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Antioxidant, anti-inflammatory, and anti-*Staphylococcal* activities of *Albizia lucidior* (Steud.) I. C. Nielsen wood extracts

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ABSTRACT: *Albizia lucidior* (Steud.) I. C. Nielsen is a leguminous tropical hardwood tree that is of great interest as a herbal medicine. In this study, both ethanolic and aqueous extracts of *A. lucidior* wood were investigated. The phenolic and flavonoid contents of the extracts were measured, and the antioxidant and anti-inflammatory activities were evaluated by inhibition of tumor necrosis factor α and nitric oxide production. Cytotoxic activity against macrophages was determined by the MTT assay, and antimicrobial activities were tested against *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), and *Staphylococcus epidermidis*, measuring the minimum inhibitory and bactericidal concentrations. The chemical composition of the extracts was analyzed by GC-MS. The aqueous extract had higher total phenolic content than the ethanolic extract. Both extracts had strong antioxidant activity in the DPPH assay, with respective IC_{50} values of 7.12 and 9.02 μg/ml. In the ABTS assay, the aqueous extract had a lower IC_{50} (9.50 μg/ml) than the ethanolic extract. The ethanolic extract significantly inhibited NO, but the aqueous extract showed no inhibitory activity. However, neither extract significantly inhibited TNF-α production. Significant antibacterial activity was found only in the ethanolic extract with an MIC and an MBC against *S. aureus* of 2.5 mg/ml and 5.0 mg/ml, respectively. From our knowledge, this is the first study of the antioxidant, anti-inflammatory, and anti-*Staphylococcal* activities of *A. lucidior* wood extracts. The significant anti-*Staphylococcal* activity should be further explored in preclinical studies.

KEYWORDS: *Albizia lucidior*, antioxidant, anti-inflammatory, anti-*Staphylococcal* activity, phenolic content, flavonoid

INTRODUCTION

Pyrexia, or fever, is an adaptive response of the body to infection (e.g., exogenous bacterial pyrogens), malignancy, or other disease states [1]. The body uses this natural function in trying to create an environment in which infectious agents or damaged tissues cannot survive. Infected or damaged tissues normally induce the formation of proinflammatory mediators, called cytokines, such as interleukins (-1β, -α, and -β) and tumor necrosis factor α, all of which increase the synthesis of prostaglandin E2 (PGE2) in the hypothalamus causing an elevation of body temperature [2].

Conventional drugs for treatment of fever and pain like nonsteroidal anti-inflammatory drugs (NSAIDs) may be associated with clinically significant side effects affecting the digestive system, kidneys, liver, central nervous system, or skin [3]. An alternative for the treatment could be herbal medicines, which also tend to have a broader range of applications, e.g., a single herbal medicine may exert anti-inflammatory, antioxidant, and antibacterial activities.

The antioxidant activity of natural substances is an area of great interest because some of them can preserve foods and protect them from the toxic effects of oxidation [4]. Besides, natural agents with free radical scavenging ability can play a significant role in the prevention or treatment of some diseases

such as brain dysfunction, cancer, heart disease, gastrointestinal disease, and immune system decline [5, 6].

In herbal medicine, a single plant species is often used to treat more than one type of diseases or conditions. However, the scientific basis for such use may be unknown and should be investigated to ascertain any potential activity against human pathogens that could be exploited clinically.

Albizia lucidior is a species of leguminous tropical hardwood tree in the Fabaceae family. Its wood is commonly used as a traditional treatment for various ailments as an antipyretic, an appetite stimulant, and a tonic; whereas the bark and wood are used as laxatives and cathartics. However, a thorough literature search reveals very limited scientific data of its medicinal properties. The purpose of this study was to assess the antioxidant, anti-inflammatory, and anti-*Staphylococcal* activities of *A. lucidior*.

