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Original Research Article

Effects of *Dipterocarpus alatus* Leaf and Bark Extracts on UVB-Protection, Collagen Stimulating Activity and Nitric Oxide Inhibition

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ABSTRACT

Dipterocarpus alatus (*D. alatus*) is used for the treatment of rheumatism in Ayurvedic medicine. In this research, the leaf and bark ethanol extracts of *D. alatus* were investigated for antioxidant activity, UV-protection on HaCaT keratinocytes, inhibition of nitric oxide formation on RAW 264.7 macrophages and collagen proliferation on normal human dermal fibroblasts (NHDFs). The total phenolic and total flavonoid contents of the leaf and bark ethanol extracts were determined using Folin-Ciocalteu assay and $AlCl_3$ assay, respectively. Cell viability of HaCaT keratinocytes, RAW 264.7 macrophages and normal human dermal fibroblasts (NHDFs) were evaluated including cell morphology, collagen production, nitric oxide inhibition. The leaf extract showed the highest phenolic content (1,327.07 ± 95.37 gGAE/100 g dry weight), flavonoid content (343.23 ± 21.36 gQE/100 g dry weight), and ferric reducing power (4.05 ± 0.34 mM $FeSO_4$/100 g dried weight), whereas the bark extract showed better DPPH and ABTS assays (IC_{50} at 13.88 ± 0.27 and 28.15 ± 0.54 µg/mL, respectively). Both leaf and bark extracts showed no toxicity on HaCaT cells and NHDFs at 1-500 µg/mL, but the leaf extract above 25 µg/mL and bark extract above 100 µg/mL showed toxicity to Raw 264.7 cells. The leaf extract at 50 µg/mL presented more UVB protective effect than the bark extract with no toxicity to UVB-induced HaCaT cells. The bark extract induced higher collagen production and nitric oxide inhibition than the leaf extract. The leaf extract of *D. alatus* showed beneficial potential for inclusion in UV protection products, whereas the bark extract is suited for wound healing products.

***Keywords*:** *Dipterocarpus alatus*; Antioxidant activity; UV protective effect; Wound healing effects; Nitric oxide inhibition.

Introduction

Plants of the genus *Dipterocarpus* have been reported to have many bioactivities including anti-bacterial, anti-oxidant, cytotoxic, anti-inflammatory, and anti-filarial activities.[1,2] Previous studies of the flavonoid content of leaf extracts from *Dipterocarpus alatus*, *Dipterocarpus costatus*, *Dipterocarpus gracilis* and *Dipterocarpus turbinatus* have identified distinct aglycone or glycoside patterns. *D. alatus* and *D. gracilis* have identical aglycone patterns (quercetin, apigenin, prodelphinidin and procyanidin), while *D. gracilis*, *D. costatus* and *D. turbinatus* have identical glycoside patterns.[3] Investigation of the chemical components in the bark extract of *Dipterocarpus hasseltii* identified resveratrol oligomers such as diptoindonesin E, (-)-ε-viniferin, laevifonol, (-)-α-viniferin, vaticanol B, (-)-hopeaphenol and a coumarin, scopoletin. Of these, hopeaphenol was found to strongly inhibit murine leukemia P-388 cells.[4]

Dipterocarpus alatus Roxb. ex G. Don, or Yang-Na, is found in about 80 percent of the forest area of Thailand. *D. alatus* has been used to treat rheumatism, diseases of the liver, and to stimulate appetite in cattle.[5]

A comparison of various parts of *D. alatus* showed the extract of the bark to have the highest antioxidant activity and the highest total phenolic content,[1] and the bark of young trees is used for rheumatism in Ayurvedic medicine.[6,7] In addition, the leaf extract of *D. alatus* contained flavonoids (luteolin-7-O-glucoside, kaempferol-3-glucoside, rutin) and phenolic acids (gallic acid, ferulic acid, and caffeic acid) as major constituents and was found to exhibit partial selective inhibition of monoamine oxidase A in unpredictable chronic mild stress-induced depression in ICR mice.[8] *D. alatus* oleoresin was also investigated and found the potential to inhibit NO production on RAW 264.7 cells and stimulation of collagen production.[9]

Most of the antioxidants found in nature are phenolic compounds that act by decreasing the levels of free radicals in cells and enhancing cellular antioxidant capacity. In addition, phenolic compounds can inhibit prostaglandin (PGE_2) and nitric oxide (NO) production.[10] Polyphenols from botanical products have presented antioxidant and anticancer potential on murine melanoma cells[11] and stimulated the production of collagen in fibroblast cells to reduce wound healing time.[12] Furthermore, polyphenols and strong antioxidants have been shown to have a photoprotective effect against ultraviolet B-induced damage in HaCaT keratinocytes.[13,14] Polyphenol sources from plants in both oral and topical form may provide protection from UV damage and sunburn and can improve skin heath.[15]

