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Article

Examination of the Antioxidant and Anti-Inflammatory Effects of Extracts from the Bark of Bangladesh Medicinal Plants

Joo Won Lee [1†], Mi-Bo Kim [2†], Seungjin Jeong [1], Hyeju Lee [1], Su Hyeon Baek [1], Md. Salah Uddin [3], Sang Woo Lee [4] and Sang Gil Lee [1,2,*]

[1] Department of Smart Green Technology Engineering, Pukyong National University, Busan 48513, Republic of Korea; moveone1995@naver.com (J.W.L.); wtw3737@gmail.com (S.J.); axxx1452@naver.com (H.L.); bmh46750@gmail.com (S.H.B.); sglee1125@pknu.ac.kr (S.G.L.)

[2] Department of Food Science and Nutrition, Pukyong National University, Busan 48513, Republic of Korea; mibokim1120@gmail.com (M.-B.K.)

[3] Ethnobotanical Database of Bangladesh, Tejgaon Dhaka-1208, Bangladesh; plantsofbd@gmail.com (M.S.U.)

[4] International Biological Material Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, 34141, Republic of Korea; ethnolee@kribb.re.kr (S.W.L.)

* Correspondence: sglee1125@pknu.ac.kr.

† These authors contributed equally to this work.

Abstract: Bangladeshi medicinal plants (BMP) have been used as traditional medicinal plants to treat chronic inflammatory diseases, but the antioxidant and anti-inflammatory effects of the bark of BMP are not yet known. The objective of this study was to determine antioxidant and anti-inflammatory activities using methanolic extracts of bark obtained from 15 medicinal spices of Bangladesh plants. The bark methanol extracts of BMP evaluated the total antioxidant activity and anti-inflammatory effect of lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. Among the 15 bark extracts of BMP, *Albizia odoratissima* (*A. odoratissima*), *Engelhardia spicata* (*E. spicata*), and *Shorea robusta* (*S. robusta*) showed the highest total phenolic contents and total antioxidant capacity by reducing free radicals scavenging activity. In particular, the three bark extracts significantly reduced the mRNA expression of LPS-induced inflammatory cytokines and inflammation-inducible enzymes in macrophages. Also, the mRNA expression of NADPH oxidase 2 was significantly suppressed by three bark extracts in LPS-induced RAW 264.7 macrophages. The results suggest that among the 15 bark extracts obtained from medicinal plant in Bangladesh, three bark extracts of *A. odoratissima*, *E. spicata*, and *S. robusta* exert total antioxidant capacity by reducing free radicals scavenging activity and inhibitory effects on LPS-stimulated inflammation in macrophages.

Keywords: Bangladeshi medicinal plants; antioxidant; anti-inflammatory

1. Introduction

Oxidative stress is caused by excess of reactive oxygen species (ROS) generated by an imbalance between ROS levels and antioxidant defense system [1]. Excessive oxidative stress is associated with the development of various chronic inflammatory diseases, including prostate cancer, atherosclerosis, and vascular disease [2]. Furthermore, chronic inflammation stimulates the secretion of inflammatory cytokines such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF-α) and the production of ROS, which triggers oxidative stress [3]. Also, to treat chronic inflammatory diseases, it is important to reduce the level of free radical expression by increasing the activity of antioxidant enzymes and suppress inflammation by reducing pro-inflammatory cytokines [4]. Therefore, intervening with antioxidants to reduce free radicals and oxidative stress is beneficial in preventing and treating chronic inflammation [5].

Natural antioxidants present in medicinal plants are responsible for their powerful redox ability to scavenge free radicals [6]. In particular, the bark parts contained in medicinal plant-based antioxidant compounds play a defensive role by preventing the generation of free radicals, which

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contribute to alleviating oxidative stress-related diseases [7]. Also, the bark of medicinal plants is known to be rich in phenolic polymers such as phenolic acid, lignin, stilbene, and tannins, which provide potent antioxidant properties [8-11]. Recent studies have highlighted that phenolic compounds derived from woody vascular plants, especially bark, are attractive biological sources for inhibiting oxidative stress [8,12]. Furthermore, phenolic compounds in the bark of medicinal plants are known to have antioxidants, anti-inflammatory, immune system enhancing, and anti-aging properties. Therefore, polyphenol compounds contained in the bark of medicinal plants are potential candidates for pharmaceutical and medical applications in treating diseases related to oxidative stress [13].