MATERIALS AND METHODS

Chemicals and reagents

All reagents were of analytical grade. The following items were purchased from Sigma-Aldrich, USA: clarithromycin, dimethylsulfoxide (DMSO), lipopolysaccharide from *E. coli* O55:B5 (LPS), thiazolyl blue tetrazolium bromide (MTT), 2,2-diphenyl-1-picryhydrazyl (DPPH), 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, aluminium chloride, potassium acetate, gallic acid, butylated hydroxytoluene (BHT), and resazurin sodium salt. Folin-Ciocalteu's reagent was purchased from Fluka, USA. Mueller Hinton Broth (MHB) and Nutrient broth (NA) were procured from RPD, Thailand. Brain heart infusion (BHI) and anaeropack were obtained from Difco, USA. Roswell Park Memorial Institute (RPMI) 1640, fetal bovine serum (FBS), trypsin-EDTA, and trypan blue were purchased from Gibco BRL, USA.

Plant materials

Albizia lucidior wood was purchased from a herbal medicine shop in Nakhon Pathom, Thailand in January 2018. The wood's botanical identification was authenticated at the Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University.

Microorganisms

Staphylococcus aureus ATCC 25923, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 20651, and *Staphylococcus epidermidis* ATCC 12228 were obtained from the culture collections of the Center of Excellence in Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University.

Preparation of the extracts

The wood was washed, dried, and ground before extraction. Two extracts, aqueous and ethanolic, were prepared according to Dechayont et al [7]. The aqueous extract was prepared by boiling the wood powder in water for 30 min, followed by filtration using Whatman #1 filter paper. The liquid was first frozen and then partially dried using a lyophilizer resulting in a viscous liquid. The ethanolic extract was prepared by soaking the wood powder in 95% EtOH for 72 h, then filtered, and partially dried in an evaporator. The whole process was repeated three times to yield the viscous ethanolic extract. Both the aqueous and ethanolic extracts were kept at −20 °C before further uses.

Total phenolic content

Total phenolics were estimated as gallic acid equivalents (GAE), expressed as mg GAE/g. Briefly, 20 µl of sample (1000 µg/ml) was added to 96 well plates followed by 100 µl of Folin-Ciocalteu reagent (diluted 10-fold). After 3 min, 80 µl of 7.5% w/v Na_2CO_3 was added, and the reaction mixture was allowed to stand for 30 min before the absorbance at 765 nm was measured [8].

Total flavonoid content

Total flavonoids were estimated as quercetin equivalents (QE), expressed as mg QE/g. Briefly, 20 µl of sample (1000 µg/ml), 60 µl of methanol, 10 µl of aluminium chloride (10% w/v), 10 µl of potassium acetate (1 M), and 120 µl of distilled water were mixed in 96 well plates. The mixture was incubated at room temperature for 30 min. Then, absorbance was determined at 415 nm [8].

Determination of antioxidant activities

DPPH radical scavenging assay

DPPH radical scavenging activity was measured using a slightly modified method of Dechayont et al [9]. DPPH solution in absolute ethanol was newly prepared before use and protected from light. A 100 µl portion of each sample solution or control was mixed with 100 µl of DPPH solution (in a 1:1 ratio) in 96-well plates, and the mixture was allowed to sit at room temperature for 30 min, protected from light. BHT was used as a positive

control. Absorbance was then measured at 520 nm. The percent inhibition of DPPH free radical scavenging activity was calculated using the following equation:

$$\%\,\text{Inhibition} = \left[(A_{DPPH} - A_{sample})/A_{DPPH}\right] \times 100$$

where A_{DPPH} = absorbance of DPPH and A_{sample} = absorbance of sample.

The percent inhibition data was plotted against the log concentration and fitted to a graph. The IC_{50} (half-maximal inhibitory concentration) value was calculated using Prism software.

ABTS radical cation decolorization assay

The ABTS radical cation decolorization assay was performed following the method of Re et al [10] with slight modification. The $ABTS^{\bullet+}$ solution was produced by the reaction of 7 mM ABTS stock solution in distilled water with 2.45 mM potassium persulfate. A 20 µl portion of each sample solution was combined with 180 µl of the $ABTS^{\bullet+}$ solution in 96-well plates, and the mixture was left to react in the dark for 6 minutes. Absorbance was measured at 734 nm, and BHT was used as a positive control.

