

An Investigation on Bioactive Constituents from *Persea declinata* (Bl.) Kosterm

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Abstract: The *Persea declinata* (Bl.) Kosterm belongs to the family Lauraceae, is another *Persea* species with potential medicinal values. It is widely distributed in Borneo, Java, Malaysia (Penang, Kelantan, Terengganu, Pahang, Selangor) and Singapore. It shares the same genus with the famously studied *Persea americana*, or better known as the avocado, however, there are no documentation of this plant being investigated chemically or biologically. This paper will discuss the antioxidant and anticancer activities of the bark crude extract and fractions of *Persea declinata* (Bl.) Kosterm through DPPH and ORAC assay, and MTT assay using human breast carcinoma cells (MCF-7), human non-small cell lung cancer cells (A549) and human normal hepatic WRL-68 cell type.

Keywords: *Persea declinata*, antioxidants, anticancer.

1. INTRODUCTION

Malaysia is a diverse country which is believed to be 130 million years old [1] with two thirds of it covered in forest [2]. There is an estimated 14,500 species of flowering plants, of which about 15% was claimed to have medicinal properties [3], and roughly about a hundred have been studied fully for their potential bioactivities and there are many more to be discovered.

Persea declinata (Bl.) Kosterm (common names: medang inai, medang tanah, kayu helah, huru manok, huru leu-ur, and meang telut) is only known for its species morphology details. It is widely distributed in Borneo, Java, Malaysia (Penang, Kelantan, Terengganu, Pahang, Selangor) and Singapore. Another member of the Lauraceae family, it shares the same genus with the popularly investigated *Persea sp.*, the avocado (*Persea americana* Mill). The avocado is widely consumed as food and for medicinal purposes. They are good antioxidants as they possess various potentially cancer preventive phytochemicals [4] and other pharmacological properties including hepatoprotective [5], anticonvulsant [6], wound healing [7], analgesic and anti-inflammatory [8] and hypoglycaemic and hypocholesterolaemic [9]. *Persea declinata* (Bl.) Kosterm on the other hand has never been investigated chemically or biologically.

In the beginning of the study, 334 plants were chosen to screen for their antioxidant activities using the DPPH assay. Out of them, 6 plants were identified to possess very low EC₅₀ values, and it was found that *Persia declinata* (Bl.)

Kosterm showed the highest activity next to ascorbic acid (standard). In this study, antioxidant and anticancer properties of *Persea declinata* (Bl.) Kosterm bark methanolic extract and fractions will be further assessed through DPPH and ORAC antioxidant assays and MTT cell culture assay.

2. MATERIALS AND METHODS

2.1. General Experimental Procedures

2.1.1. Plant Material

The bark of *Persia declinata* (Bl.) Kosterm was collected from Dungun, Terengganu, Malaysia. The plant species was identified by Mr. Teo Leong Eng with a Voucher specimen (No. KL 5068) and was deposited in the herbarium of the Chemistry Department, University of Malaya.

2.1.2. Cell Culture

All the cells that used in this study were obtained from American Type Cell Collection (ATCC) and maintained in a 37°C incubator with 5% CO₂ saturation. MCF-7 human breast carcinoma cells, HepG2 human hepatocellular carcinoma cells and WRL-68 normal hepatic cells were maintained in Dulbecco's modified Eagle's medium (DMEM). Whereas A549 non-small cell lung cancer cells were maintained in RPMI medium. Both medium were supplemented with 10% fetus calf serum (FCS), 100 units/mL penicillin, and 0.1 mg/mL streptomycin.

2.1.3. DPPH Assay

The DPPH assay was performed in a 96-well microplate according to the method reported by Orhan *et al.* (2007)[10] and Brem *et al.* (2004)[11]. Ascorbic acid was used as the positive control and methanol as the negative control. The

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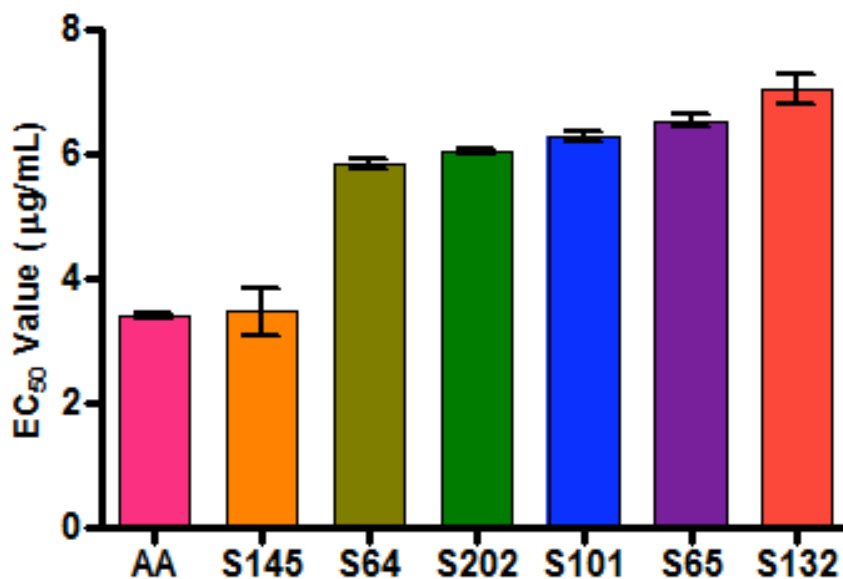