This study investigated the antioxidant activity of *D. alatus* leaf and bark extracts and their UV-protective properties in HaCaT keratinocytes, ability to inhibit nitric oxide formation in RAW 264.7 macrophages, and effect on collagen proliferation in normal human dermal fibroblasts (NHDFs). The results could provide support for the development of bark or leaf Yang-Na extracts for incorporation in novel herbal heath products.

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Materials and Methods

Plant materials

The leaf and bark of *D. alatus* were collected in January 2017 from the grounds of the Faculty of Medicine, Khon Kaen University, Thailand. The *D. alatus* plant was identified by Suppachai Tiyaworanant and voucher specimens (KKPSH010102992 to KKPSH010102996) were deposited in the Faculty of Pharmaceutical Sciences, Khon Kaen University. The collected plant material was air-dried in darkness at 50°C for 24 h. Dried *D. Alatus* leaves and bark were then cut and mashed to dry powder.

Chemicals

2,2-diphenyl-1-picrylhydrazyl(DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), vitamin C (ascorbic acid) and Folin-Ciocalteu reagent, 2,2'-Azobis (2-amidinopropane) dihydrochloride and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) were purchased from Sigma Chemical Co., USA. The RAW 264.7 murine macrophage cell lines were obtained from Dr. Pramote Mahakunakorn (Faculty of Pharmaceutical Sciences, Khon Kaen University). The HaCaT keratinocyte cell lines were obtained from Dr. Natsajee Nualkaew (Faculty of Pharmaceutical Sciences, Khon Kaen University). The normal human dermal fibroblasts were purchased from ATCC (PCS-201-012, USA). Fetal Bovine Serum (FBS), 1% (v/v) penicillin–streptomycin, and Dulbecco's Modified Eagle's Media (DMEM) containing 10% FBS and 1% (v/v) penicillin–streptomycin were purchased from Gibco, USA.

Sample extraction

Powdered *D. alatus* leaf or bark samples (100 g) were macerated with 400 mL of 95% ethanol. After 24 h, the mixture was filtered through Whatman® Filter Paper No. 1 and the extraction was repeated using the residue with an equal volume of solvent. The filtrates were combined and evaporated to dryness under vacuum at 45°C using a rotary evaporator followed by freeze drying to give the crude extracts.

Total phenolic content determination

Total phenolic content was determined by Folin-Ciocalteu assay.[16] A 20 µL aliquot of extract sample was added to 80 µL of 7.5% sodium carbonate and 100 µL of Folin-Ciocalteu reagent at room temperature. The absorbance was measured at 765 nm after 30 min and results were expressed as gallic acid equivalents (GAE/g extract sample).

Total flavonoid content determination

Total flavonoid content was determined with the $AlCl_3$ assay. A 100 µL aliquot of extract sample was added to 100 µL of 2% aluminium chloride at room temperature. The absorbance was measured at 415 nm after 60 min. Quercetin was used as the standard (QE/g extract sample).[16]

Antioxidant assays

DPPH, ABTS and FRAP assays were performed for determination of antioxidant activity.[17,18] Firstly, the DPPH radical ($DPPH^{\bullet}$) was dissolved in MeOH (0.2 mM). Then 50 µL of extract sample was mixed with DPPH reagent. The mixture was incubated for 30 min in the dark, and absorption was measured at 517 nm. Secondly, $ABTS^{\bullet+}$ was produced by the reaction of equal volumes of 7 mM ABTS solution and 2.4 mM potassium persulfate for 16 h, in the dark and at room temperature. The stock solution was then diluted (10-fold) in DI water. Then 50 µL of sample was mixed with diluted ABTS reagent. The mixture was incubated for 30 min in the dark, and absorption was measured at 700 nm. Finally, FRAP reagent was prepared by mixing 0.3 mM acetate buffer with 10 mM TPTZ and 20 mM Ferric chloride (10:1:1). Then 50 µL of sample was mixed with FRAP reagent. The mixture was incubated for 30 min in the dark, and absorption was measured at 593 nm. The absorbance was compared with standard ferrous sulfate to obtain the FRAP value. The negative control (blank) was 200 µL of ABTS reagent and Trolox was used as a positive control. The DPPH and ABTS radical scavenging capacity (%inhibition) was calculated as [1-(absorbance of sample/absorbance of control)] ×100 and the FRAP assay results are reported as mM $FeSO_4$ equivalent/100 g extract. All antioxidant assays were performed three times.

