



Antioxidant and anti-tumor activities of pectinesterase inhibitor fractions from jelly fig (*Ficus awkeotsang* Makino) achenes

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Abstract

The aims of this study are to observed antioxidant activities of jelly fig fractions (ferrous ion chelating activity, DPPH radical scavenging activity, and reducing power) and anti-proliferative activity against K562 myeloid leukemia cells. The jelly fig fractions were obtained by separating polyphenol and protein from pectin esterase inhibitor (PEI) extract into crude polyphenol (CP) and protein fraction (PR). CP was further purified by liquid chromatography into purified polyphenol (PP). The results showed that PP fraction had the highest antioxidant activities and anti-proliferative activity against K562 myeloid leukemia cells, while PR fraction had the lowest. All fraction of jelly fig had higher ability (lower EC₅₀ values) in DPPH radical scavenging activity than ferrous ion chelating activity. Comparing the IC₅₀ of anti-proliferative activity of different fractions of jelly fig after 24 h treatment, PP fraction had the lowest value (27.27 µg/mL), followed by CP (85.89 µg/mL), PEI (144.47 µg/mL) and PR (too low to be determined). Polyphenol of jelly fig showed promising bioactivities against oxidation and leukemia cell proliferation.

Keywords: Jelly fig, Antioxidant, Anti-proliferation, K562 myeloid leukemia cells, Polyphenol.

Introduction

Leukemia is the most common cause of cancer death among males under age forty and females under age twenty¹. As the consumer awareness on healthy natural products, natural compounds derived from foods can act as promising strategies to devitalize malignant cells through apoptosis. Jelly fig (*Ficus awkeotsang* Makino) achenes are commonly used to make jelly curd, a local popular summer drink in Taiwan. Pectin esterase inhibitor (PEI) is an extract from jelly fig (*Ficus awkeotsang* Makino) achenes which can eliminate the activity of pectin esterase enzyme. The potential of PEI as anti-tumor agent against cancer cell has been reported²⁻⁴. However, antioxidant activities and anti-proliferative activity against myeloid leukemia cells of different fractions from PEI have not been observed. Therefore, this study aims to observe the antioxidant activities (ferrous ion chelating activity, DPPH radical scavenging activity and reducing power) and anti-proliferative activity against myeloid leukemia cells of jelly fig fractions to ensure which fraction caused its bioactivities.

Materials and methods

Preparation of jelly fig's fractions: Jelly figs were purchased from Taoyuan District office, Kaohsiung City. The pectinesterase inhibitor (PEI) was prepared from jelly figs as reported previously⁵. In brief, jelly fig achenes that had been rinsed repeatedly in 15 volumes (w/v) of 4% NaCl solution were rinsed again twice with 20 volumes (w/v) of distilled water to

wash out residual salt. After drying at 50°C in an oven, PEI were extracted from achenes by using 15 volumes (w/v) of distilled water for 6 h followed by centrifugation (20000 ×g, 30 min) to collect the supernatant. Collected supernatant were then lyophilized and stored at 4°C prior to further extraction and analysis.

The lyophilized PEI was extracted with 70% alcohol for 24 h at 4°C twice to precipitate protein (PR) and the supernatant collected was crude polyphenol (CP) fraction. CP was further separated for its polyphenol with Sephadex LH-20 chromatogram (H₂O-MeOH, 0-100%) and 80 tubes (each tube/minute) were collected. Collected tubes were further purified by HPLC using C-18 column (15 cm x 4 mm) with mobile phase of methanol: acetonitrile: water (55: 30: 15) to collect purified polyphenol fraction (PP).

Ferrous ion chelating activity: Different lyophilized fractions and EDTA (ethylenediaminetetraacetic acid) were dissolved in methanol. The Fe²⁺-chelating ability was determined according to the method of Huang *et al.*⁷. The sample was mixed at a ratio of 10:1:2 of sample: 2 mM FeCl₂: 5mM ferrozine.

The mixture was shaken and left to stand at room temperature for 10 min. The resulting solution was measured at the absorbance of 562 nm. The chelating ability (%) of the sample was calculated using the following equation: [1-Absorbance of sample/ Absorbance of control]×100%.

DPPH radical scavenging activity: Different lyophilized fractions and vitamin C (ascorbic acid) were dissolved in methanol. The reaction was started by addition of 1.0 ml solution of 200 μ M DPPH solution in methanol into 3 ml of sample or ascorbic acid⁶. The reaction mixture was kept at 30⁰C for 30 min and the absorbance was measured at 517 nm. Radical scavenging activity (%) was calculated using the following equation: $(1 - (\text{Absorbance of sample} / \text{Absorbance of control})) \times 100\%$.

