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Research Article

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Cell proliferation activities *in vitro* model of Thai mimosaceous extracts

T. Tunsaringkarn[1], J. Suwansaksri[2], A. Rungsiyothin[1] and A. Palasuwan[3*]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand
[2]Department of Clinical Chemistry, [3]Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand

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ABSTRACT

The proliferation and inhibition (anti-proliferation) of human white blood cells in vitro model from 21 Thai mimosaceous extracts were investigated. The results showed that plant extracts of Albizia lebbeck (L.) Benth. branch, Adenanthera pavonina L. leaves, Cathormion umbellatum (Vahl) Kosterm. leaves, Albizia lebbeckoides (DC.) Benth. bark, Albizia lebbeckoides (DC.) Benth. branch and Acacia catechu (L.f.) Willd. branch stimulated white blood cell proliferation at 50.88, 40.10, 34.34, 30.83, 24.81 and 24.81 percent respectively, while plant extracts of Albizia procera (Roxb.) Benth. bark, Entada rheedii Spreng. pericarp, Acacia farnesana (Linn.) Welld. Twig, Parkia speciosa Hassk. pericarp and Acacia catechu (L.f.) Willd. leaves inhibited white blood cell proliferation at 24.56, 20.05, 17.67, 17.17 and 14.54 percents respectively at concentration 25 µg/ml. This study found that tannin contamination which was found in all plant extracts had cell proliferation activity 14.79% at concentration 25 µg/ml. However, there was no correlation between cell proliferation activity and tannin concentration in plant extracts. These findings suggest that tannin may interfere the cell proliferation activity in plant extracts and should be considered for future study in vivo model test of their activities.

Key words: Cell proliferation, mimosaceous plant, plant extract, *in vitro*

INTRODUCTION

Cell proliferation is increased in cell number as a result of cell growth and division. The accurate assessment of cell number and cell proliferation is useful in many high content assays and is a key readout in cytotoxic cell and apoptosis applications. Cell proliferation is also a very sensitive indicator of cell stress since it requires intact cell structures and function [1]. The biology of cell division, differentiation, and apoptosis is exceedingly similar in both normal and cancer cells [2]. Fundamentally, cancer is a disease of uncontrollable accumulation of clone cells. Abnormal cell proliferation is necessary, although often insufficient, for tumorigenesis. It is increased in tumor cell number, and thus tumor burden that ultimately accounts for the adverse effects on the host or human. Indeed, the goal of most current cancer therapy is to reduce the number of tumor cells and to prevent their further accumulation [3]. Many cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents such as phenolic acid, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinines which have antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects [4]. Tannin is a substance found in many different plants. Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. The most abundant polyphenols are the condensed tannins, found in virtually all families of plants in the tree bark, wood, fruit, fruitpod, leaves, and roots and also in plant gall comprising up to 50% of the dry weight of leaves. Tannins have benefits on antiseptic, antiviral [5], antibacterial [6], anti-parasitic effects [7] (Kolodziej et al, 2005) and anti-inflammatory and antiulcer activities [8]. Although tannins showed strong antioxidant property for possible therapeutic applications [9], the high dose of tannins was toxic for human and animal by protein precipitation [10,11]. Our previous study reported that some Thai medicinal plant such as mimosaceous plants

showed high antioxidant and α-glucosidase inhibition activities [12]. In addition, the recently study indicated that Thai mimosaceous plants which contained high antioxidant activity as demonstrated by Heinz body inhibition activity [13]. Therefore, the tannin concentration of all extracts should be investigated in parallel. The screening data of Thai mimosaceous plant extracts on human blood cell proliferation is still lacked. Therefore, the aim of this study was to investigate twenty-one Thai mimosaceous plant extracts on the activation and inhibition of human white blood cell proliferation compared to tannin concentration for future therapeutic applications.

EXPERIMENTAL SECTION

Sample preparation

Twenty one Thai mimosaceous plants were separated in part of stem, bark, branch, leaves and twig and dried in air. They were grounded and keep in room temperature and ethanol extracted by soxhlet's apparatus for 8 hours or solvent colorless. The solvent was evaporated by rotary evaporator and stored at 4^{o}C until cell proliferation analyses.