Anti-inflammatory activity

Nitric oxide-inhibitory effect

Mouse macrophage leukemia-like cells (RAW 264.7, ATCC TIB-71TM) were induced with lipopolysaccharide (LPS) to release inflammatory mediators, including nitric oxide and tumor necrosis factor-alpha (TNF-α). Griess reagent was used for determination of the nitrite, a stable end product of nitric oxide (NO) in the cell culture supernatants, and the TNF-α production was measured using a commercial kit (ImmunoTools, Germany) following the manufacturer's protocols. The inhibitory effect on NO production was evaluated using the method of Suthisamphat et al [11]. Approximately 100 000 cells were seeded into each well of 96-well plates along with Dulbecco's Modified Eagle Medium (DMEM) and incubated for 24 h. The medium was then removed and replaced with fresh medium. LPS was added for a final concentration of 5 ng/ml. The *Albizia lucidior* extracts of various concentrations were also added. After 24 h of further incubation, the ability of the extract to inhibit NO in the supernatant was detected by adding 100 µl of Griess reagent and reading the absorbance at 570 nm. The percent inhibition was calculated using the following equation:

$$\%\,\text{Inhibition} = [(A - C) - (B - D)]/(A - C) \times 100$$

where A = control, with no sample but in the presence of LPS; B = sample, in the presence of LPS; C = blank of A, in the absence of sample and LPS; and D = blank of B, with sample but in the absence of LPS.

IC_{50} values were calculated from the percent inhibition of concentrations using the Prism program.

TNF-α inhibitory effect in RAW 264.7 cells by ELISA

RAW 264.7 cells were incubated with 5 ng/ml of LPS and the extract for 24 h. Then TNF-α levels in the supernatants were determined using an ELISA kit (ImmunoTools) following the manufacturer's instructions [12].

Cytotoxicity by MTT method

Cell viability was determined using the MTT assay. Macrophage cells (RAW 264.7) were divided into two experimental groups: (1) the control group consisting of only added medium and (2) the sample group having added medium and the *A. lucidior* extracts in various concentrations. The two experimental groups were incubated in 96-well plates for 24 h. A 10 µl portion of MTT solution (5 mg/ml) was then added to each well, and incubation proceeded for another 4 h at 37 °C. Next, 100 µl of dimethyl sulfoxide (DMSO) was added to each well to dissolve any deposited formazan. The optical density (OD) of each well was measured at 570 nm using a microplate reader. Percentage of cell survival was calculated using the equation:

$$\%\,\text{Cell viability} = (Ae/Ac) \times 100$$

where Ae and Ac are the absorbance of the extract and the control groups, respectively.

Antimicrobial activity

Microorganism and culture conditions

Staphylococcus aureus ATCC 25923, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 20651, and *Staphylococcus epidermidis* ATCC 12228 were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *A. lucidior* extracts. Prior to each experiment, the cultures were incubated through three successive generations by letting them grow for three successive nights in nutrient agar (NA).

Determination of minimum inhibitory concentration (MIC)

The MICs of the *A. lucidior* extracts were determined in 96-well microplates plates using microdilution as-

say, a slightly modified method of Sarker et al [13]. This technique relies on resazurin, an oxidation-reduction indicator used for evaluating cell growth, particularly in the cytotoxic activity assay [14]. Broth cultures of *S. aureus*, MRSA, and *S. epidermidis* were prepared and then incubated for 18–24 h, depending on the individual bacteria. When the cultures reached the required density of 0.5 McFarland Standard, each was diluted with broth at a ratio of 1:200. Two-fold serial dilutions of the wood extracts were prepared directly in sterile 96-well plates as follows. Each of the viscous extracts (aqueous and ethanolic) was diluted 1:1 with broth. All the 96 wells of the plate were filled with 50 μl of bacterial suspension. Then, 50 μl of the diluted extract was added to the first row of the plate. After mixing, the 100 μl of the first row contents were pipetted to the second row. This two-fold dilution procedure continued for a total of five rows. The plates were then incubated again for the same bacterium-specific times as before. Next, 10 μl of resazurin was added to each well and incubated for an additional 2 h. Color changes were assessed visually. The MIC was defined by the lowest concentration of the extract that did not result in a color change from purple to pink or no color. Measurements were made in triplicate, and the mean value was used.