Bangladesh is well known for its wide range of medicinal plants and herbal medicines among South Asian countries. Furthermore, it is estimated that more than 500 species of medicinal plants grow in Bangladesh, of which approximately 250 are utilized in traditional medicine preparations [14]. Bangladesh medicinal plants have traditionally been used for therapeutic purposes including diarrhea, stomachache, dysentery, wounds, and diabetes. However, no studies have been conducted to compare the overall antioxidant and anti-inflammatory effects of bark parts of medicinal plants in Bangladesh. Therefore, this study aims to provide wide applicability to new sources by demonstrating antioxidant and anti-inflammatory effects using bark extracts of medicinal plants used in Bangladesh.

2. Materials and Methods

2.1. Chemical reagents

Sodium carbonate (Na_2CO_3) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Folin-Ciocalteu's reagent, gallic acid, ascorbic acid (vitamin C), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) phosphate-buffered solution (PBS), citric acid, sodium acetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeSO_4{\cdot}7H_2O$, and $FeCl_2$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was purchased from Roche (Basel, Switzerland). For cells experiments, Dulbecco's Modified Eagle's Medium-high glucose (DMEM), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and lipopolysaccharide (LPS) from Escherichia coli O26:B6 were purchased from Sigma-Aldrich. Dulbecco's phosphate-buffered saline (DPBS) was purchased from WelGENE (Daegu, South Korea). Penicillin-streptomycin solution (P/S) was purchased from Cytiva-Hyclone Laboratories, Inc. (Logan, UT, USA). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Sample preparation

Fifteen bark methanol extract powders from medicinal plant in Bangladesh were obtained from International Biological Material Research Center (IBMRC) in the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The medicinal plants were collected from various regions of Bangladesh between March and October 2015 and identified by Md. Salah Uddin, a taxonomist at the Ethnobotanical Database of Bangladesh (Table 1). A voucher specimen of the plant was deposited in the herbarium of KRIBB. The bark extract stock solutions were prepared to 1000 mg/mL with methanol. For analysis, the working solution was diluted to the indicated concentration using methanol.

Table 1. The list of 15 types of medicinal plants from Bangladesh.

Voucher number[1)]	Species	Family name	Traditional uses
FBM269-007	*Albizia odoratissima* (L. f.) Benth	Fabaceae	Cough, diabetes, and burning sensation

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FBM274-026	*Araucaria columnaris* (J.R. Forst.) Hook	Araucariaceae	Ulcers and wounds
FBM269-003	*Bruguiera sexangula* (Lour.) Poir	Rhizophoraceae	Diarrhoea and tumour
FBM269-041	*Dipterocarpus turbinatus* Gaertn	Dipterocarpaceae	Gonorrhoea, gleet, and rheumatism
FBM274-001	*Engelhardia spicata* Lesch. ex Blume	Juglandaceae	Scabies and skin disease
FBM266-002	*Haldina cordifolia* (Roxb.) Ridsdale	Rubiaceae	Diarrhoea and dysentery
FBM269-039	*Saraca declinata* Miq	Fabaceae	Cure piles
FBM268-013	*Saraca thaipingensis* Cantley ex King	Fabaceae	Relives pain and improve skin complexion
FBM269-038	*Shorea robusta* Gaertn	Dipterocarpaceae	Diarrhoea
FBM274-007	*Sonneratia apetala* Buch.-Ham	Sonneratiaceae	Pain and kill worm
FBM269-033	*Talipariti tiliaceum* (L.) Fryxell	Malvaceae	Boils, wounds, cuts, and swellings
FBM269-037	*Vitex peduncularis* Wall. ex Schauer in A. DC	Verbenaceae	Anal fissure, diabetes, gout, and malaria
FBM269-043	*Wrightia arborea* (Dennst.) Mabb	Apocynaceae	Treating bleeding from cutting wound
FBM269-002	*Xylocarpus moluccensis* (Lam.) M. Roem	Meliaceae	Dysentery, stomachache, and candidiasis
FBM274-029	*Zanthoxylum spinosum* (L.) Sw	Rutaceae	Insect bite and rheumatism