Reducing power: Reducing power was carried out from the method which was mentioned by Canabady-Rochelle *et al.*⁸. Amount of 70 μ l sample solution or vitamin C (ascorbic acid) was mixed with 35 μ l of potassium ferricyanide 1% (w/v) and incubated for 20 min at 50⁰C. Afterwards, 135 μ l of distilled water, 33 μ l of 10% (w/v) trichloroacetic acid (TCA), and 27 μ l of 0.1% (w/v) ferric chloride (FeCl₃) were added into the solution and the solution was incubated for 10 min at room temperature. The absorbances of the solutions were measured at 700 nm.

Culture of K562 myeloid leukemia cells: K562 myeloid leukemia cells (ATCC: CCL-243) purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan) was cultured using RPMI-1640 medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin-streptomycin mixture. The cultures were incubated at 37⁰C in a 5% CO₂ incubator.

Cell viability measurement: Cultures of K562 were treated with different concentrations of PEI, CP, PP, and PR at 37⁰C in a humidified 5% CO₂ incubator for 24-72 h. Untreated cells were used as negative control. Viable cells were counted using 0.04% trypan blue exclusion dye in a hemocytometer. Growth inhibition (%) was calculated by the equation of:

$(1 - (\text{viable cell number of treatment group} / \text{viable cell number of control group})) \times 100\%$. From this calculation of growth inhibition at different concentrations, IC₅₀ was determined. IC₅₀ is the concentration required to inhibit 50% of cell viability.

Statistical analysis: Results were presented as mean \pm standard deviation (SD) of three replications. Statistical analysis was assessed by the ANOVA with Duncan's post hoc using SPSS program with *p*-value of less than 0.05.

Results and discussion

Antioxidant activities of different fraction of jelly fig achenes are shown in Figure-1. In general, polyphenol fraction of jelly fig had the highest antioxidant activities, including ferrous ion chelating activity (Figure-1A), DPPH radical scavenging activity (Figure-1B), and reducing power (Figure-1C), while protein fraction had the lowest antioxidant activity mentioned above. Moreover, polyphenol fraction of jelly fig had almost the same capacities compared to their positive controls.

The EC₅₀ values of different fraction of jelly fig are shown in Table-1. The lower the EC₅₀ values, the higher the antioxidant activity as it represent the concentration needed to have 50% of antioxidant capacity. For reducing power, the antioxidant capacity is not shown in percentage, so the EC₅₀ value represents the concentration that the absorbance at 700 nm is 0.5. The EC₅₀ of protein fraction (PR) cannot be determined due to its limited antioxidant activities. For ferrous ion chelating activity and DPPH radical scavenging activity, purified polyphenol (PP) fraction and positive control had the lowest EC₅₀ values, followed by crude polyphenol (CP) and pectin esterase inhibitor (PEI). For reducing power, EC₅₀ of PP had lowest EC₅₀ value, followed by positive control, CP and PEI. All fraction of jelly fig had higher ability (lower EC₅₀ values) in DPPH radical scavenging activity than ferrous ion chelating activity.