Cell assays

HaCaT keratinocyte cell lines (HaCaT cells)

For the cytotoxicity of the leaf and bark extracts, HaCaT cells were seeded in DMEM containing 10% FBS and 1% penicillin-streptomycin at a density of 1.5×10^5 cells/well in 24-well plates and incubated for 24 h at 37°C under 5% CO_2. The medium was replaced and 100 µL of extract samples in media at different concentrations (1-1,000 µg/mL) were added and plates were incubated for 24 h at 37°C under 5% CO_2. The medium was removed and cell viability was determined by MTT assay. A 100 µL aliquot of 0.5 mg/mL MTT in phosphate buffer saline (PBS) pH 7.4 was added to the cells and plates were incubated for 3 h at 37°C under 5% CO_2. Crystals of formazan were solubilized in 100 µL dimethyl sulfoxide (DMSO). The absorbance was measured at 550 nm by microplate readers (Bio-Rad Model 680, Tokyo, Japan) and medium from non-treated cells was used as control.[19] Percent cell viability was calculated using the following equation:

$$\%\text{Cell viability} = (A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 550 nm.[9]

For UVB protection assay, cells were pretreated with different concentrations of samples and incubated for 24 h at 37°C under 5% CO_2. The medium was removed and then cells were rinsed with PBS pH 7.4. After that, cells were covered with a thin PBS layer and irradiated with UVB (120 mJ/cm^2) by UVB lamp (Philips, Poland) for 10 min. The 1 mL of medium was added and cells were maintained in regular culture conditions which were incubated for 24 h at 37°C under 5% CO_2 until analysis. Images of cell morphology was performed by inverted microscope (Hollywood international group, Thailand) and cell viability was evaluated by MTT assay and percent cell viability was calculated as mentioned above. using the following equation:

$$\%\text{Cell viability} = (A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 550 nm.[9]

Normal human dermal fibroblasts (NHDFs)

The cytotoxicity of leaf and bark extracts on NHDFs were determined. NHDFs were cultured in DMEM medium with 10% FBS and 1% penicillin-streptomycin in incubator for 24 h at 37°C under 5% CO_2. Cell viability of NHDFs was performed with MTT assay.[19] NHDFs were seeded into 96-well plates at a density of 1×10^4 cells/well. Then 100 µL of extract samples in media at different concentrations (1-1,000 µg/mL) were added and incubated for 24 h at 37°C under 5% CO_2. The media were removed and 100 µL of extract samples in media at different concentrations (1-1,000 µg/mL) was added. Then treated cells were incubated for 24 h at 37°C under 5% CO_2. The medium was removed and the cell viability was determined by MTT assay. Percent Cell viability was calculated as the following equation:

$$\%\text{Cell viability} = (A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 550 nm.

The removed medium from all cell treatments were further determined for collagen content by Serius red method.[9] The 100 µL medium was mixed with 100 µL of 0.5 M acetic acid and 1 mL of Direct red solution in saturated picric acid. The mixture was sonicated for 30 min and then centrifuged at 10,000 rpm for 5 min. The supernatant was removed and further added 1 mL of 0.1 M HCl. The mixture was again centrifuged at 10,000 rpm for 5 min. The supernatant was

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removed and added 1 mL of 0.5 N NaOH. The absorbance was measured at 550 nm. The collagen content was determined by comparison with standard collagen type I.[9]

RAW 264.7 Murine Macrophages (RAW 264.7 cells)

The cytotoxicity of the leaf and bark extracts on the RAW 264.7 cells were determined by the MTT assay method.[9] RAW 264.7 cells were cultured in DMEM medium with 10% FBS and 1% penicillin-streptomycin for 24 h at 37°C under 5% CO_2. RAW 264.7 cells were seeded into 96-well plates at a density of 1×10^4 cells/well. The medium was removed and cells were treated with 100 µL of extract samples in media at different concentrations (250-1,000 µg/mL). The treated cells were incubated for 24 h at 37°C under 5% CO_2. Subsequently, the medium was removed and cells were performed for cell viability by MTT assay. The percent cell cytotoxicity was calculated using the following equation below:

$$\%\text{Cell viability} = (A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 550 nm.

