxb. (Smilacaceae) (SG), or *Pygmeopremna herbacea* Prain et Burkill (Verbenaceae)(PM). Three *in vitro* bioassay were used to compare activities of these plants such as cytotoxic , antioxidant and antimicrobial activities.The extract procedures used were similar to those practiced by Thai traditional doctors (water and ethanolic extraction). The SRB assay used to test cytotoxicity against two types of human liver cancer cell line (HepG2). The lipid peroxidation of liposomes assay was used to test for antioxidant activity of the 5 plant extracts. Disc diffusion method were used for antimicrobial activity. The results found that The ethanolic extracts of DB showed the highest cytotoxic activity against Hela (IC_{50}=28.060±6.338 μg/ml) and the ethanolic extracts of DM showed the highest cytotoxic activity against HepG2 (IC_{50}=18.539±2.371 μg/ml). The water extracts of these plants showed no cytotoxic activity against two types of human cancer cell line. The ethanolic extract of *Dioscorea membranacea* rhizome showed highest antioxidant activity (EC_{50}= 8.09 μg/ml). and also showed the highest activity against with *S. aureus* , *B.subtilis* and *E. floccosum*

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

Introduction

In Thai traditional medicines, herbal drugs named "Hua-Khao-Yen" have been long used as common ingredients in several preparations, including those used in the treatments of lymphopathy, dermopathy, venereal diseases, leprosy, and cancers. Interestingly, despite their close resemblance, the drugs available in traditional drug stores throughout the country are in fact rhizomes from different plant species from at least 3 genera and 5 species, *Dioscorea membranacea* Pierre (Dioscoreaceae) (DM), *D. burmanica* Prain ex Burkill (Dioscoreaceae) (DB), *Smilax corbularia* Kunth (Smilacaceae) (SC), *S. glabra* Roxb. (Smilacaceae) (SG), and *Pygmeopremna herbacea* Prain et Burkill (Verbenaceae)(PM). Among these, we found that the EtOH extract from the rhizome of *D. membranacea* Pierre was potently cytotoxic against various cancer cell lines, including COR-L23, LS-174T, MCF-7, and SVK-14[1]. However, no report about antimicrobial, antioxidant and cytotoxic activities of these plants against liver and cervical cancer cells.

The aim of this study was to compare antimicrobial, antioxidant and cytotoxic activities of five plant extracts against two types of human liver (HepG2) and cervical cancer cells.

Methods

Plant material and Preparation of extract

The part of plants, which were reported to be used against anticancer by folk doctors in Thailand, were collected from all parts of Thailand. Place of collection were all of part of Thailand, *Dioscorea membranacea* Pierre. (Chumporn), *Dioscorea burmanica* Prain ext Burkill. (Chuntaburee), *Smilax corbularia* Kunth.(Chiengmai), *Smilax glabra* Roxb.(Loui), *Pregmeopremna herbacea* Prain et Burkill (Ubonrajatanee). Authentications of plant materials were carried out at the herbarium of the Department of Forestry Bangkok, Thailand where the herbarium vouchers have been kept.

The plants material were dried at 50^{o}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.

Antioxidant Assay by Lipid peroxidation of liposome

This assay are foolowed the method of Uchiyama and Mihara[2]. This assay used liposomes which were prepared from a bovine brain extract suspension in phosphate buffered saline (PBS) (5mg/ml), 0.1 ml $FeCl_3$, 0.1ml ascorbic acid (1mM), 0.5 ml PBS and 0.1 ml of ethanolic or water extract to be assessed. Propyl gallate ($1x10^{-4}$M) is positive control. All test tubes were incubated at 37°C for 20 minutes. The lipid peroxidation of liposomes should occur within that incubation period, unless the test substance exerted a protective antioxidant effect. The extract was prepared as 0.5, 0.1, 0.05, 0.01 and 0.05 mg/ml. Four replicates were carried out for each mixture. The TBA test was performed after the 20 minute incubation at the end of this incubation period at 85°C, thiobarbituric acid should have formed a coloured adduct with malonaldehyde and measure by spectroscopy at 532 nm .

In vitro assay for cytotoxic activity

The cytotoxicity assay was carried out using sulphorhodamine B (SRB) assay[3] (Skehan *et al*., 1990). Two different types of human cell lines were used i.e. liver carcinoma (HepG2) and cervical carcinoma (Hela) . 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 IC_{50} 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 [4](Suffness and Pezzuto, 1990) crude extract with an IC_{50} values < 30 μg/ml were considered active.

Antimicrobial assay [5,6]

In the preliminary studies, all extracts were evaluated for antibacterial activity by disc diffusion method. All extracts were tested against two types of gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two type of gram negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*) and two type of fungi (*Candida albicans* and *Epidermophyton floccosum*). The active plant extracts were diluted to determine the minimum inhibitory concentration (MIC).