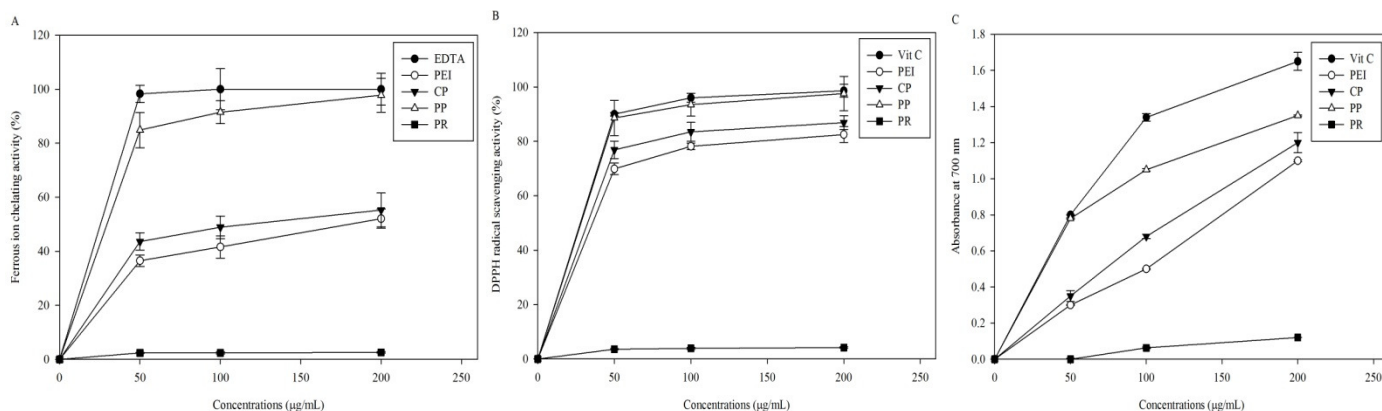


Figure-1: Ferrous ion chelating activity (A), DPPH radical scavenging activity (B), and reducing power (C) of pectin esterase inhibitor (PEI), crude polyphenol (CP), purified polyphenol (PP), and protein (PR) fractions from jelly fig. Positive controls used were EDTA (ethylenediaminetetraacetic acid) for ferrous ion chelating activity and Vit C (ascorbic acid) for DPPH radical scavenging activity and reducing power. Each values represents as mean \pm SD.

Anti-tumor activity of jelly fig achenes fraction was determined using cell culture approach by measuring their ability to inhibit the proliferation of K562 myeloid leukemia cells. The results of proliferation inhibition of different jelly fig achenes fractions at different sample treatment times are shown in Figure-2. In

general, the longer the treatment time, the higher the proliferation inhibition. PR had the lowest proliferation inhibition activity (Figure-2D) compared to PEI (Figure-2A), CP (Figure-2B) and PP (Figure-2C).

Table-1: EC₅₀ values (µg/mL) of antioxidant capacities of pectin esterase inhibitor (PEI), crude polyphenol (CP), purified polyphenol (PP), and protein (PR) fractions from jelly fig.

Fractions	EC ₅₀ values of ferrous ion chelating activity ¹	EC ₅₀ values of DPPH radical scavenging activity ¹	EC ₅₀ values of reducing power ²
Positive control ³	0.05 ± 0.04 ^b	3.43 ± 0.76 ^b	33.73 ± 1.20 ^c
PEI	184.92 ± 53.49 ^a	12.98 ± 7.49 ^a	93.67 ± 0.66 ^a
CP	140.04 ± 84.25 ^a	6.20 ± 2.04 ^{a,b}	71.81 ± 2.92 ^b
PP	3.35 ± 5.15 ^b	1.10 ± 1.74 ^b	20.04 ± 0.56 ^d
PR	ND ⁴	ND ⁴	ND ⁴

¹EC₅₀ values mean effective concentration required to obtain a 50% antioxidant effects. ²EC₅₀ values mean the concentration that the absorbance at 700 nm is 0.5, ³Positive controls used were EDTA (ethylenediaminetetraacetic acid) for ferrous ion chelating activity and ascorbic acid for DPPH radical scavenging activity and reducing power. ⁴ND mean not determined, ^{a-d}Values (mean ± SD) with different letters were significantly different at p<0.05.