Minimum bactericidal concentration

The MBCs were determined using the well plates from the end of the MIC assay. The contents of all the MIC assay wells with no visible growth were transferred to agar plates, incubated at 37 °C for 24 h, and then assessed for bacterial growth. The quadrant with the lowest concentration of wood extract that showed no growth was the MBC. The assay was done in triplicate, and the mean value was used. Extract concentrations were recorded as mg/ml.

Statistical analysis

All experiments were carried out in triplicate and presented as means ± SEM (standard error of the mean).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the wood ethanolic extract of *A. lucidior* was analyzed by the GC-MC technique using Bruker Scion 436-GC model coupled with a single quadruple mass spectrophotometer comprising of a CP-8410 autosampler. A fused silica capillary column, SCION-5MS (5% phenyl/95% dimethyl poly siloxane) 30 m × 0.25 mm × 0.25 μm, was run on helium gas with a flow rate of 1.2 ml/min. The sample (1 μl) was injected in the split ratio of 1:50 and run for 56 min throughout the experiment. The initial temperature of 80 °C was gradually increased up to 250 °C at a rate of 5 °C/min, and finally raised up to 310 °C at a rate of 20 °C/min. Identification of components were confirmed by comparison of the mass spectra with reference data from the National Institute Standard and Technology (NIST) library.

RESULTS AND DISCUSSION

In the present study, phytochemical investigation of different species belonging to the genus *Albizia* afforded different classes of secondary metabolites such as saponins, terpenes, alkaloids, and flavonoids [15]. Reports on other *Albizia* species showed that the methanolic extract of *Albizia procera* leaves possessed antioxidant activity (DPPH assay) with an IC_{50} of 43.43 μg/ml [16]. The ethanolic extract of *Albizia myriophylla* wood was reported for potential antioxidant and anti-inflammatory activities (both *in vitro* and *in vivo*). Using the DPPH, ABTS, and FRAP assays, it was demonstrated that the isolated compounds from the extract (indenoic acid and 8-methoxy-7,3 ,4 -trihydroxyflavone) had potent free radical scavenging effects [17]. The methanolic extract of *A. adianthifolia* leaves exhibited high content of phenolics (30.17 mg gallic acid/g) and high radical scavenging property (using DPPH assay) of 58.19% [18].

The extraction yields of *A. lucidior* (Table 1) for the aqueous and the ethanolic extracts were 2.97 and 2.14%, respectively. Mean total phenolic and flavonoid contents of the aqueous extract were 139.97 ± 1.91 mg GAE/g and 165.98 ± 7.98 mg QE/g; while those for the ethanolic extract were 116.19 ± 1.22 mg GAE/g and 221.62 ± 9.69 mg QE/g, respectively. The aqueous extract showed greater scavenger activity than the ethanolic extract in the ABTS and DPPH assay (Table 1). To the best of our knowledge, our research is the first publication on the antioxidant activity of *A. lucidior* wood extracts.

The determination of anti-inflammatory activities of the aqueous and ethanolic extracts of *A. lucidior* wood, by the NO and TNF-α assays revealed that only the ethanolic extract had anti-inflammatory activity, inhibiting NO production with an IC_{50} of 58.81 μg/ml (Table 1 and Fig. 1). Both extracts, at various concentrations, showed no cytotoxicity activity with viability percentage exceeding 70%.

Table 1 The percentage yield, total phenolic content, flavonoid content, antioxidant activities, and inhibition of NO and TNF-α productions of LPS-stimulated RAW 264.7 cells ($n = 3$) of *A. lucidior* wood extract by decoction and maceration methods.[†]

Extract	% Yield	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	IC_{50} (µg/ml) DPPH assay	ABTS assay	Inhibition of NO	Inhibition of TNF-α
Aqueous	2.97	139.97 ± 1.91	165.98 ± 7.98	7.12 ± 0.45	9.50 ± 1.22	> 100	> 100
95% EtOH	2.14	116.19 ± 1.22	221.62 ± 9.69	9.02 ± 0.93	27.44 ± 1.20	58.81 ± 0.90	> 100
BHT	–	–	–	16.23 ± 0.36	5.21 ± 0.56	–	–

[†] RAW 264.7 cells were stimulated by 5 ng/ml of LPS. The concentrations of extracts were used to calculate IC_{50} that displayed no cytotoxic (cell viability >70%).