Cell proliferation activity

PBMC (Peripheral Blood Mononuclear Cell) preparation

Human whole blood (10 ml) was gently washed with 20 ml of PBS (phosphate buffered saline, 1x) solution and centrifuged at 800 g, 25 °C for 15 min. After discarded the supernatant, the washed blood were re-suspended by PBS (1x) solution. The whole mixture was gently overlaid upon 20 ml of lymphoprep™ solution, prior placed in 50 ml-centrifuge tube. The tube was centrifuged (800 g, 25°C) for 20 min and the mixture was separated into 4 layers from top to bottom as, plasma, PBMC, lymphoprep™, and red blood cells, respectively. The PBMC layer were carefully transferred into a new 15 ml-centrifuged tube and then washed with 2 x 10 ml of PBS (1x) by gently mixing and centrifugation at 300 g 25°C, for 10 min. The pellet residue was re-suspended with 0.5 ml of working RPMI-1640 medium.

Cell culture in herb extracts

The PBMC was cultured in 100 µl of RPMI-1640 working media (1% pen – strep solution and 20% fetal bovine serum in RPMI-1640) and each herb extract was added 25 µg/ml of the medium in each well. The PBMC was prior calculated to add in for about 100,000 cells/well. The micro plate was incubated at 37°C 5% CO_2 for 72 hr, and was gently mixed 2 times a day.

XTT cell proliferation test

After incubation for 72 hr, the cultured well was added by XTT working solution 20 µl/well, gently mixed and incubated further for 4 hr in the refrigeration (4°C). The absorbance at 450 nm and 690 nm were recorded using ELISA reader. Calculation of the inhibition percentage was done as followed:

$$\Delta OD = OD_{450\ nm} - OD_{690\ nm}$$

$$\% \text{ Inhibition} = \frac{[\Delta OD_{control} - \Delta OD_{unknown}] \times 100}{\Delta OD_{control}}$$

where:

OD = Optical Density

Control = The well cultured without herb extracts

Tannin Analysis

Tannin analysis was conducted following the modified method by Hagerman and Bulter (1978) [14]. All plant extracts were dissolved in 10% ethanol and 20 µl of the solution mixed in micro plate with 25 µl of ferric chloride (1.6 mg/ml), and added total volume to 200 µl by buffer. Then, the mixtures were incubated at 37^{o}C for 10 min and stood for 5 min and then quantified by measuring by spectrophotometer at 510 nm.

RESULTS AND DISCUSSION

The scientific name and used part of mimosaceous plants, yield and tannin concentration of plant extracts were shown in Table 1. Most of high yield plant extracts were bark and leaves. The plant extracts at concentration of 25 µg/mL showed that *Albizia procera* (Roxb.) Benth. bark, *Entada rheedii* Spreng. pericarp, *Acacia farnesana* (Linn.) Welld. twig, *Parkia speciosa* Hassk. pericarp, *Acacia catechu* (L.f.) Willd. leaves, *Archidendron jiringa* I.C. Nielsen seed coat, *Parkia speciosa* Hassk. seed coat, *Xylia xylocarpa* (Roxb.) Taub. stem and *Xylia xylocarpa* (Roxb.) Taub. bark had inhibitory cell proliferation at 24.56, 20.05, 17.67, 17.17, 14.54, 12.66, 12.16, 7.39 and 5.01 percents respectively while they had tannin contamination at 50.00, 250.00, 58.00, 250.00, 167.00, 83.00, 350.00, 400.00 and 350.00 mg/g respectively (Figure 1). In addition, *Albizia lebbeck* (L.) Benth. branch, *Adenanthera pavonina* L.