Nitric oxide production by RAW 264.7 cells was assayed by a nitric oxide scavenging activity assay method.[9] RAW 264.7 cells were seeded into 96-well plates at a density of 1×10^4 cells/well in 100 µL for 24 h at 37°C under 5% CO_2. Then the medium was removed and cells were treated with 100 µL of extract samples in media at different concentrations (1-1,000 µg/mL) that contained LPS at 1 µg/mL of final concentration. The treated cells were incubated for 24 h at 37°C under 5% CO_2. After that, 100 µL of medium was removed and added 100 µL of Griess reagent (0.1% naphthylethylenediamine dihydrochloride (NED), and 1% sulphanilamide in 5% phosphoric acid, 1:1). Then the absorbance at 520 nm was measured after 30 min. Percent nitric oxide inhibition was calculated using the following equation:

$$\%\text{NO inhibition} = [1 - (A_{sample}/A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of non-treated cell, and A_{sample} is the absorbance of sample at 520 nm.

Chromatographic characterization of Yang-Na leaf and bark extracts

High-Performance Liquid Chromatography (HPLC) was performed using a C18 reversed-phase column (4.6 mm × 250 mm, 5 µm; Luna®, USA) with UV detection at 254 nm. The mobile phase was a gradient system of 0.01% acetic acid in DI water (A) and 0.01% acetic acid in acetonitrile (B) with a flow rate of 1.0 mL/min and an injection volume of 20 µL. The gradient system was performed as: 0-10 min 85% of mobile phase A; 10-20 min, 40% of mobile phase A; and 20-30 min, 100% of mobile phase B.

Statistical analysis

All experiments were performed in triplicate and results are expressed as mean value ± standard deviation (SD). Data analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post-test by SPSS version 26.0 (KKU software). The statistical significance value was $p<0.05$.

Results and Discussion

Total phenolic and total flavonoid content and antioxidant activity

The percent yield of the leaf extract (LE) and bark extract (BE) of *D. alatus* were 8.66 ± 0.31 and 1.71 ± 0.12 %, respectively. The total phenolic content (TPC) and total flavonoid content (TFC) of LE and BE were 1,327.07 ± 95.37 and 414.04 ± 11.35 mg GAE/100 g dry weight and 343.23 ± 21.36 and 18.94 ± 1.59 mg QE/100 g dry weight, respectively. LE had significantly higher TPC and TFC than BE ($p < 0.05$). For antioxidant activity, LE showed more ferric reducing activity in the FRAP assay (4.05 ± 0.34 mM $FeSO_4$ equivalent/100 g dry weight) than BE, but BE exhibited more radical scavenging activity than LE in the DPPH and ABTS assays (IC_{50} of 13.88 ± 0.27 and 28.15 ± 0.54 µg/mL, respectively) (Table 1).

Cytotoxicity and UV protection in HaCaT cells

The leaf and bark extracts showed no cytotoxic effects to HaCaT cells at 1-100 µg/mL and increasing cytotoxicity from 250 µg/mL. HaCaT cells treated with leaf extract at 1-10 µg/mL and bark extract at 50 µg/mL showed more cell proliferation than 25 µg/mL ascorbic acid (Figure 1).

For the UV-protection study, HaCaT cells pre-treated with 10-50 µg/mL of leaf extract prior to being exposed to UVB 120 mJ/cm^2 of UVB radiation had significantly increased percentage cell viabilities compared to control UVB-treated cells (Figure 2). Furthermore, leaf extract showed a dose-dependent response in the protection of HaCaT human keratinocytes. The HaCaT cells pre-treated with 50 µg/mL of leaf extract prior to UVB exposure showed the highest cell viability and retained the cell morphology of ascorbic acid treated cells and controls. In contrast, the bark extract caused cell death (Figure 3). Therefore, Yang-Na leaf extract presented the possibility to protect HaCaT cells from UVB irradiation more than Yang-Na bark extract. The leaf extract at 50 µg/mL was non cytotoxic and preserved cell morphology as well as the control (non UVB treated cells) and vitamin C. Whereas the cells pre-treated with the bark extract followed by UVB treatment exhibited cell death and unusual and different cell morphology compared to the control and vitamin C treated cells (Figure 3). In terms of the TPC and TFC content, the ethanol extract of the leaf was higher than the bark extract. Plant phenolic compounds and flavonoids were reported to prevent UVB absorption, which causes skin cancer, and this was correlated to their antioxidant activity.[20] In addition, the phenolic and flavonoid contents from *Cucumis melo* L. were found to correlate with the Sun Protection Factor (SPF).[21] Furthermore, natural antioxidants could protect skin from UV radiation, which shows potential for use in topical applications.[22]

Cell viability and collagen content in NHDFs

Leaf and bark extracts of *D. alatus* at concentrations 1-500 µg/mL showed no cytotoxicity to NHDFs (Figure 4). Treatment of NHDFs with 500 and 1,000 µg/mL of bark extract showed collagen content 2 and 3 times higher than the control. Whereas the leaf extract did not affect the collagen content (Figure 5). Therefore, the bark extract has collagen stimulating effect on NHDFs.