Results and discussion

Water and ethanolic extracts from five 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 *Dioscorea membranacea* possessed the highest antioxidant activity by DPPH assay with an EC_{50} value of 8.1±1.21 µg/ml, followed by the ethanolic extract of *Dioscorea burmanica* and *Smilax glabra* (EC_{50} = 16.13 ±3.70, 32.91± 2.02µg/ml respectively). Two ethanolic extract of *Dioscorea membranacea and Dioscorea birmanica* exhibited high cytotoxic against both cancer cells

Table 1 % yield, antioxidant as EC_{50} (µg/ml) ± SEM, cytotoxic as IC_{50} (µg/ml) ± SEM of extracts from five plants called Hua-Khao-Yen against cancer cell lines at exposure time 72 hours (n=3)

Plant extract	% yield	Antioxidant	Cytotoxic	
		Lipid Peroxidation	HepG2	Hela
DM(W)	21.9	742.17± 0.05	>100	>100
DM(Et)	3.4	8.10±1.21	18.539±2.37	32.435±3.152
DB (W)	15.0	334.57±0.05	>100	>100
DB(Et)	3.1	16.13 ±3.70	33.67±0.37	28.060±6.338
SC(W)	9.2	185.65 ± 0.05	>100	>100
SC(Et)	12.03	46.87± 7.50	85.78±1.45	>100
SG(W)	7.2	94.95 ± 0.01	>100	>100
SG(Et)	6.4	32.91± 2.02	>100	>100
PM(W)	6.4	2470.73±0.90	>100	>100
PM(Et)	7.2	754.12 ± 2.40	>100	>100

Note: DM=*Dioscorea membranacea*, DB=*Dioscorea burmanica*, SC=*Smilax corbularia*, SG=*Smilax glabra*, PM=,*Pygmaeopremna herbacea*, W.= Water extract, Et.= Ethanolic extract

Table 2: Antibacterial and antifugal activity screening of the crude extract of all kinds of Hua-Khao Yen at concentration of extract 25mg/ml

Plant meterial	Inhibition zone diameter (mm)					
	E.c.	*B.s.*	*S.a.*	*P.a.*	*C.a.*	*E.f.*
DM(E)	-	14±0.2	14±0.1	-	-	+
DM(W)	-	-	-	-	-	+
DB(E)	-	-	9	-	-	+
DB(W)	-	-	-	-	-	+
SMC(E)	-	10±0.3	13±0.5	-	-	-
SMC(W)	-	-	13±0.0	-	-	+
SMG(E)	-	10±0.1	12±0.4	-	-	-
SMG(W)	-	-	-	-	-	-
PM(E)	-	-	-	-	-	-
PM(W)	-	-	-	-	-	-
Ampicillin(0.003mg/ml)	16±0.0	25.0±0.1	30±0.3	-	NT	NT

Note: Diameter of inhibition zones in millimeters are means of triplicates;-no inhibition,+ inhibit fungi growing ;*E.c.: Escherichia coli; B.s:.Bacillus subtilis; S.a.: Staphylococcus aureus; P.a.:Pseudomonas aeroginosa.; C.a:Candida albican; E.f:Epidermotophytum floccosum* NT= not test, DM : *Dioscorea membranacea*, DB: *Dioscorea birmanica*, SMC: *Smilax corbularia*, SG: *Smilax glabra*, PM: *Pygmaeopremna herbacea*, DMC, DMM, and DMW are chloroform , methanol and water fraction of *Dioscorea membranacea*; E: ethanolic extract, W: water extract.

The results of antibacterial and antifungal screening shown in table 2 indicate that the ethanolic extract of the two genera of *Dioscorea* and *Smilax* showed antibacterial effects

against gram positive bacteria (*S.aureus* and *B.subtilis*) with *D. membrancea* having the highest activity. The result of screening, which was confirmed testing for minimum inhibition concentration (MIC) (Table 3) found that the ethanolic extract of *Dioscorea membranacea* showed the highest antimicrobial activity against gram positive bacteria (*S. aureus*) and fungi (*E. floccosum*). For the two *Smilax* species it was found that the ethanolic extract of *Smilax glabra* exhibited higher antibacterial against *S. aureus, B. subtilis* and *E. coli* than *S. corbularia.* Conversely the water extract of *Smilax corbularia* showed higher antimicrobial activity than *Smilax glabra* and it exhibited antifungal activity against with *E. floccosum*

Table 3: MIC (mg/ml) for extracts of all kinds of Hua-Khao-Yen on antibacterial and antifungal activity

Plant extracts	*S.aureus*	*B.subtilis*	*E.coli*	*C.albicans*	*E.floccosum*
DM(E)	<1.25	<1.25	2.5	>10	<1.25
DB(E)	<1.25	<2.5	5	>10	>10
SC(E)	<1.25	5	2.5	>10	>10
SC(W)	<1.25	5	5	>10	<1.25
SG(E)	<1.25	<5	2.5	>10	>10
SG(W)	<1.25	>10	5	>10	>10

Note: MIC was regarded as the lowest concentration showing no bacterial growth after 24 hours and fungal growth after 7 days.

Conclusion

Cytotoxic activity screening of five species extracts by using the SRB assay was carried out against three human cell lines i.e. HepG2 and Hela. The results found that the ethanolic extracts of Dioscorea birmanica showed the highest cytotoxic activity against cervical cancer and the ethanolic extracts of Dioscore membranacea showed the highest cytotoxic activity against liver cancer cells. The water extracts of all plants showed no cytotoxic activity against two types of human cancer cell line. The ethanolic extract of *Dioscorea membranacea* rhizome showed highest antioxidant activity and also showed the highest activity against with *S. aureus*, *B.subtilis* and *E. floccosum*

This result conclude that *Dioscorea membranacea* showed cytotoxic against cancer cell and also have antioxidant and antimicrobial activity. Thus *this plant was supported* for using of folk doctors for treatment cancer.

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