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Cytotoxic and antioxidant activities of the ethanolic extract from *Dioscorea birmanica* prain & burkill

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Abstract

The objective of this research is to investigating on antioxidant and cytotoxic activities against cancer cells of *Dioscorea birmanica* Prain & Burkill extract which Thai traditional medicine used to treat cancer patients . Cytotoxic activity was tested against two type of lung cancer cell line (CORL23 and A549) and one type of normal lung cell (MRC5) by sulforhodamine B assay The antioxidant activity evaluated by DPPH assay. The results were found that the ethanolic extract of *Dioscorea birmanica* Prain & Burkill showed high cytotoxic activity against lung cancer cells ; A 549 and COR-L23 (IC_{50}= 7.45± 0.31, 8.71 ± 0.29 µg/ ml respectively) but no cytotoxic activity against normal cancer cells MRC5 (IC_{50}= 94.76. ± 1.25 µg/ ml).

The antioxidant activity of the ethanolic extract of this plant was evaluate as EC_{50} as 9.35 ± 0.62µg/ml . Six fractions were isolated by vaccuum liquid chromatography by ordering polarity of solvent (hexane, hexane: chloroform 1:1, chloroform, chloroform:methanol 8:2, chloroform :methanol 1:1 and methanol were code as F1-F6 respectively). It found that F4 showed the highest cytotoxic against A595 and F5 showed the highest activity against COR-L23 (6.07 and 16.44 µg/ml respectively). Two these fraction less active against normal cell. F3 showed the highest antioxidant activity followed by F4 and F5 (11.5, 14.9 and 15.5 respectively). Thus it is concluded that F4 and F5 should be continuous isolated cytotoxic and antioxidant compounds for discovery anticancer drug from this plant in the future

Keywords: cytotoxic activity, antioxidant activity, *Dioscorea birmanica* Prain & Burkill, lung cancer cell line

Introduction

Malignant disease is the leading cause of death in Thailand [1]. There are several types of cancer which are the cause of death in worldwide, lung cancer is the first leading in worldwide. Plant-based systems have a long history of use in traditional health care [2]. World Health Organization estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care and at least 119 chemical substances derived from 90 plant species can be considered as important drugs currently in use in one or more countries [3] (Farnsworth *et al.,* 1985). 74% of these 119 compounds were discovered as a result of chemical studies directed at the isolation of the active substances from plants basic used from traditional medicine [4]. Therefore, the usage of ethnopharmacology, or traditional use, is channeled for discovery of new biologically-active molecules[2]. Thai traditional medicine was used to treat cancer patients for long time ago. From the selective interview southern folk doctor was found that *Dioscorea birmanica* is a one plant to use for treat cancer patients [5]. Thus it should be continue investigation for cytotoxic activity against lung cancer cells which is a biggest problem for people in worldwide.

The objective of this investigation is to test cytotoxic activity against two type of lung cancer and comparison with lung normal cell and also test antioxidant activity. The

bioassay guide fractionation would be used to led to isolate cytotoxic compounds against lung cancer cells and antioxidant activity.

Materials and methods

Plant materials and preparation of extracts

The rhizome of *Dioscorea birmanica* Prain & Burkill were washed and then dried at 50 ◦C, powdered and extracted by methods corresponding to those used by traditional doctors. Dried ground plant material (1kg) was macerated with 95% ethanol for 3 days and the marc was macerated 2 times filter and then concentrate to dryness by evaporator.The extracts of plant were calculated percentage of yield and dissolved in dimethyl sulfoxide (DMSO) before bioassay.

Bioassay guild fractionation from *Dioscorea birmanica* Prain & Burkill extracts

The dried powder rhizomes of *Dioscorea birmanica* Prain & Burkill were macerated with 95% ethanol, and then concentrated under reduced pressure to obtain 11.13% of ethanolic extract. The crude soluble fraction was separated by quick column chromatography over silica gel eluting with a gradient system of increasing polarity as follow, using hexane (1000 ml), hexane:chloroform (1:1) (1000 ml), chloroform (1000 ml), chloroform:methanol (8:2), chloroform:methanol (1:1) and methanol (1000 ml) respectively and drying by rotary evaporation. Each fraction was dried and evaporated to yield 0.36 0.63, 2.85,,9.77,,50.02,and 25.67%, respectively, ,these fractions being denoted as DB1, DB2 ,DB3,DB4,DB5 and DB6. All of these fraction and the ethanolic extract of this plant were tested cytotoxic and antioxidant activities for leading to discovery a cytotoxic compound against lung cancer cells .

In vitro assay for cytotoxic activity

Cell culture

Two different types of human cancerous cell lines as the large cell lung carcinoma (COR-L23) and non small cell lung carcinoma (A549) , normal human lung cell as normal lung fibroblast cell line (MRC-5) were used to test in this study. COR-L23 and A549 are cultured in RPMI 1640 medium supplement with 10% heated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin [6]. MRC-5 is cultured in DMEM culture medium containing 10% heated fetal bovine serum and 1% of 10,000 U penicillin and 10 mg/ml streptomycin. The cells are maintained at 37^{o}C in an incubator with 5% CO_2 and 95% humidity.