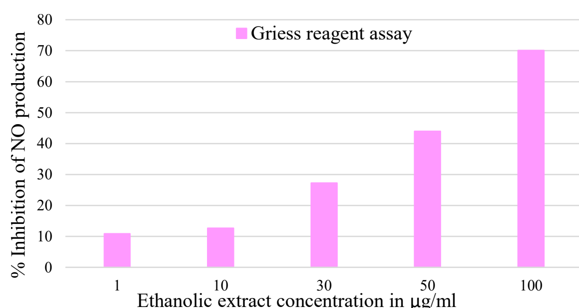

Fig. 1 The inhibition of nitric oxide production by different concentrations of the ethanolic extract of *A. lucidior* wood.

In consistent with a previous report, the ethanolic extract of *A. myriophylla* wood inhibited the release of nitric oxide production with IC_{50} value of 13.8 µg/ml [17]. In another study, methanolic extract from leaves of *A. anthelmintica* revealed anti-inflammatory activity by decreasing the production of inflammatory mediators, PGE2 and leukotrienes, via inhibition of COX-1, COX-2, and 5-LOX, with IC_{50} values of 4.11, 0.054, and 1.74 µg/ml, respectively [19]. Our findings suggest that the moderate *in vitro* anti-inflammatory activity of the ethanolic extracts may act mostly through the NO production pathway. However, further investigations for other mechanisms are needed.

In our experiments, only the ethanolic extract of *A. lucidior* wood inhibited *Staphylococcus* species with MICs values of 2.5–5 mg/ml (Table 2) in line with what reported by Duraipandiyan et al. At concentrations of 1.5, 2.5, and 5 mg of methanolic extract of *A. procera* stem bark, there was dose-dependent inhibition of *S. aureus* and *S. epidermidis* with inhibition zones of 9, 11, and 13 mm, respectively [20]. On the other hand, the aqueous extract was less effective against all three pathogens. Antimicrobial susceptibility testing of different fractions of methanolic extract of *A. procera* leaves at a concentration of 800 µg/disc inhibited *S. aureus* with inhibition diameter range of 7–12 mm [21]. Recently, the methanolic extract of *A. adianthifolia* roots and bark were reported to have antimicrobial activity against multi-drug resistant Gram-negative bacteria [22].

Based on the literature reviews and our results, the use of different extraction solvents yields different compounds with different properties, e.g., aqueous extracts contain phenolic compounds which possess greater antioxidant potential compared with organic extracts, whereas only ethanolic extracts contain active compounds with anti-inflammatory and anti-microbial activities.

The GC-MS analysis showed retention times of different chemical components (Fig. 2), and 39 compounds were identified being phenols, sterols,

Table 2 Antimicrobial activity of *A. lucidior* wood extracts and antibiotic drugs against *S. aureus*, MRSA, and *S. epidermidis* ($n = 3$).

Treatment	MIC[*], MBC[*] *S. aureus*	MRSA	*S. epidermidis*
Ethanolic extract (mg/ml)	2.5, 5	2.5, >5	5, >5
Aqueous extract (mg/ml)	>5, >5	>5, >5	>5, >5
Ampicillin (µg/ml)	7.8×10^{-4}, >100	>100, –	1.56, >100
Norfloxacin (µg/ml)	1.56, >100	7.8×10^{-4}, >100	3.9×10^{-4}, 1.56

[*] = mg/ml for extracts and µg/ml for antibiotic drugs.

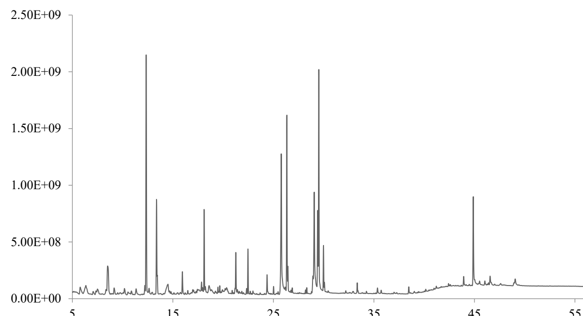

Fig. 2 GC-MS chromatogram of the ethanolic extract of *A. lucidior* wood.

and volatile oils. Likewise, different compounds were found from the phytochemical analysis of the ethanolic extract of the aerial parts of *A. procera*: carbohydrates and glycosides, phytosterols, triterpenoids, flavonoids, phenolic compounds, tannins, and saponin [23]. Table 3 shows the five highest peaks on the chromatogram of 2-methoxy-3-(2-propenyl) (14.57%), n-hexadecanoic acid (12.34%), (E)-9-Octadecenoic acid ethyl ester (11.94%), hexadecanoic acid, ethyl ester (8.94%), and cis-vaccenic acid (8.88%) as well as all the other compounds.