[1)] The voucher number is a number assigned by the IBMRC to identify methanol extract sample.

2.3. Total phenolic content

The total phenolic content (TPC) of methanol extracts from the bark of 15 medicinal plants of Bangladesh was measured using the Folin-Ciocalteu method [15]. Briefly, 10 µL of bark extract were mixed with 10 µL of Folin & Ciocalteu's reagent and 130 µL of distilled water in a 96-well plate. After incubating for 6 min at room temperature, 100 µL of 7% Na_2CO_3 solution was added into mixture and incubated for 90 min at room temperature. The absorbance was measured at 750 nm using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific, MA, USA). TPC of each sample was expressed as mg gallic acid equivalents (GAE)/g dry weight.

2.4. ABTS radical scavenging activity

ABTS radical scavenging assay was investigated using a little modified method [16]. To make ABTS radical reagent, 1.0 mM AAPH, and 2.5 mM ABTS were dissolved in PBS and reacted at 80°C water bath for 40 min in the dark. The ABTS radical solution was filtered using a 0.45 µM PVDF syringe filter and diluted with PBS to adjust the absorbance of 0.700 ± 0.020 at 734 nm. A total 10 µL

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of bark extract was mixed with 240 μL of ABTS radical solution in a 96-well plate, followed by incubation at 37℃ for 10 min. The absorbance was measured at 734 nm by a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific). The inhibition percent of $ABTS^+$ was calculated according to the following equation: scavenging effect (%) = (1-(A_{sample} /$A_{control}$) x 100%.

Where $A_{control}$ is the absorbance of control reaction and A_{sample} is the absorbance of the sample with $ABTS^+$. The antioxidant capacity in samples was expressed as half-maximal inhibitory concentration (IC_{50})that indicates the concentration of the sample required to scavenge 50% of radicals.

2.5. DPPH radical scavenging assay

DPPH radical scavenging activity of bark extracts was measured according to the slightly modified method [16]. DPPH radical solution (0.1 mM) was prepared by mixing DPPH and 80% methanol. The solution was diluted with 80% methanol to adjust the absorbance of 0.700 ± 0.020 at 517 nm using a Shimadzu spectrophotometer (Shimadzu). A total of 5 μL of bark extract were mixed with 245 μL of DPPH solution and incubated for 30 min at room temperature. The absorbance was measured at 517 nm using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific). The antioxidant capacity is expressed as percentage inhibition, calculated using the following formula: scavenging effect (%) = (1-(A_{smaple} /$A_{control}$) x 100%. Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample at 517 nm. The DPPH radical scavenging capacity of bark extracts was calculated as IC_{50}.

2.6. Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) of bark extracts was measured according to a slightly modified method [17]. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution at ratio of 10:1:1 (v/v), respectively. After mixing FRAP solution, it was kept at 37°C until use. Six μL of each bark extract was mixed with 200 μL of FRAP solution and incubated at 37°C for 4 min in a 96-well microplate. The absorbance was measured at 593 nm using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific). The standard curve linear range was between 0 and 1.0 mM $FeSO_4$. FRAP of bark extract was expressed as mM $FeSO_4$ equivalent (FSE)/g dry weight.

2.7. Cell culture

Murine RAW 264.7 macrophages (Korean Cell Line Bank (KCLB), Seoul, South Korea) were cultured in DMEM supplemented with 10% FBS, 1% P/S in a humidified incubator at 37℃ with 5% CO_2 atmosphere.