Fig. (1). EC₅₀ Values of 6 extracts screened for DPPH inhibiting activity. AA, Ascorbic Acid as Control. Results are means ± SD of 2 replicates.

absorbance was determined at 517 nm using a Microplate Absorbance Reader after 30 min of incubation at room temperature. The formula to calculate the percentage of DPPH free radical inhibition activity and to determine the concentration of each sample required to quench 50% of the DPPH free radical activity (EC₅₀ value) was according to Enayat and Banerjee (2009)[12].

2.1.4. ORAC Assay

Samples were dissolved and diluted with PBS, and serial dilutions for the Trolox standards were prepared accordingly. ORAC assay was performed according to Huang *et al.* (2002)[13], in a 96-well black microplate. The positive control was quercetin and the negative control was blank solvent/PBS. Finally, the fluorescence was measured with an excitation wavelength of 485 nm and an emission wavelength of 535 nm using Plate Chameleon™ V Multilabel Counter (Hidex, Turku, Finland). Data were collected every 2 min for a duration of 2 h. The quantification of the antioxidant activity was based on the calculation of the area under the curve (AUC), as proposed by Cao and Prior (1999)[14]. The results were expressed as µM of Trolox Equivalents (TE) per 20 µg/mL of sample.

2.1.5. MTT Assay

A549, MCF-7 and WRL-68 cells were used to determine the inhibitory effect of *Persea declinata* (Bl.) Kosterm extract and fractions on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. For measurement of cell viability, cells were seeded at a density of 1×10^5 cells/mL in a 96-well plate and incubated for 24 hours at 37 °C, 5% CO₂. Next day, cells were treated with the test agents and incubated for another 24 hours. After 24 hours, MTT solution at 2 mg/mL was added for 1 hour. Absorbance at 570 nm were measured and recorded. Results were expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agent. The potency of cell growth inhibition for each test

agent was expressed as an EC₅₀ value, defined as the concentration that caused a 50% loss of cell growth. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells.

3. RESULTS AND DISCUSSION

3.1. DPPH Assay

DPPH Assay was used to initially screen *Persea declinata* (Bl.) Kosterm methanolic extract for antioxidant properties. From other 6 plant methanolic extracts tested, *Persea declinata* (Bl.) Kosterm, which was labeled S145 then, showed highest DPPH inhibition percentage, comparable to ascorbic acid, which was used as the positive control. As shown in Fig. (1), 6 plant extracts with EC₅₀ closest to ascorbic acid (AA) (3.408 ± 0.06 µg/ml) were identified, as S145 being the closest to AA with an EC₅₀ value of (3.468 ± 0.54 µg/ml), followed by S64 (5.847 ± 0.14 µg/ml); S202 (6.0495 ± 0.05 µg/ml); S101 (6.2875 ± 0.09 µg/ml); S65 (6.54 ± 0.15 µg/ml) and S132 (7.041 ± 0.32 µg/ml).

For this reason, S145 was fractioned using the Preparative High Performance Liquid Chromatography (HPLC) into 6 fractions, to roughly determine which fraction of the plant that gives this activity, and possibly isolate the responsible compound/s. B, C, D and E each represent a major peak isolated from the HPLC profile, whereas A is the combination of remaining fractions collected before fraction/peak B until fraction/peak E, and F is the combination of fractions obtained after fraction/peak E.

The fractions undergo another screening for DPPH inhibition activity. The dose responses in DPPH assay, as shown in Fig. (2), with the positive control AA having an EC₅₀ value of 3.449 ± 0.114 µg/mL, the crude extract showed EC₅₀ value of 3.515 ± 0.083 µg/mL, whereas fraction F has the lowest EC₅₀ value (4.883 ± 0.044 µg/mL), followed by C (5.212 ± 0.057 µg/ml), B (6.210 ± 0.067 µg/ml), A (7.703 ± 0.220 µg/ml), then E (75.679 ± 0.112 µg/ml) and lastly D (93.755 ± 0.488 µg/ml).