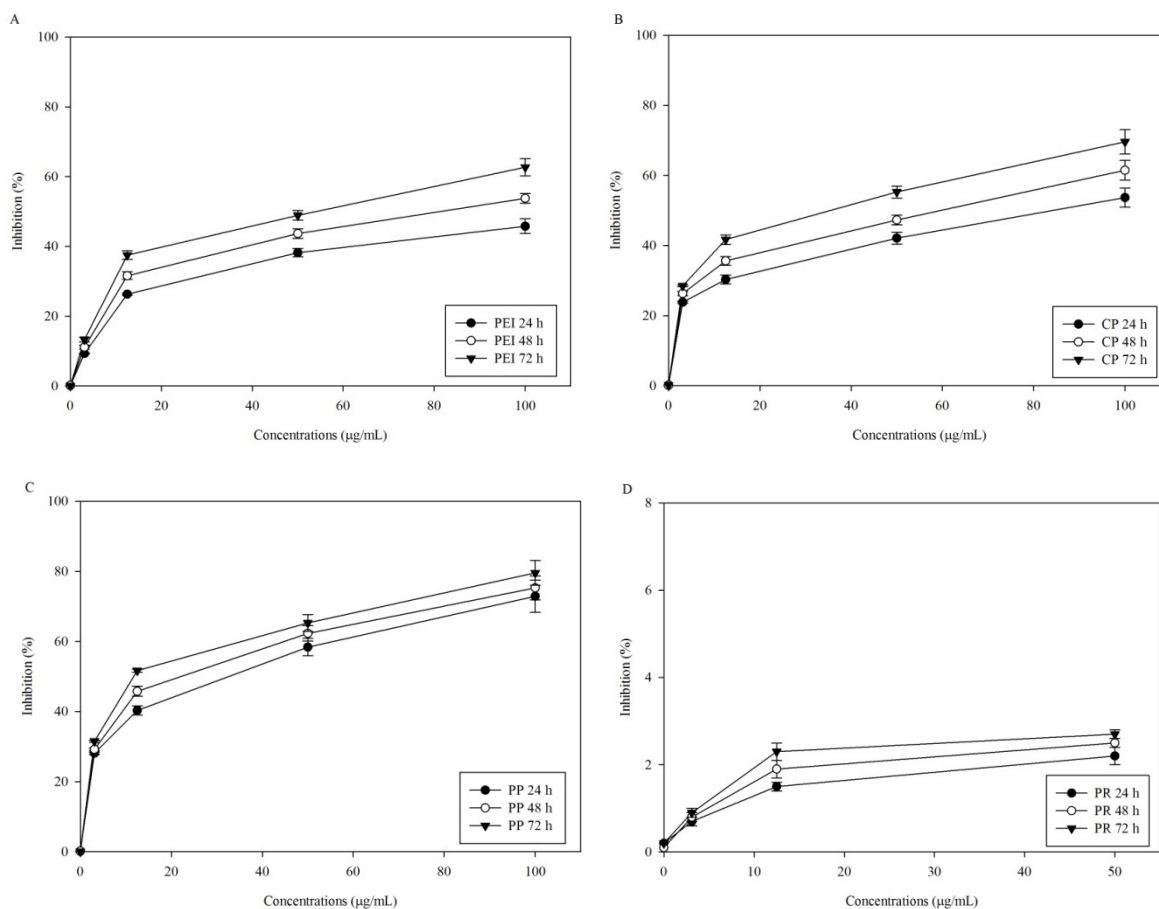


Figure-2: Proliferation inhibition of different jelly fig fractions (A) pectin esterase inhibitor (PEI), (B) crude polyphenol (CP), (C) purified polyphenol (PP), (D) protein (PR) on K562 myeloid leukemia cells. Each value represent as mean ± SD.

The IC₅₀ value represents the concentration needed to cause proliferation inhibition for 50%. The lower the value, the higher the proliferation inhibition owned by the fraction. The IC₅₀ values of different fractions of jelly fig at different treatment time are shown in Table-2. Comparing among different fraction, PP fraction had the lowest IC₅₀ which also indicate that it had the highest anti-proliferative activity against myeloid leukemia cells. CP and PEI fraction had lower anti-proliferative activity compared to PP, but PR fraction had the least anti-proliferative activity, which cause its IC₅₀ value cannot be determined.

Table-2: IC₅₀ values of growth inhibition of pectin esterase inhibitor (PEI), crude polyphenol (CP), purified polyphenol (PP), and protein (PR) fractions from jelly fig on K562 myeloid leukemia cells.

Incubation time	PEI	CP	PP	PR
24 h	144.47± 9.30 ^a	85.89± 16.94 ^b	27.17± 3.96 ^{d,e,f}	ND
48 h	75.95± 12.63 ^b	49.98± 7.62 ^c	19.16± 2.20 ^{e,f}	ND
72 h	38.39± 4.77 ^{c,d}	29.08± 3.93 ^{d,e}	12.97± 0.56 ^f	ND

^{a-c}Values (mean ± SD) with different letters are significantly different (p<0.05). *ND is referred as “not determined”.

It was suspected that protein fraction (PR) of pectin esterase inhibitor (PEI) caused the anti-proliferative activity against U937 monocytoid leukemia cells⁴. However, this study showed that it was purified polyphenol (PP) had role of anti-proliferative activity against K562 myeloid leukemia cells. This anti-proliferative activity may also supported by antioxidant activities, including radical scavenging and chelating ability. Polyphenol activity against oxidation and proliferation of cancer cell has been well known. However, the type of polyphenol which have role in these bioactivities is needed to be observed further.

Conclusion

In conclusion, polyphenol fraction had the highest antioxidant capacities (ferrous ion chelating activity, DPPH radical scavenging activity, and reducing power) and anti-proliferative activity against K562 myeloid leukemia cells. All fraction of

jelly fig had higher ability in DPPH radical scavenging activity than ferrous ion chelating activity as shown in lower EC₅₀ value. When comparing the antioxidant activities and anti-proliferative activity of different fractions of jelly fig, they all revealed a consistent order: purified polyphenol > crude polyphenol > pectin esterase inhibitor > protein.

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