2.8. Cell viability

Cell viability was measured using MTT assay. RAW 264.7 macrophages were treated with various concentration (0-100 μg/mL) of bark extracts for 24 hr. MTT solution (500 μg/mL) was added to the cells and incubated at 37°C for 1 hr. After discarding culture media, the formazan crystals were dissolved by dimethyl sulfoxide. Absorbance at 570 nm was measured using BioTek Cytation 5 Image reader (Winooski, VT, USA). Cell viability revealed no significant toxicity at various concentration (0-5 μg/mL) of bark extracts (data not shown). Further experiments were conducted at bark extract concentration under at μg/mL.

2.9. Quantitative real time PCR (qRT-PCR)

To The mRNA expression levels of specific genes were evaluated by qRT-PCR. RAW 264.7 macrophages were pre-treated with 1.25, 2.5, and 5 μg/mL of bark extract or vehicle for 6 hr, and then stimulated with or without 100 ng/mL of LPS for 3 hr. Total RNA extract from macrophages, synthesis of cDNA, and qRT-PCR were performed as previously described [18]. The primers for RT-

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PCR were manufactured by Macrogen Co., (Seoul, South Korea), and the sequences are listed in Table 2.

Table 2. Primers for real-time PCR amplification of gene expression.

Primer	F/R	Sequences (5' to 3' direction)
IL-6	F	CCC ACC AAG AAC GAT AGT CA
	R	CTC CGA CTT GTG AAG TGG TA
IL-1β	F	GTC ACA AGA AAC CAT GGC ACA T
	R	GCC CAT CAG AGG CAA GGA
TNF-α	F	GGC TGC CCC GAC TAC GT
	R	ACT TTC TCC TGG TAT GAG ATA GCA AAT
iNOS	F	AAT CTT GGA GCG AGT TGT GG
	R	CAG GAA GTA GGT GAG GGC TTG
COX2	F	GCC TAC TAC AAG TGT TTC TTT TTG CA
	R	CAT TTT GTT TGA TTG TTC ACA CCA T
NOX2	F	CCC TTT GGT ACA GCC AGT GAA GAT
	R	CAA TCC CGG CTC CCA CTA ACA TCA
GAPDH	F	GGT GGT CTC CTC TGA CTT CAA CA
	R	GTT GCT GTA GCC AAA TTC GTT GT

2.10. Statistical analysis

All analyses were repeated in triplicate. One-way ANOVA and Tukey's post hoc test were performed using GraphPad 9.0 (GraphPad Software La Jolla, CA, USA). *P* values less than 0.05 were considered statically significant. All data were expressed as mean ± standard deviation.

3. Results

3.1. Total phenolic content

Phenolic compounds from medicinal plants are characterized by a benzene ring substituted with hydroxyl groups and exhibit a variety of biological properties, including antioxidant, anti-inflammatory, antiproliferative and antibacterial activities [16,19]. TPC was first performed to investigate which bark extract from 15 Bangladeshi medicinal plants had the highest phenolic content (Table 3). TPC result for 15 bark extracts ranged from 17.05 to 369.18 mg GAE/g dry weight. Among 15 types of bark extracts from Bangladesh medicinal plants, *Albizia odoratissima* (369.18 ± 26.47), *Engelhardia spicata* (309.50 ± 70.70), *Xylocarpus moluccensis* (357.78 ± 41.41), and *Shorea robusta* (243.08 ± 93.64) showed the highest TPC. On the other hand, *Wrightia arborea* (17.05 ± 2.44) and *Vitex peduncularis* (22.20 ± 0.37) exhibited the lowest TPC.

Table 3. TPC and total antioxidant activity of the bark extracts of 15 medicinal plants from Bangladesh.