Table 1: The yield, total phenolic and total flavonoid contents, and antioxidant activity of leaf (LE) and bark extract (BE) of *D. alatus*

Sample	%Yield	TPC (mg GAE/100g DW)	TFC (mg QE/100g DW)	IC_{50} (µg/mL)		FRAP (mM $FeSO_4$/100 g DW)
				DPPH	ABTS	
LE	8.66 ± 0.31	1,327.07 ± 95.37^b	343.23 ± 21.36^b	40.06 ± 1.33^c	34.22 ± 0.29^d	4.05 ± 0.34^c
BE	1.71 ± 0.12	414.04 ± 11.35^a	18.94 ± 1.59^a	13.88 ± 0.27^b	28.15 ± 0.54^c	2.24 ± 0.02^a
Vit C	-	-	-	6.51 ± 0.19^a	1.81 ± 0.01^a	-

LE is Yang-Na leaf extract, BE is Yang-Na bark extract and Vit C is Vitamin C or ascorbic acid. Results are expressed as mean ± SD (n = 3). $^{a, b, c, d}$ letters indicate significant differences in the same column at $p < 0.05$ (Post hoc test by Tukey).

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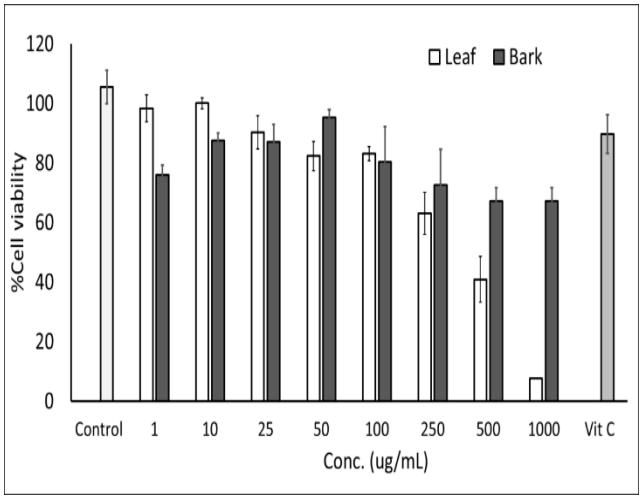

Figure 1: Cytotoxicity of leaf and bark extracts (1-1,000 µg/mL) on HaCaT cells compared with Vitamin C 25 µg/mL and control (non-UVB treated cells).

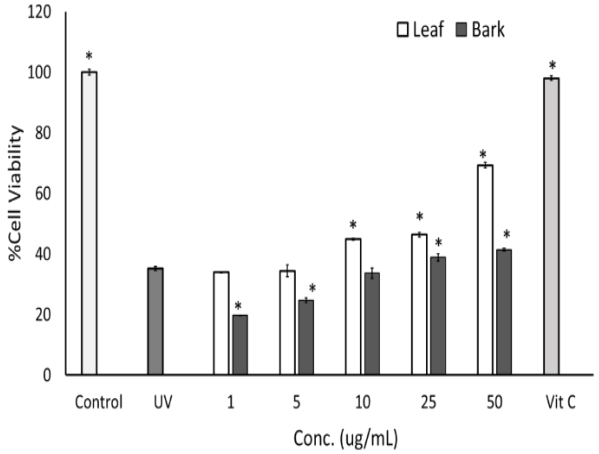

Figure 2: The effect of leaf and bark extracts on the viability of HaCaT cells exposed to 120 mJ/cm^2 of UVB radiation. Values represent mean ± SD of three replicates.
* Indicate significant differences by Dunnett's test compared with UVB treatment cells (UV), $P < 0.001$.