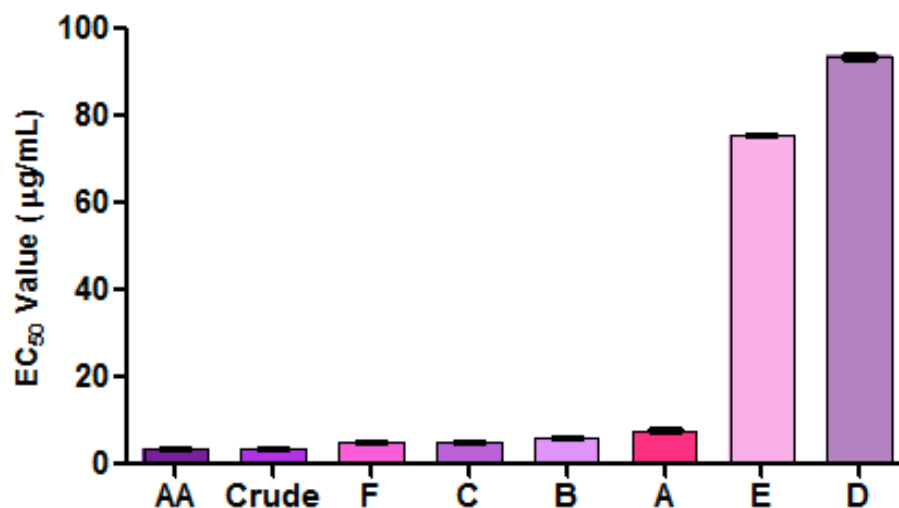


Fig. (2). EC₅₀ values of crude extract and fractions subjected to DPPH assay. AA, Ascorbic Acid as Control. Results are means \pm SD of 2 replicates.

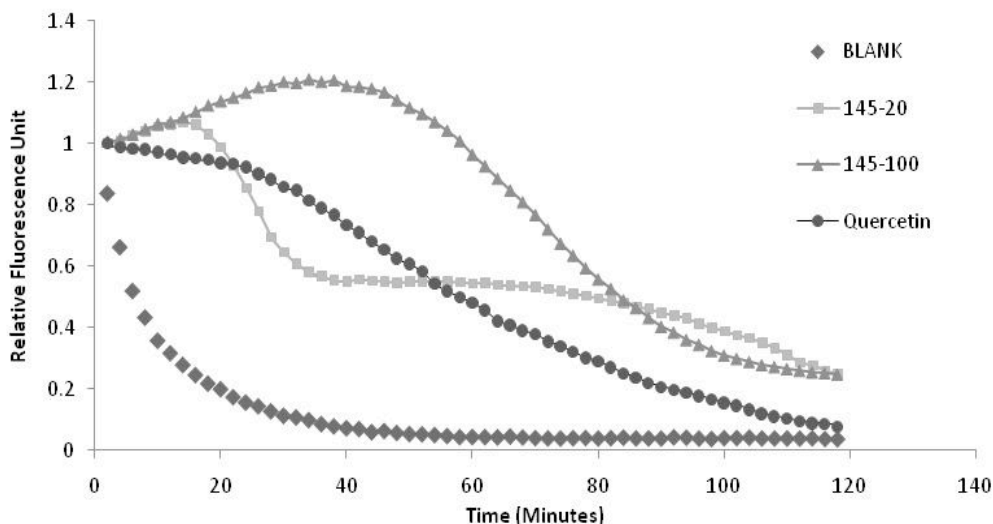


Fig. (3). The fluorescence decay curves of S145 at 100µg/ml and at 20µg/ml, compared to Blank and Quercetin (5µg/ml) by ORAC assay.

3.2. ORAC Assay

Fig. (3) shows the fluorescence decay curves or AUC of crude S145 at 100 µg/mL and at 20 µg/mL compared to the blank (PBS) and Quercetin at 5 µg/mL. The curves show that S145 at both concentrations have much protective effect on the fluorescence of the fluorescein over time. A Trolox calibration curve was used to calculate the equivalent concentration of Trolox; S145 at a concentration of 100µg/ml and 20µg/ml give equivalent antioxidant activity to 97.98 ± 5.69 µM and 68.69 ± 0.22 µM of Trolox, respectively. Quercetin, on the other hand, at 5µg/ml is equivalent to 59.99 ± 8.58 µM of Trolox.