Species	TPC (mg GAE/g)	ABTS (IC^{50}, μg/mL)	DPPH (IC^{50}, μg/mL)	FRAP (mM FSE/g)
Albizia odoratissima (L. f.) Benth	369.18 ± 26.47[a]	68.84 ± 4.79[a,b,c]	181.39 ± 1.91[a]	5.62 ± 1.03[a]

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Araucaria columnaris (J.R. Forst.) Hook	112.38 ± 27.92^{d,e}	151.49 ± 7.28^{g}	880.92 ± 14.56^{e}	1.58 ± 0.70^{c,d}
Bruguiera sexangula (Lour.) Poir	208.88 ± 34.75^{b,c}	93.37 ± 5.06^{e}	231.28 ± 5.89^{a,b,c}	4.10 ± 0.64^{a,b}
Dipterocarpus turbinatus Gaertn	203.74 ± 42.48^{b,c}	63.42 ± 1.59^{a}	281.40 ± 0.61^{c}	1.35 ± 0.30^{c,d}
Engelhardia spicata Lesch. ex Blume	309.50 ± 70.70^{a}	60.80 ± 4.31^{a}	192.59 ± 2.42^{a,b}	5.50 ± 0.73^{a}
Haldina cordifolia (Roxb.) Ridsdale	49.88 ± 6.64^{d,e}	442.50 ± 14.73^{h,i}	2711.83 ± 17.98^{g}	0.21 ± 0.03^{d}
Saraca declinata Miq	138.66 ± 15.38^{c,d}	78.22 ± 2.92^{c,d}	240.12 ± 4.05^{a,b,c}	2.74 ± 0.81^{b,c}
Saraca thaipingensis Cantley ex King	125.31 ± 31.03^{d}	112.72 ± 7.28^{f}	270.39 ± 4.74^{b,c}	0.52 ± 0.04^{d}
Shorea robusta Gaertn	243.08 ± 93.64^{a,b}	63.78 ± 1.49^{a,b}	211.87 ± 1.52^{a,b,c}	3.21 ± 0.30^{b}
Sonneratia apetala Buch.-Ham	184.52 ± 36.48^{b,c}	79.33 ± 4.42^{d}	292.06 ± 18.29^{c,d}	3.47 ± 0.60^{b}
Taliparititi tiliaceum (L.) Fryxell	54.73 ± 0.98^{d,e}	432.41 ± 39.83^{h}	2603.58 ± 85.53^{f}	1.25 ± 0.74^{c,d}
Vitex peduncularis Wall. ex Schauer in A. DC	22.20 ± 0.37^{e}	1625.98 ± 248.27^{k}	18796.98 ± 408.54^{j}	0.21 ± 0.09^{d}
Wrightia arborea (Dennst.) Mabb	17.05 ± 2.44^{e}	1054.52 ± 46.56^{j}	16687.88 ± 772.52^{i}	0.24 ± 0.11^{d}
Xylocarpus moluccensis (Lam.) M. Roem	357.78 ± 41.41^{a}	74.57 ± 2.50^{b,c,d}	344.33 ± 14.33^{d}	3.52 ± 1.13^{b}
Zanthoxylum spinosum (L.) Sw	55.24 ± 18.68^{d,e}	465.64 ± 16.17^{i}	4042.37 ± 43.29^{h}	1.52 ± 0.75^{c,d}

The values are expressed as the means ± SD (n=3). Different letters in the same column indicated significant difference (P<0.05).

3.2. Total antioxidant capacity

The total antioxidant capacity of 15 bark extracts was investigated through ABTS, DPPH, and FRAP analysis. ABTS and DPPH are expressed as IC_{50} of each antioxidant activity (Table 3). Among 15 bark extracts, the highest IC_{50} values for ABTS radical scavenging activity were observed from *Engelhardia spicata* (60.80 ± 4.31 µg/mL) followed by *Dipterocarpus turbinatus* (63.42 ± 1.59), *Shorea robusta* (63.78 ± 1.49), and *Albizia odoratissima* (68.84 ± 4.79). For DPPH radical scavenging activity, *Albizia odoratissima* showed the lowest IC_{50} values among 15 bark extracts as 181.39 ± 1.91 µg/mL, followed by *Engelhardia spicata* (192.59 ± 2.42), *Shorea robusta* (211.87 ± 1.59), *Bruguiera sexangula* (231.28 ± 5.89), and *Saraca declinate* (240.12 ± 1.52). In the case of FRAP assay, *Albizia odoratissima* and *Engelhardia spicata* also showed the highest reducing power as 5.62 ± 1.03 and 5.50 ± 0.73 mM FSE/g dry weight, respectively. Therefore, three bark extracts of *Albizia odoratissima*, *Shorea robusta*, and *Engelhardia spicata* showed the highest total antioxidant activity among 15 bark extracts in both ABTS, DPPH, and FRAP assays.