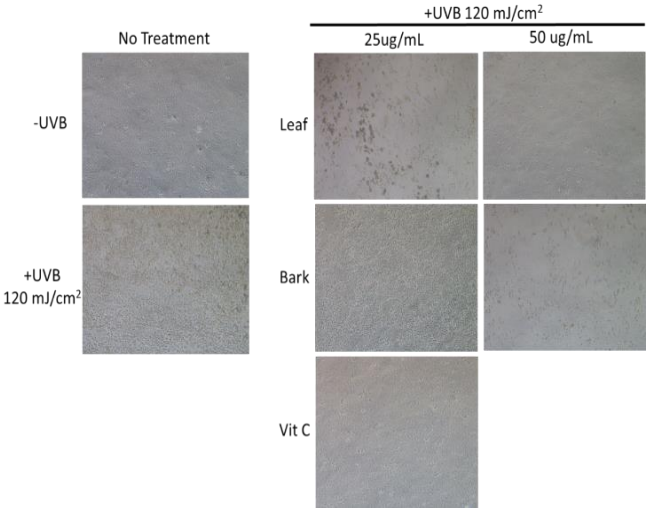

Figure 3: Cell morphology of HaCaT cell line with or without treatment with Vitamin C (25 µg/mL), 25 and 50 µg/mL of bark and leaf extracts upon exposure to 120 mJ/cm^2 for 24 h. Images of cell morphology(40X) was performed by inverted microscope (Hollywood international group, Thailand).

The chemical constituents found in the bark of the genus Dipterocarpus are mostly sesquiterpenes and triterpenes and other compounds such as oligostilbenoids, coumarin compounds, resveratrol compounds and phytosterol. The bark of *D. alatus* is used in traditional medicine for rheumatism and diseases of the liver.[23] Resveratrol which is found in the bark extract, can stimulate the proliferation of fibroblasts and contribute to the increase in the concentration of collagen.[24] Therefore, the bark extract showed more collagen production effect than leaf extract.

Cell viability and nitric oxide inhibition in RAW 264.7 cells

From the cytotoxicity study (Figure S1), the leaf and bark extract at 250-1,000 µg/mL were found to have no cytotoxicity on Raw 264.7 cells. In this research, the leaf and bark extracts at 1-1,000 µg/mL were treated on LPS-induced RAW 264.7 cell lines compared with control (non treated cells) and 1 mM L-NAME (positive control). The bark extracts at 25, 50, 100 µg/mL increased the viability of LPS-induced RAW 264.7 cells compared to the leaf extract at the same concentration. In contrast, the leaf extract was cytotoxic to RAW 264.7 cells from 50 to 1,000 µg/mL (Figure 6). The percentage NO inhibition of LPS-induced RAW 264.7 cells increased dose-dependently for both the leaf and bark extracts from 1-250 µg/mL (Figure 7). The bark extract showed a higher NO inhibition (31.20-45.45%) than the leaf extract (15.4-35.87%). The bark extract at 25, 50 and 100 µg/mL showed no significant difference in NO inhibition. While the positive control (L-NAME) provided 70.28% of NO inhibition (Figure 7).

A traditional medicine prepared from *Dipterocarpus tuberculatus* Roxb. is used for many diseases, especially inflammation. In a previous study, *Dipterocarpus tuberculatus* Roxb. ethanol extract dose-dependently inhibited the expression of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β in LPS-treated RAW264.7 cells.[25] Similarly, the leaf and twig ethanol extracts of *D. tuberculatus* were found to suppress LPS-mediated inflammatory responses for NO and PGE2 from macrophages in a dose-dependent manner.[26] In addition, *D. alatus* methanol leaf, bark and twig extracts showed anti-inflammatory effects such as inhibition of NO, PGE2, IL-1β and TNF-α production in LPS-stimulated RAW 264.7 macrophages.[27] In the current study, both the *D. alatus* leaf and bark extracts could produce NO inhibition effects on Raw 264.7 cells. The ethanol extract that was used in this research might have a different chemical composition from the methanol extract because of the different polarities of the solvents. However, the ethanol extract might provide less phenolic compounds than methanol extracts.[28] Ethanol was preferred as the extraction solvent in this research since it is less toxic to human and animal cells and ethanol is acceptable for further health product development. In this research, the ethanol bark extract produced higher percentage NO inhibition than the leaf extract at the same concentration. Therefore, the ethanol bark extract of *D. alatus* could have more possibility for NO inhibition which might be involved in its anti-inflammatory effects. According to traditional use, the bark of Dipterocarpus is commonly used for rheumatism in many countries. However, there is lack of information about the anti-inflammatory effects of *D. alatus*. This research should be further studied to confirm the mechanism for its pharmaceutical or health product applications.

