Original article **P03**

Study on cytotoxic and antioxidant activities of *Pseuderathemum platiferum*

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Abstract:

The aim of this investigation was to determine the total phenolic content, cytotoxic and antioxidant activities of *Pseuderanthemum palatiferum* extracts (Payawanon in Thai). It was used to treat various diseases in Veitnam. Its leaves which were used to treat cancer were extracted by different methods and got 11 crude extracts. These extracts were tested cytotoxic activity against two types of lung cancer cells (COR-L23 and A549) and liver cancer cells (HepG2) by SRB assay. They were also tested antioxidant activity by DPPH assay and determine total phenolic content using the Folin–Ciocalteu method.. They were found that the dry leaves extract by macerated with 95%EtOH showed cytotoxic activity against COR-L23 and A549 (IC_{50} = 31.70 and 40.68 µg/ml respectively). The dry leaves extract by decoction method exhibited the highest antioxidant activity (EC_{50} value of 5.95 µg/ml). The total phenolic content of dry leaves extract by soxhlet extraction with methanol showed the highest content followed by fresh leaves extract by maceration with 95%EtOH and fresh leaves extract by decoction (GAE = 137.17, 127.58, 126.75 mg/g, respectively). In the conclusion, *P*. palatiferum have non specific cytotoxic effect against lung cancer and also showed high total phenolics content and high antioxidant activity. Thus, these results suggest that this plant could be supported ethnomedical used to treat cancer .

Keywords: *Pseuderanthemum palatiferum*, Cytotoxicity, SRB assay, Antioxidant activity, Phenolic content

Introduction

Pseuderanthemum palatiferum (Nees) Radlk is a shrub or small tree occurring in Northern Veitnam.(Cuong and Quynh, 1999) It belong to the family of the Acanthaceae.(HO *et al*., 2000) This plant is a medicinal plant in Vietnam used by some population to treat various diseases such as digestive disorders, stomach trouble, large intestine inflammation, bleeding injury, kidney and liver diseases and cancer. (Dieu *et al*., 2005) Its leaves also is vegetable . The phytochemical investigation of *P. platiferum* leaves found that its compounds were flavonoids, apigenin, triterpenoids saponin, β-sitosterol, stigmasterol, kaempferol, and salicylic acid. (Hung *et a*l., 2004). The objective of this study, we aims to study on cytotoxic activity against lung cancer cells and also investigate antioxidant activity by DPPH assay and determine total phenolic content.

Methodology

Plant material

Pseuderanthemum palatiferum (Nees) Radlk were collected from Amphor Wichenburi, Petchaboon Province, Thailand. Authentication of plant materials were carried out at the herbarium of the Department of Forestry Bangkok, Thailand where the herbarium vouchers have been kept to specify plant and species identified.

Preparation of sample

The method of extracts were devided 3 methods. The dry leaves were dried at 50°C, powdered and macerated with 95 % ethanol. It was filtrated and concentrated to dryness

under reduced pressure to be ethanolic extracts (EtOH). The dried leaves were also extracted by soxhlet apparatus using ordering polarity solvent as hexane, chloroform and methanol for 8 hours each. They were concentrated by evaporator. The decoction was also used for the dry leaves extract . They were prepared according to folk medicine. It were boiled in distilled water and the final volume reduced to1/3volume then filtered and dried by the lyophilizer. The fresh leaves were extracted by the method of dry leaves extracts above . Another method for fresh leaves which differ dry leaves extract method, they were blended, filtrate and dry by lyophillizer The percentage of yields were showed in Table 1. The water extracts were dissolved in sterile water and the ethanolic extracts were dissolved in DMSO and all stock solution were filtrated by sterile filter paper (0.2μm) before cytotoxic testing.

In vitro Assay for Cytotoxicity

Human cell lines

Two type of lung cancer cells such as human large cell lung carcinoma (COR-L23) and carcinomic human alveolar basal epithelial cells (A549) were used to test. Human hepatocellular liver carcinoma cell line (HepG2) was also studied. COR-L23, A549 were cultured in RPMI 1640 medium supplement with 10% heated foetal bovine serum, 50 IU/ml penicillin and 50 μg/ml streptomycin. HepG2 were cultured in Minimum Essential Media (MEM) with Earle Salt (without glutamine medium supplement) with 10% heated foetal bovine serum, 50 IU/ml penicillin and 50 μg/ml streptomycin and 1% non-essential amino acid and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity (Keawpradub *et al., 1999*). According to their growth profiles, the optimal plating densities of CORL23, A549 and HepG2 were determined $1x10^3$, $1x10^3$ and 3 x 10^3 cells/well respectively to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number when analyzed by SRB assay

Cytotoxicity assay

The brief of the assay , the cell pellets were resuspensed and viable cells were counted to give a final concentration of $1x10^3$, $1x10^3$ and $3x10^3$ cells/well for CORL23, A549 and HepG2 .100 μl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24h the cells were treated with the extracts. Each extract was initially dissolved in an amount of DMSO for the ethanolic extracts and sterile distilled water for the water extracts. The extracts were diluted in medium to produce 5 concentrations and 100 μl of each concentration was added to each well of the plates in 6 replicates to obtain final concentrations of 100, 50, 10, 1, 0.1 μg/ml for extract . The final mixture 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 times of 72 hours. as indicated. At the end of each exposure time, the medium was removed. The wells were then washed with medium, and 200 μl of fresh medium were added. The plates were incubated for recovery period of 6 days and cell number were analyzed by SRB assay (Skehan et al 1990). The IC_{50} values were calculated from the Prism program obtained by plotting the percentage of survival versus the concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines [10] extracts with IC_{50} values < 20 μg /ml were considered active.