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3.3. Correlation between TPC and total antioxidant capacity

Pearson correlation analysis was conducted to characterize the relationship between TPC and three antioxidant assays such as ABTS, DPPH, and FRAP assay (Table 4). TPC assay had a significantly positive correlation with FRAP assay (r=0.872, P<0.01). Also, ABTS assay showed the highest significant correlation with DPPH assay (r=0.972, P< 0.01) compared to other assays correlation. However, ABTS and DPPH assay had a negative correlation with TPC and FRAP assays (ABTS&TPC; r= - 0.664, P<0.05, ABTS&FRAP; r= - 0.599, P<0.050, DPPH&TPC; r= - 0.604, P<0.05, DPPH &FRAP; r= - 0.549, P<0.05).

Table 4. Pearson's correlation between TPC and three antioxidant capacity results in the bark extracts of 15 medicinal plants from Bangladesh.

Antioxidant activity	TPC	ABTS	DPPH	FRAP
TPC	1	-0.664 (P<0.01)	-0.604 (P<0.05)	0.872 (P<0.01)
ABTS		1	0.972 (P<0.01)	-0.599 (P<0.05)
DPPH			1	-0.549 (P<0.05)
FRAP				1

3.4. Comparison of antioxidant capacities by heatmap analysis

To compare TPC and total antioxidant capacity of bark extracts of 15 medicinal plants in Bangladesh, heat map analysis was performed (Table 5). The heat map replaced the different levels of TPC and antioxidant capacities with red color for the highest level, light red for lower levels toward average level in white. Among 15 bark extracts, *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* showed the highest of TPC and antioxidant capacities. Among the rest, *Bruguiera sexangular*, *Dipterocarpus turbinatus*, *Sonneratia apetala*, and *Xylocarpus moluccensis* exhibited significantly outstanding TPC and antioxidant capacities. On the other hand, *Vitex peduncularis* and *Wrightia arborea* revealed the lowest levels of TPC and total antioxidant capacity among 15 bark extracts.

Table 5. Heatmap analysis of TPC and antioxidant capacity for the 15 bark extracts of medicinal plants from Bangladesh.

Species	TPC	ABTS	DPPH	FRAP
Albizia odoratissima (L. f.) Benth				
Araucaria columnaris (J.R. Forst.) Hook				
Bruguiera sexangula (Lour.) Poir				
Dipterocarpus turbinatus Gaertn				
Engelhardia spicata Lesch. ex Blume				
Haldina cordifolia (Roxb.) Ridsdale				
Saraca declinata Miq				
Saraca thaipingensis Cantley ex King				
Shorea robusta Gaertn				
Sonneratia apetala Buch.-Ham				
Talipariti tiliaceum (L.) Fryxell				
Vitex peduncularis Wall. ex Schauer in A. DC				
Wrightia arborea (Dennst.) Mabb				

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Xylocarpus moluccensis (Lam.) M. Roem
Zanthoxylum spinosum (L.) Sw **Low**