Chromatographic characterization

The HPLC chromatograms from the *D. alatus* leaf extract (Figure 8A) and bark extract (Figure 8B) showed different HPLC fingerprints caused by the different chemical compositions. The standard mixture chromatogram (Figure 8C) presents the peaks for gallic acid (1), chlorogenic acid (2), catechin (3), caffeic acid (4), resveratrol (5), quercetin (6) and apigenin (7) at retention times of 3.33, 4.55, 5.19, 6.31, 9.62, 10.05 and 11.19 min, respectively. Gallic acid (1), chlorogenic acid (2) and apigenin (7) were observed in both the leaf and bark extracts. Caffeic acid (4) and quercetin (6) were detected in the leaf extract. Resveratrol (5) was only detected in the bark extract. However, there were also some peaks that did not match with the standards mixture chromatogram.

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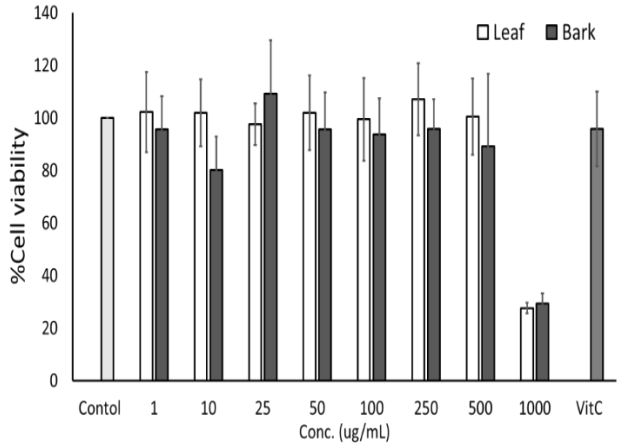

Figure 4: Percent cell viability on NHDFs of leaf and bark extracts of *D. alatus* concentration 1, 10, 25, 50, 100, 250, 500, 1000 µg/mL and Vitamin C 25 µg/mL. Data are representatives of three replicates and shown as mean ± SD.

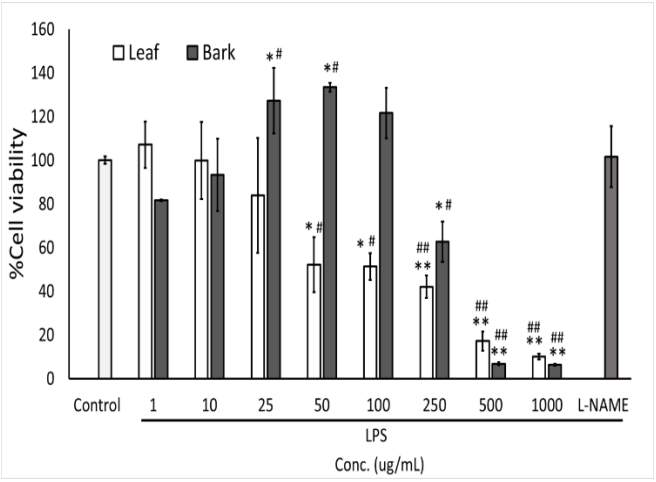

Figure 6: Percent cell viability on LPS induced Raw 264.7 cell lines of leaf and bark extracts of *D. alatus* concentration 1, 10, 25, 50, 100, 250, 500, 1,000 µg/mL. The significant differences by Dunnett's post-hoc test are indicated as *, **, #, ## when *p <0.05, **p < 0.001 (compare with control) and #p <0.05, ##p< 0.001 (compared with L-NAME).

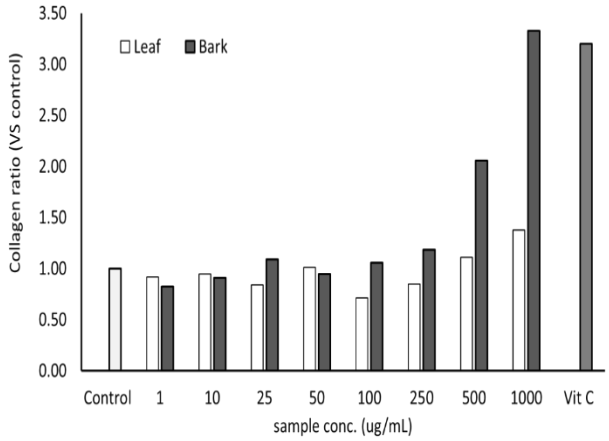

Figure 5: Collagen content ratio of 25 µg/mL Vitamin C (positive control), leaf and bark extracts of *D. alatus* concentration 1, 10, 25, 50, 100, 250, 500, 1000 µg/mL compared with control (medium from non-treated NHDFs)