leaves, *Albizia lebbeckoides* (DC.) Benth. bark, *Cathormion umbellatum* (Vahl) Kosterm. leaves, *Albizia lebbeckoides* (DC.) Benth. branch, *Acacia catechu* (L.f.) Willd. branch, *Pithecellobium dulce* Benth. bark, *Xylia xylocarpa* (Roxb.) Taub. leaves, *Samanea saman* (Jacq.) Merr. leaves, *Cathormion umbellatum* (Vahl) Kosterm. bark, *Samanea saman* (Jacq.) Merr. branch, and *Entada rheedii* Spreng. seed coat had cell proliferation activity at 50.88, 40.10, 34.34, 30.83, 24.81, 19.80,17.67, 17.17, 14.79, 14.79 and 9.77% respectively which they had tannin concentration at 6.70, 1.70, 150.00, 250.00, 17.00, 33.00, 13.00, 167.00, 17.00, 167.00, 42.00 and 400.00 mg/g respectively (Figure 2). All plant extracts were found tannin contamination which it has activated cell proliferation 14.79% at concentration 25 μg/ml.

Table 1 Scientific name, Thai name, study part of plants and percentage yield of plant extracts

Scientific name of plants	Used Part	%Yield of Plant Extractions[a] (w/w)	Tannin concentration (mg/g plant extracts)
Acacia catechu (L.f.) Willd.	Branch	2.70	33.00
Acacia catechu (L.f.) Willd.	Leaves	16.38	167.00
Albizia lebbeckoides (DC.) Benth.	Bark	11.04	150.00
Albizia lebbeckoides (DC.) Benth.	Branch	3.93	17.00
Acacia farnesana (Linn.) Welld.	Twig	15.52	58.00
Adenanthera pavonina L.	Leaves	20.72	1.70
Albizia lebbeck (L.) Benth.	Branch	3.40	6.70
Albizia procera (Roxb.) Benth.	Bark	8.18	50.0
Archidendron jiringa I.C. Nielsen	Seed coat	9.01	83.00
Cathormion umbellatum (Vahl.) Kosterm.	Bark	11.41	167.00
Cathormion umbellatum (Vahl.) Kosterm.	Leaves	11.25	250.00
Entada rheedii Spreng.	Seed coat	4.72	400.00
Entada rheedii Spreng.	Pericarp	1.60	250.00
Pithecellobium dulce Benth.	Bark	3.94	133.00
Parkia speciosa Hassk.	Pericarp	not scale weight	250.00
Parkia speciosa Hassk.	Seed coat	19.17	350.00
Samanea saman (Jacq.) Merr.	Branch	4.46	42.00
Samanea saman (Jacq.) Merr.	Leaves	17.88	17.00
Xylia xylocarpa (Roxb.) Taub.	Bark	22.69	400.00
Xylia xylocarpa (Roxb.) Taub.	Leaves	19.44	167.00
Xylia xylocarpa (Roxb.) Taub.	Stem	4.10	350.00