3.5. Effect of anti-inflammatory on three bark extracts in LPS-stimulated RAW 264.7 macrophages

Compounds with antioxidant properties are known to have anti-inflammatory abilities [5]. Since three bark extracts of medicinal plants such as *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* showed higher TPC and total antioxidant capacity than the other bark extracts, we further investigated the anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages. LPS significantly increased the mRNA expression of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α, which were significantly abolished by all three bark extracts (Figure 1a). Also, the three bark extracts significantly reduced the mRNA expression of inflammation-inducible enzymes such as inducible NO synthase (iNOS) and cyclooxygenase-2 (COX2) was significantly reduced in LPS-stimulated RAW 264.7 macrophages (Figure 1b). Furthermore, the mRNA expression of NADPH oxidase 2 (NOX2), an enzyme that generates ROS, was significantly increased by LPS in RAW 264.7 macrophages while three bark extracts significantly suppressed the LPS-induced NOX2 mRNA expression (Figure 1c).

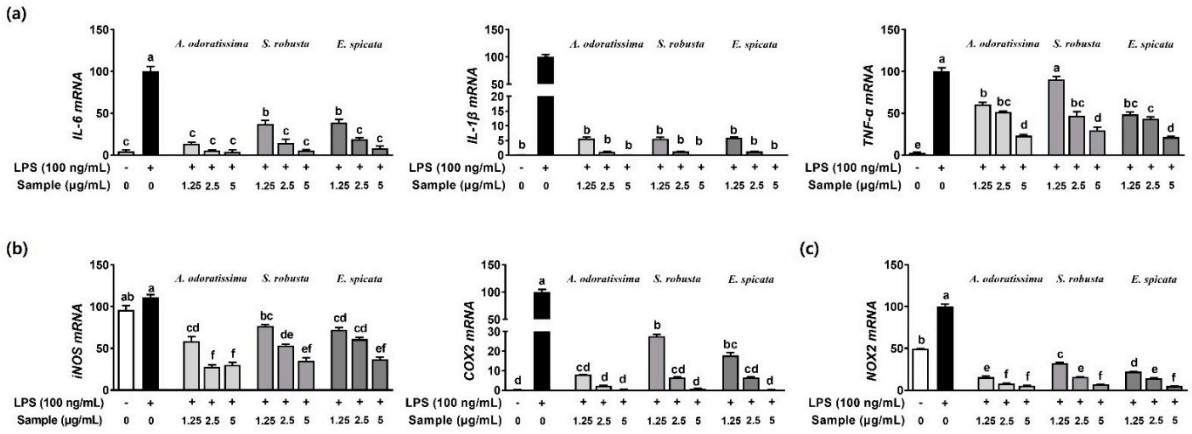

Figure 1. Anti-inflammatory effects of three bark extracts of *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* in LPS-stimulated RAW 264.7 macrophage. RAW 264.7 macrophages were pretreated with various concentrations (1.25, 2.5, 5 μg/mL) of the three species of bark extracts for 6 hr and then stimulated with 100 ng/mL of LPS for 3 hr in absence or presence of three bark extracts. The mRNA expression of (a) pro-inflammatory cytokines, (b) inflammation-inducible enzymes, and (c) NOX2 was determined using qRT-PCR. GAPDH was used as internal controls. Different letters indicate significant difference (p<0.05) based on ANOVA with Tukey's post hoc analysis.

4. Discussion

Phenolic compounds in medicinal plants have the ability to provide antioxidant and anti-inflammatory effects, which help to alleviate oxidative stress and inflammation at the cellular level [20]. Studies have demonstrated that Bangladeshi medicinal plants exert antioxidant capacity and inhibit pro-inflammatory cytokine secretion [21-24]. However, the effects of bark extract of Bangladeshi medicinal plant on antioxidant and anti-inflammatory functions have not been confirmed. In this study, we found that extracts derived from the bark of three medicinal plants in Bangladesh, namely *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta*, exhibited the highest total antioxidant capacity compared to 15 different types of bark extracts. Also, these three bark extracts showed anti-inflammatory properties by reducing the expression of pro-inflammatory cytokines and inflammation-inducing enzymes in LPS-stimulated RAW 264.7 macrophages.