Evaluation of the antioxidant activity

The ability of the extract to annihilate the DPPH radical was determined by the method of Yamasaki *et al*, (1994). In this assay expressed as EC_{50} (μg/ml) according to the DPPH radical scavenging assay using BHT as a standard. Samples for testing were prepared by dissolution in absolute ethanol (1, 10, 50, and 100 μg/ml in final conc.). A sample solution was mixed with the same volume of 6 x 10^{-5} M DPPH in absolute ethanol. After 30 min incubation in darkness and at ambient temperature, the absorbance was recorded at 520 nm.

(The changes in color from purple to a residual pale yellow color). The percentage inhibition was calculated using Eq.1. Estimated EC_{50} values are presented as the average of quadruplicate analyses (Yamasaki *et al*., 1994). The data are mean ±SD. Determination was done in triplicate.

$$\text{Percentage of inhibition} = [Abs_{control} - Abs_{sample}/Abs_{control}] \times 100 \quad \text{(Eq.1)}$$

The scavenging activity of the *Pseuderanthemum palatiferum* extracts is shown in Table 2.

Determination of total phenolic content

The content of total phenolic content in samples was determined by the Folin–Ciocalteu's (FC) reagent (Miliauskas *et al*., 2004) using gallic acid as standard. This method is based on the reduction of a phosphowolframate–phophomolybdate complex by phenolics to blue reaction products. For the preparation of calibration curve were dilution of 5, 10, 20, 40, 80 and 100 µg/ml. Aqueous gallic acid solutions were mixed with 100µg/ml FC reagent and 20µg/of sodium carbonate. Preparation of the sample were dilution of 500 µg/ml. Sodium carbonate of 20µg/ml and 100mg/ml of Folin–Ciocalteu's reagent were added. The absorbance of the standards and samples was measured at 765 nm after 30 min of reaction at room temperature. The results were expressed as mg of gallic acid equivalents (GAE). All tests were run in triplicate and averaged. The amounts of total phenolics in the studied herbs are shown in Table 2.

Results

Table 1. Percentage yield and IC_{50} of cytotoxicity assay of *Pseuderanthemum palatiferum* extracts.

Part of used	Methods	Extracts	% yeild	IC_{50}(µg/ml)		
				COR-L23	A549	HepG2
Fresh leaves	Blended	Water	2.53	>100	>100	>100
	Decoction		1.93	36.05± 0.1	64.94± 0.6	>100
	Maceration	95%EtOH	1.66	71.45± 0.3	78.23± 1.1	>100
	Soxhlet extraction	Hexane	0.13	48.61± 0.6	>100	52.84± 1.4
		Cholofrom	1.92	>100	>100	>100
		Methanol	0.66	>100	>100	>100
Dry leaves	Decoction	Water	26.42	>100	>100	>100
	Maceration	95%EtOH	9.10	31.70± 0.9	40.68± 1.5	65.63± 2.5
	Soxhlet extraction	Hexane	1.59	>100	>100	>100
		Cholofrom	2.15	>100	>100	>100
		Methanol	7.79	>100	>100	>100

Discussion

Its dry leaves extract by decoction showed the highest percentage of yield (26.42%). Cytotoxic activity of its dry leaves extract by maceration with 95%EtOH showed the best cytotoxic effect against COR-L23 and A549 (IC_{50} = 31.70 and 40.68 µg/ml respectively) but it show less cytotoxic effect against HepG2 (IC_{50} = 65.63 µg/ml). It showed as specific activity for lung cancer treatment better than liver cancer. Dry leaves extract by decoction exhibited the highest antioxidant activity, followed by dry leaves extract by soxhlet extraction with methanol (EC_{50} = 5.95 and 10.95 µg/ml, respectively) while BHT showed less antioxidant activity than two these extracts (EC_{50} = 11.35 µg/ml) . The total phenolic contents were observed in dry leaves extract from soxhlet extraction with methanol, fresh

leaves extract by maceration with 95% EtOH and followed by fresh leaves extract by decoction (GAE = 137.17, 127.58, 126.75 mg/g, respectively)

Table 2. Antioxidant activity by DPPH assay (EC_{50} μg/ml) and the total phenolic content of the *Pseuderanthemum palatiferum* extracts (n=3)

Part of used	Methods	Extracts	DPPH assay EC_{50} (μg/ml)	total phenolic content GAE(mg/g)
Fresh leaves	Blended	Water	>100	29.07±1.47
	Decoction		12.32±0.54	126.75±3.33
	Maceration	95%EtOH	9.14±0.99	127.58±1.20
	Soxhlet extraction	Hexane	12.44±1.90	26.39±7.33
		Chloroform	>100	28.71±2.02
		Methanol	>100	29.80±5.14
Dry leaves	Decoction	Water	5.95±4.59	53.57±4.81
	Maceration	95%EtOH	53.22±2.61	65.18±12.20
	Soxhlet extraction	Hexane	15.57±1.72	31.17±4.96
		Chloroform	34.42±7	64.42±8.36
		Methanol	10.95±0.52	137.171±1.71
BHT			11.35±0.20	-

Conclusion

We have reported the *in vitro* antioxidant and cytotoxic activities of *P. palatiferum* in Thailand. The most of the antioxidant activity and total phenolic content were fresh leaves maceration with 95%EtOH. While dry leaves maceration with 95%EtOH shown inhibition to the growth of a human lung cancer cell line. Thus the result is preliminary screening for supplements seem to protect against cancer and health promotion.

Acknowledgements

This study was financially supported by Faculty of Medicine, Thammasat University.

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