Cytotoxic assay

The sulforhodamine B (SRB) assay was used in this study to estimate cell numbers indirectly by staining total cellular protein with the SRB. The protocol was based on that originally described by Skehan, et al. (1990) [7] In brief, cells at the exponential growth phase were detached with 0.25% trypsin-EDTA to make single cell suspensions. The viable cells were counted by trypan blue exclusion using a haemocytometer and diluted with medium to give a final concentration of $1x10^4$, $1x10^4$and $5x10^4$ cells/ml for COR-L23, A549 and MRC-5 respectively. 100 μl/well of these cell suspensions would be seed in 96-well microtiter plates and incubate to allow for cell attachment. After 24h the cells were treated with various concentrations of the extracts. The extracts were diluted in medium to produce the required concentrations 100 μl/well of each concentration would be added to the plates to obtain final concentrations of 1, 10, 50, 100 μg/ml for the extract and 0.1, 1, 10, 50 μM for pure compound, the final mixture would used for treating the cell contained not more than 1% of the solvent, the same as in solvent control wells. The plates were incubated for selected exposure time of 72 hours. At the end of each exposure time, the medium would be

removed. The wells were washed with medium, And 200 µl of fresh medium were added to each well. The plates were incubated for a recovery period for 72 hours. On the seventh day of culture period, cells were fixed by 100 µl of ice-cold 40% trichloroacetic acid (TCA) per well, incubated at 4°C for 1 hour in the refrigerator and washed 5 times with tap water to wash non viable cells, so viable cells were fixed as monolayer in each well. 50µl of SRB solution (0.4% w/v in 1% acetic acid) was added to each well and left in contact with the cells for 30 min; then the plates were washed 4 times with 1% acetic acid until only dye adhering to the cells are left. The plates dry and 100 µl of 10 mM Tris base (tris (hydroxy methyl) aminomethane, pH 10.5) was added to each well to solubilize the dye. To shake the plates gently for 20 minutes on a gyratory shaker. Read the absorbance (OD) of each well (4 replicate) on Microplate reader at 492 nm as an indication of cell number. To measure cell survival as the percentage absorbance compared with the control (non-treated cells). The IC_{50} values calculated from the Prism program obtained by plotting the percentage of survival versus the concentrations, interpolated by cubic spine.

***In vitro* assay for Antioxidant activity**

DPPH radical scavenging assay [8] pipetted sample solution in each concentration 100 µl in 96-well plate, added DPPH solution 100 µl in each sample and mixed.(Final concentration of sample 100, 50, 10, 1, 0.5 µg/ml). The absorbance (A) was measured at 520 nm. Calculated by formula %inhibition = [(Acontrol – Asample) / Acontrol] × 100 and EC_{50} value calculated by linear regression analysis by prism program.

Results and discussion

The percentage of yield and antioxidant activities exhibited in table 1. The cytotoxic activity against all cells was showed in table 2 **.**

Table 1. The percentage yields and antioxidant activity by DPPH assay of the extracts (n=3)

Code of Extract	Solvent	%yield	Antioxidant by DPPH assay as EC50 (µg/ml)±SEM
DBE	ethanol	11.13	9.35 ± 0.62
F1	Hexane	0.36	>100
F2	Hexane:Chloroform 1:1	0.63	>100
F3	Chloroform	2.85	11.50 ± 0.59
F4	Chloroform:methanol 8:2	9.77	14.85 ± 1.40
F5	Chloroform:methanol 1:1	50.22	15.51 ± 0.30
F6	Methanol	25.67	45.33 ± 1.7
BHT			10.35±0.40

The ethanolic extract of *Dioscorea birmanica* Prain & Burkill showed high activity against A 549 and CORL23 (IC_{50} = 7.45 and 8.71 respectively) but this extract showed no cytotoxic activity against normal lung cell (IC_{50} = 94.76 µg/ml). These results showed that this extract showed specific cytotoxic activity against only cancer cell but not kill normal cells. In addition, the ethanolic extract also showed high antioxidant activity because it showed higher antioxidant activity than BHT as positive control. This extract is good two points for discovery cancer drug which should kill only cancer cells but not kill normal cells and also good for being antioxidant product in the future. F4 and F5 showed high potentcy against CORL23 and A545 and less active for normal cell MRC5. F4 showed the highest cytotoxic against A595 and F5 5 showed the highest activity against CORL23 (6.07 and 16.44 µg/ml respectively). Two these fraction less active against normal cell. F3 showed the

highest antioxidant activity followed by F4 and F5 (11.5, 14.9 and 15.5 respectively). F4 and F5 should be continuous isolated cytotoxic and antioxidant compounds

Table2 Cytotoxic acrtivity showed as IC_{50} value (µg/ml) and SEM of *Dioscorea birmanica* Prain & Burkill extract and its fraction against lung cancer cell line (COR L-23 and A 549) and normal cell line (MRC -5) at exposure time (72 hrs) (n = 3)

Code of Extract	Solvent	Cytotoxic activity IC_{50} µg/ml ±SEM		
		COR L23	A549	MRC5
DBE	ethanol	8.71 ± 0.29	7.45 ± 0.31	94.76 ± 1.25
F1	Hexane	96.75 ± 1.25	100 ± 0	100 ± 0
F2	Hexane:Chloroform 1:1	75.66 ± 1.48	100 ± 0	100 ± 0
F3	Chloroform	67.52 ± 2.045	23.01 ± 0.43	83.51 ± 1.20
F4	Chloroform:methanol 8:2	20.22 ± 1.67	6.07 ± 0.25	38.77 ± 1.63
F5	Chloroform:methanol 1:1	16.44 ± 1.23	6.14 ± 0.08	85.57 ± 1.70
F6	Methanol	100 ± 0	89.34 ± 1.74	100 ± 0

Conclusion

It was concluded that the ethanolic extract of this plant showed cytotoxic activity against human lung cancer cell but no cytotoxic against normal lung cells and it also showed high antioxidant activity . From bioassay guide fractionation , fraction which isolated by Chloroform and methanol showed potential for find out cytotoxic and antioxidant compounds for discovery anticancer drugs and antioxidant product from this plant in the future

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