Phenolic compounds are known to have superior antioxidant ability due to particular chemical structures, which contain conjugated double bonds and hydroxyl groups [25]. In the present study, three bark extracts, *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* exhibited the highest

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TPC among 15 types of bark extracts of Bangladesh medicinal plants, which was considered to have the highest phenolic compounds content formed by conjugated double bonds and hydroxyl groups. Also, three bark extracts showed the highest DPPH and ABTS radicals scavenging activity and FRAP activity among 15 types of bark extracts. The strong antioxidant properties of three bark extracts are partly due to their high phenolic compound content, which exerts a scavenging effect on ROS and interface with oxidative free radicals [26]. Furthermore, studies have demonstrated that high abundance of phenolic compounds and strong antioxidant capacity of three bark extracts are closely related to traditional uses to improve body's immune system against ROS. The bark extract of *Albizia odoratissima*, which has been traditionally used in Bangladesh to treat diseases such as coughing, bronchitis, and diabetes, has been reported to contain flavonoids and terpenoids [27]. Similarly, the bark extract of *Shorea robusta*, which is known for medicinal use in treating scabies and skin diseases in Bangladesh, contains major components such as flavonoids and tannin. [28,29]. Also, phenolic derivatives such as flavonoids, triterpenes, diarylheptanoids, and aromatic acid/esters were isolated from the genus *Engelhardia* [30].

Multiple inflammatory triggers, such as overproduction of ROS during oxidative metabolism, have been documented to initiate the inflammatory cascade, leading to the production and release of proinflammatory cytokines [31]. Furthermore, elevated production of ROS resulting from oxidative stress can induce the oxidation of amino acids, lipid peroxidation, and oxidative damage to DNA, thereby triggering an inflammatory response [32]. In this study, the three bark extracts, including *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* significantly suppressed the mRNA expression of inflammatory cytokines and inflammation-inducible enzymes in LPS-stimulated macrophages. Also, three bark extract exhibited the highest phenolic content with total antioxidant capacity by reducing ABTS and DPPH free radicals scavenging activity and FRAP capacity. The high antioxidant capacity of phenolic compounds can reduce oxidative stress-mediated inflammation through the inhibition of nuclear factor (NF)-κB-mediated proinflammatory cytokines [31]. Therefore, the strong antioxidant capacity and high phenolic content of the three bark extracts likely contribute to suppressing LPS-induced inflammation in RAW 264.7 macrophages by reducing oxidative stress. In particular, three bark extracts completely suppressed LPS-induced the mRNA expression of NOX2 in macrophages. NOX2 is known to regulate the production of ROS within macrophages, which stimulates the inflammatory process by secreting pro-inflammatory cytokines [28]. Although this study did not evaluate the effect of three bark extracts on ROS production in macrophages, three bark extracts were observed to potentially reduce the elevated ROS production by inhibiting NOX2 gene expression in LPS-induced RAW 264.7 macrophages. Finally, since the three bark extracts exhibited strong antioxidant and anti-inflammatory abilities, it is necessary to find phenolic compounds in the three bark extracts that scavenge free radical activity and inhibit pro-inflammatory cytokines.

5. Conclusions

In conclusion, this study provides that three bark extracts including *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* among the 15 bark extracts of medicinal plants from Bangladesh have the strongest antioxidant abilities and anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages. The three bark extracts of *Albizia odoratissima*, *Engelhardia spicata*, and *Shorea robusta* exhibited the strong total antioxidant capacity with high amount of phenolic compounds contents, which contribute to suppress the LPS-stimulated inflammation in RAW 264.7 macrophages. Constantly, three bark extracts significantly reduced LPS-induced the mRNA expression of pro-inflammatory cytokines and inflammation-inducible enzymes. However, further studies are required to investigate phenolic compounds of three bark extract from medicinal plant in Bangladesh that potent antioxidant and anti-inflammatory properties. Therefore, our findings suggest that the three bark extracts from medicinal plants in Bangladesh have great potential as valuable nutraceutical resources for both the prevention and treatment of chronic inflammatory diseases.

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