The extract of *Albizia procera* (Roxb.) Benth. bark showed the highest cell proliferation inhibition (24.05%) while it contained low tannin concentration (6.7 mg/g). This finding supported the previous suggestion that 12-17% of tannin content of mimosaceous plant bark [15, 16] which is related to rheumatism [17] and hemorrhage [18]. It is considered useful in treating these problems. *Albizia procera* (Roxb.) Benth. bark is medicinal plants used for tonic and longevity with high antioxidant activity and xanthine oxidase inhibition [19]. In addition, inhibition of xanthine oxidase has been suggested for the treatment of hepatitis and brain tumor [20]. The previous study showed saponin in *Albizia procera* (Roxb.) Benth. bark and showed strong cytotoxicity against A2780 mammalian cell line [21,22].This bark is generally given with salt to water for buffalo drink as in Thai traditional medicine. In India, the leaves are poulticed onto ulcers [16]. *Entada rheedii* Spreng. pericarp also showed high cell proliferation inhibition (20.05%) with high tannin concentration at 250.00 mg/g while the previous study of *Entada rheedii* Spreng. seed extracts showed anti-tumor activity [23]. The extracts of *Acacia farnesana* (Linn.) Welld. twig and *Parkia speciosa* Hassk. pericarp had nearly cell proliferation inhibition activity at 17.67 and 17.17% but the extract of *Parkia speciosa* Hassk. pericarp had higher tannin contamination (250.00 mg/g) than that of *Acacia farnesana* (Linn.) Welld. twig (58.00 mg/g). The tannins are widely distributed in almost all plant foods, species which according to World Health Organization more than 21,000 plants are being in use for medicinal purpose all around the world. But total phenolic content was mostly found to be more than tannin in methanol extract [24]. For the reason, tannins had toxic at high dose [11] and had activation of cell proliferation activity 14.75% at concentration 25 μg/mL (Figure 2). Some plant extracts had much higher tannin concentration, therefore it might interfere cell proliferation activity. It might interact with active ingredients in the extracts because some plant extracts such as *Entada rheedii* Spreng. seed coat and *Cathormion umbellatum* (Vahl) leaves had higher tannin contamination but they were lower cell proliferation activity than *Albizia lebbeck* (L.) Benth. branch and *Adenanthera pavonina* L. leaves. On the other hand, tannin contamination of all plant extracts was much higher than those of tannin test (25 μg/mL), it may have toxic effects at high concentration to human or animals. However, there was no statistical correlation between the percentage of cell proliferation activation and tannin concentration as same as no correlation between the percentage of cell proliferation inhibition and tannin concentration in this study. The extracts of *Albizia lebbeck* (L.) Benth. branch and *Adenanthera pavonina* L. leaves showed high cell proliferation activity at 50.88 and 40.10% with low tannin contamination at 6.7 and 1.7 mg/g., but *Entada rheedii* Spreng. seed coat showed low cell proliferation activity at 9.77% with high tannin contamination at 400.00 mg/g. As the same reason, *Albizia lebbeck* (L.) Benth. branch and *Adenanthera pavonina* L. leaves may useful for wound healing. The previous study showed that biochemical contents of *Albizia lebbeck* (L.) Benth were responsible for anti-helmintic activity [25] and treatments of cough, abdominal tumor, flu, lung problems, gingivitis, inflammation [26,27]. The Extracts of *A. lebbeck* stem

bark contain tannins, flavonoids, anthraquinones, saponins, steroids, terpenoids, and coumarins which ethanolic extracts and petroleum ether were tested against four models of inflammation in rats [28, 29]. In addition, the bark of *A. lebbeck* has been reported to a possess depressant of central nervous system. [30]. *Albizzia lebbeck* is traditionally important medicinal plant in many ayurvedic preparations [31].

Adenanthera pavonina L. has been used as traditional medicine for the treatment of asthma, boil, diarrhea, gout, inflammation, rheumatism, tumor and ulcer, and also used as a tonic, antibacterial and antifungal agents [32-36]. There are lack of phytochemical screening, antioxidant and cell proliferation activities from plant extracts for providing unlimited opportunities new drug [37]. The future development of the pharmacognostic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal medicine.