S145 fractions were also subjected to ORAC. Fig. (4) indicates the antioxidant capacity of the 6 fractions of S145. Results are expressed as the equivalent concentration of Trolox that gives the same level of antioxidant activity as the samples at 20 µg/mL. Fraction A had the greatest peroxy radical scavenging ability in this method, with ORAC value of 241.4 ± 4.00 µM TE/20 µg/mL, followed by Fraction F, B, D, C and E with respective ORAC values of $139.22 \pm$

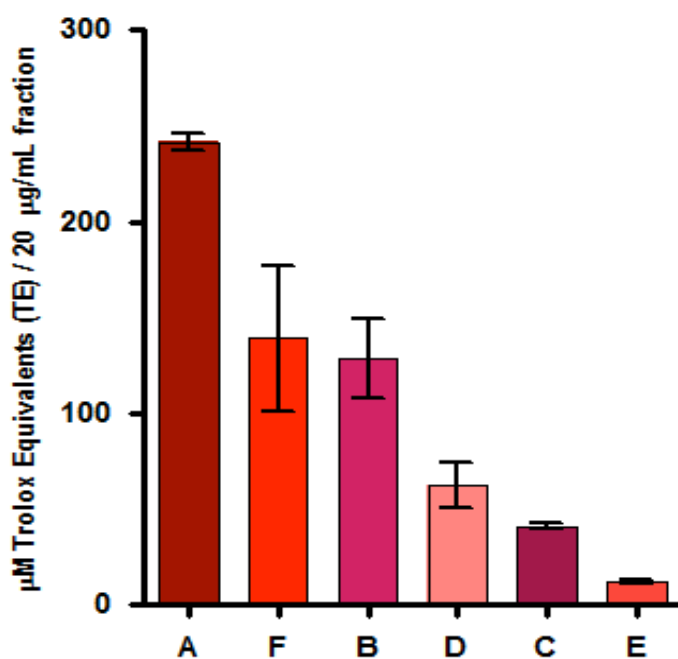
38.00 , 128.4 ± 20.61 , 62.44 ± 11.86 , 40.81 ± 1.47 and 11.99 ± 1.29 µM TE/20 µg/mL.

3.3. MTT Assay

To evaluate the cytotoxic activity, crude extract and fractions (from preparative HPLC) of S145 were tested with a series of different doses on human breast carcinoma cells (MCF-7), human non-small cell lung cancer cells (A549) and human normal hepatic WRL-68 cell type, respectively. After 24 hours, cell viability was determined by the MTT assay. Test agents induced cell cytotoxicity in a concentration dependent manner. These dose titration curves allowed to determine EC₅₀ for the test agents towards different cell lines (Table 1). The crude showed it was toxic to all 3 cell lines tested, being most toxic in MCF-7 cells (EC₅₀ 9.800 ± 2.21 µg/mL). Fraction A was most toxic to WRL-68 (EC₅₀ 33.17 ± 5.73 µg/mL), whereas fraction B and D were most toxic to MCF-7 (EC₅₀ 31.05 ± 3.98 µg/mL) and A549 (EC₅₀ 38.55 ± 3.64 µg/mL) respectively. These results indicate that cell lines differ in their sensitivity to the same test agent, which may be determined by multiple cell type-specific signaling cascades and transcription factor activities.

Table 1. Effect of *Persea Declinata* (Bl.) Kosterm (S145) Extract and Fractions on Cells Expressed as EC₅₀ Values in 24 Hours MTT Assay. Results are means \pm SD of 3 Replicates. NC, no Cytotoxicity

Test Agent	EC ₅₀ \pm S.D (μ g /ml)		
	A549	MCF-7	WRL-68
Crude Extract	23.32 \pm 2.13	9.800 \pm 2.21	97.90 \pm 3.45
Fraction A	55.00 \pm 3.54	NC	33.17 \pm 5.73
Fraction B	42.61 \pm 4.53	31.05 \pm 3.98	53.90 \pm 3.46
Fraction C	NC	NC	53.96 \pm 1.36
Fraction D	38.55 \pm 3.64	NC	272.0 \pm 7.90
Fraction E	369.0 \pm 5.87	41.12 \pm 3.67	130.2 \pm 5.83
Fraction F	90.12 \pm 5.51	166.3 \pm 5.42	334.4 \pm 8.73

**Fig. (4).** ORAC values of 6 fractions of S145 at 20 μ g/ml. Results are means \pm SD of 3 replicates.

4. CONCLUSION

Persea declinata (Bl.) Kosterm locally known as Medang Inai has never been studied before, phytochemically and biologically. Hence, this study was aimed to investigate the biological activities of the plant such as antioxidants and cytotoxicity activities. This study demonstrated that crude extract and fractions from *Persea declinata* (Bl.) Kosterm possessed interesting anti-oxidant and cytotoxic activities which can be further investigated and researched.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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