In a previous research, n-hexadecanoic acid was the main compound found in the *A. amara* leave extracts, and it was shown to have wound healing properties [24]. Using HPLC-PDA-ESI-MS analysis, mainly flavonoids and galloyl glucoside derivatives related to antioxidant and anti-inflammatory activities were found in the methanolic extract of *A. anthelmintica* leaves [19]. Thus, our study is a first report of wood of *A. lucidior* components analysis using GC-MS technique.

Table 3 Phytocomponents identified in the ethanolic extract of *A. lucidior* wood by GC-MS.

NO.	R/T[†]	Compound	Molecular formula	MW (g/mol)	Percentage
1	5.784	3-Acetylthymine	$C_7H_8N_2O_3$	168	1.04
2	8.518	Tridecane	$C_{13}H_{28}$	184	0.34
3	9.154	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	0.66
4	10.182	Massoilactone	$C_{10}H_{16}O_2$	168	0.64
5	10.878	Safrole	$C_{10}H_{10}O_2$	162	0.39
6	11.345	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.69
7	12.214	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	0.60
8	12.345	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	14.57
9	13.377	Heptadecane	$C_{17}H_{36}$	240	6.23
10	13.449	Vanillin	$C_8H_8O_3$	152	0.39
11	15.948	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl-	$C_{10}H_{18}O_3$	186	1.36
12	17.851	Butyrovanillone	$C_{11}H_{14}O_3$	194	0.73
13	17.956	2-Methyl-Z,Z-3,13-octadecadi	$C_{19}H_{36}O$	280	0.31
14	18.114	Heptadecane	$C_{17}H_{36}$	240	4.02
15	18.234	3-Heptafluorobutyroxytetrade	$C_{18}H_{29}F_7O_2$	410	0.20
16	19.484	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	182	0.34
17	19.667	aR-Turmerone	$C_{15}H_{20}O$	216	0.38
18	21.264	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	2.79
19	22.335	Oleic Acid	$C_{18}H_{34}O_2$	282	0.32
20	22.472	Eicosane	$C_{20}H_{42}$	282	2.16
21	24.373	Lidocaine	$C_{14}H_{22}N_2O$	234	1.09
22	25.026	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.36
23	25.784	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.34
24	26.337	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	8.94
25	26.446	Tetracosane	$C_{24}H_{50}$	338	1.33
26	26.879	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.25
27	28.322	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.27
28	28.932	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	1.23
29	29.064	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	8.88
30	29.398	9(E),11(E)-Conjugated linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	4.51
31	29.519	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	11.94
32	29.988	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	2.16
33	30.079	Hexacosane	$C_{26}H_{54}$	366	0.40
34	33.346	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	0.55
35	35.358	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	$C_{28}H_{34}N_2O_3$	446	0.40
36	38.475	(E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene	$C_{16}H_{16}O_4$	272	0.39
37	43.936	Campesterol	$C_{28}H_{48}O$	400	0.50
38	44.890	.beta.-Sitosterol	$C_{29}H_{50}O$	882	5.64
39	49.553	Cholest-4-en-3-one	$C_{27}H_{44}O$	384	0.66

[†] R/T = retention time.

CONCLUSION

The wood of *A. lucidior* is commonly used as a traditional treatment for various ailments such as fever and general fatigue. Our study has shown that the ethanolic extract of *A. lucidior* exerted significant anti-free-radical activity and moderate inhibition of NO production, but anti-*Staphylococcal* activity was poor. These encouraging results support the use of *A. lucidior* in traditional medicine for the treatment of non-specific fever. The extracts should be further investigated to see if they may have a clinical application outside of the traditional medicine.

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