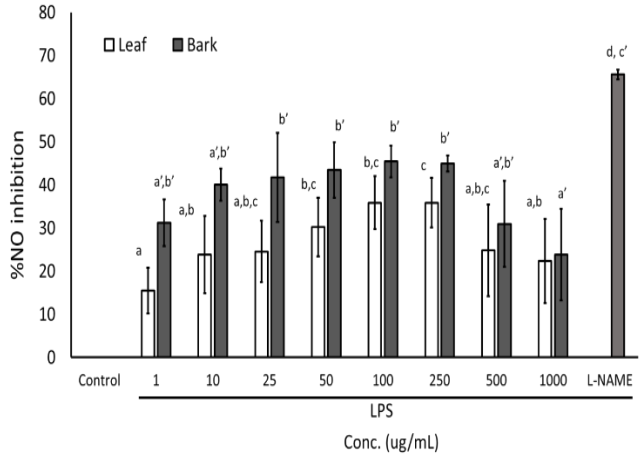

Figure 7: Percent Nitric oxide inhibition of leaf and bark extracts of *D. alatus* concentration 1, 10, 25, 50, 100, 250, 500, 1000 µg/mL and 200 µM L-NAME (positive control). Data are representatives of three replicates and shown as mean ± SD. a, b, c, d and a', b', c', d' letters indicate significant differences in the same column at p-value < 0.05 (Post hoc test by Duncan).

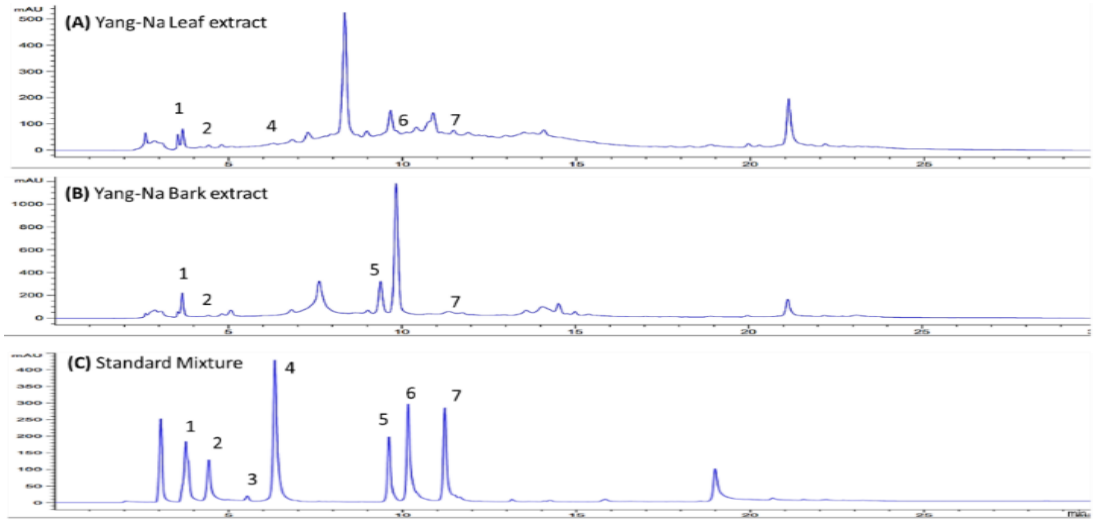

Figure 8: HPLC fingerprint chromatograms of Yang-Na leaf extract of *D. alatus* **(A)**, Yang-Na bark extract of *D. alatus* (**B**) and mixture of 7 standards include Gallic acid (1), Chlorogenic acid (2), Catechin (3), Caffeic acid (4), Resveratrol (5), Quercetin (6), and Apigenin (7) (**C**) in HPLC-UV analysis which the mobile phase system was used 0.01% Acetic acid in DI water and 0.01% Acetic acid in Acetonitrile (1:1) and detected at 254 nm

Conclusion

This study investigated the biological activity of ethanol extracts from *D. alatus* or Yang-Na leaves and bark. The leaf extract was found to contain more phenolic compounds, which provided a high antioxidant effect. The leaf extract also showed a higher cell protective activity against UVB. In contrast, the bark extract exhibited more collagen stimulating effects on NHDFs together with higher percentage NO inhibition on LPS-induced RAW 264.7 cells. The increased collagen production and anti-inflammatory activity of the bark extract might be a valuable potential product for wound healing activity. Therefore, the leaf extract should be further studied for incorporation in UV protective products and the bark extract is an interesting candidate for development of wound healing products or skin care cosmetics. Nevertheless, both *D. alatus* extracts should be further studied for other activities and to confirm their related mechanisms, which could provide valuable information for natural health product applications in the future.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The author hereby declares that the work presented in this article is genuine and that any liability for claims relating to the content of this article will be borne by them.

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