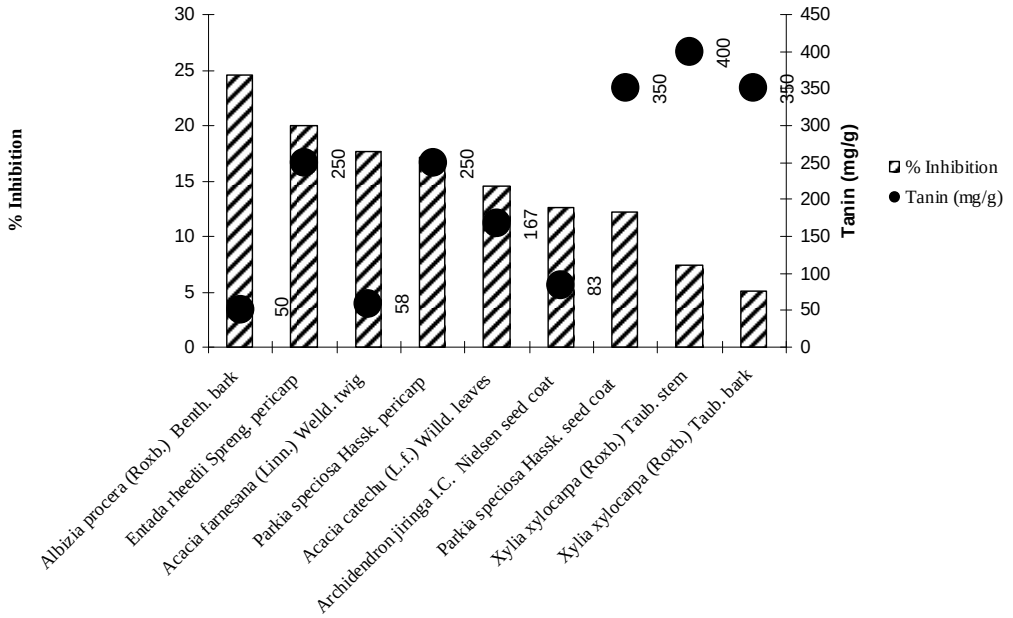

Figure 1 Inhibitory cell proliferation of plant extracts

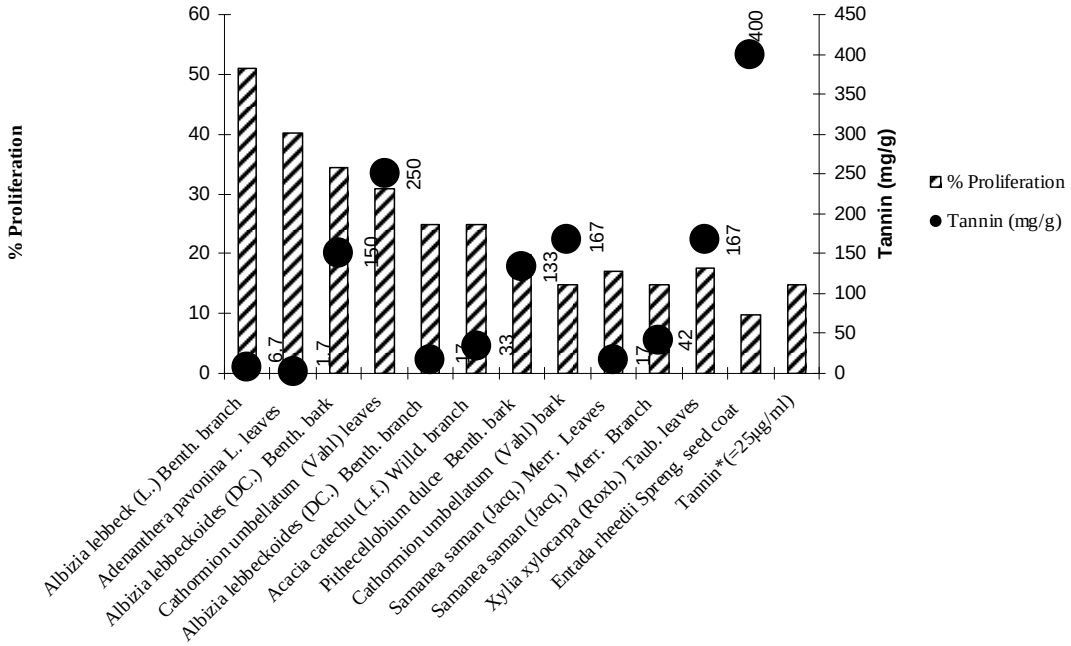

Figure 2 Activation cell proliferation of plant extracts

CONCLUSION

This study indicated that *Albizia lebbeck* (L.) Benth. branch and *Adenanthera pavonina* L. leaves could inhibit white blood cell proliferation while they had low tannin contamination. This benefit may useful for therapeutic cancer application in the future. Moreover, the extracts of *Albizia lebbeck* (L.) Benth. branch and *Adenanthera pavonina* L. leaves showed high cell proliferation activity which may benefit for wound healing. This study also suggested that all plant extracts should separate tannin contamination for future study of plant extract activities.

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