AJSTR ASEAN JOURNAL OF SCIENTIFIC AND TECHNOLOGICAL REPORTS
ONLINE ISSN: 2773-8752

Research article

Evaluation of Antibacterial, Antiinflammatory Activities and GC-MS Profiling of *Millingtonia hortensis* Linn. Leaf and Stem Bark Extracts

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Citation:
Deethae, A.; Sritapunya, T.; Khetnaoanurak, S.; Jinwan, W. Evaluation of antibacterial, antiinflammatory activities and GC-MS profiling of *Millingtonia hortensis* Linn. leaf and stem bark extracts. *ASEAN J. Sci. Tech. Report.* **2023**, *26*(3), 72-80. https://doi.org/10.55164/ajstr.v26i3.249339.

Article history:
Received: May 30, 2023
Revised: June 26, 2023
Accepted: June 27, 2023
Available online: June 28, 2023

Abstract: The present study aimed to evaluate the antibacterial and antiinflammatory activity of *Millingtonia hortensis* Linn. leaf and stem bark extracts and identify the bioactive compounds by GC-MS analysis. This study determined the antibacterial activities using the agar disc diffusion technique. The results revealed the ethanol extract of *M. hortensis* Linn. stem bark was the highest potential inhibitory against gram-positive bacteria *(Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus,* and *Bacillus cereus).* The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed. The highest inhibitory effect on *Enterococcus faecalis, Enterococcus faecium,* and *Bacillus cereus* with MIC and MBC values of 500 mg/ml. This investigation also indicated the *M. hortensis* Linn. leaf and stem bark extracts had no cytotoxicity against macrophage RAW264.7 cell line and strong antiinflammatory properties. The results of antiinflammatory activity showed that the ethanol extract of *M. hortensis* Linn. leaf and stem bark extracts were the most effective in inhibiting nitric oxide secretion with IC_{50} values of 0.25 and 0.41 mg/ml, respectively. GC-MS profiling analyzed the ethanol extract of *M. hortensis* Linn. stem bark, which had the highest antibacterial and antiinflammatory activity. There were three bioactive substances: 9,12-Octadecadienoic acid, ethyl ester, trans-13-Octadecenoic acid, and oleic acid. The natural functions were antiinflammatory, dermatitigenicity, antioxidants, and antifungal activities. Therefore, the results suggest that the ethanol extract of *M. hortensis* Linn. stem bark had the highest potential usefulness for treating gram-positive bacteria infection and inflammation-induced ailment. This research will be helpful toward the better acceptability of this extract in therapeutics.

Keywords: Antibacterial activity; antiinflammatory activity; GC-MS; bioactive substance

1. Introduction

The several properties of medicinal plants have been helpful in various categories of human ailments and conditions. Traditional herbal medicine is used worldwide for healthcare management [1]. The acceptance of herbal medicine is increasing because of its safety, no side effects, efficacy, and low

cost. Mainly, medicinal plants produce a diverse range of bioactive substances as a global source of therapeutic compounds to play a main role in several mechanisms of human health [2,3].

Millingtonia hortensis Linn. (Syn *Biognonia suberosa* Roxb.) is an important herb in South Asia, ranging from Thailand, India, Southern China, and Burma. It belongs to the family of Bignoniaceae [4]. It is a tall deciduous tree, a height of between 18 and 25 meters. It has corky stem bark, a straight trunk, and a few branches. In winter, the tree blooms with fragrant white flowers [5]. The leaf is large, dark green, has no odor and tastes slightly bitter. The stem bark is dark brown colored and has a characteristic odor. In traditional uses, flower buds are used in the treatment of asthma, sinusitis, and tonic. Its leaf is used for anticancer, antimicrobial, and anti-asthmatic activity. The stem bark also has great medicinal value as a treatment for throat ailments, coughs, and hangovers [6]. The phytochemical reports revealed some bioactive compounds from *M. hortensis* flowers, such as millingtonine, hispidulin, scutellarein, scutellarein-5-alactoside, and hortensin. The natural compounds from its leaf and stem bark were strong antimicrobial substances such as tannins, β-carotene, dinatin, and rutinosid [7]. In prior reports, the acetone extract of *M. hortensis* leaf had larvicidal activity against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. The different of stem bark extracts showed anthelmintic activity against the adult earthworm *Pheretima posthuma* [8,9,10]. The methanol extract of *M. hortensis* leaf was more potent than fluconazole against *Candida krusei* and *Saccharomyces cerevisiae* [11]. The efficacy of acetone and methanol extract of *M. hortensis* stem bark were reported in their natural compounds as Dl-alpha-tocopherol, Vitamin E, Squalene, Bicyclo[4.1.0]heptane,7-pentyl-, Methyl 6,9-octadecadienoate could be the potential for the treatment of dapsone resistance of leprosy [12].

Inflammation is one of the physical responses of living tissues to toxins, damage, injuries with chemical stimuli, and contains infections. Inflammatory reactions result from the immune response against irritants and pathogens, and activated macrophages such as RAW 264.7 cells are involved in the process of inflammatory mechanism [13]. The inflammation is generally acute but can become chronic, leading to many diseases. The activated macrophages produce increased expression of inflammatory mediators such as nitric oxide (NO), prostaglandins 2 (PGE2), and tumor necrosis factor-alpha (TNF-α). Nitric oxide is a pro-inflammatory mediator that induces inflammation due to its overproduction in abnormal tissue responses. Therefore, NO inhibitors represent crucial therapeutic occurrences in managing inflammatory diseases because NO is involved in the pathogenesis of inflammatory disorders [13].

However, the herbal source comes into focus as natural components with effective antiinflammatory agents. In a prior report, the potency of the aqueous extract of *M. hortensis* stem bark revealed an antiinflammatory effect by carrageenan-induced paw edema on rats [14]. There is no information on the chemical components and antiinflammatory effects of *M. hortensis* leaf and stem bark extract. Therefore, the present investigation aimed to find scientific data for evaluating bioactive substances from *M. hortensis* leaf and stem bark extract that affected antibacterial and antiinflammatory activities.

2. Materials and methods

2.1 Plant materials and preparations

The leaf and stem bark of *M. hortensis* were collected at Chiang Mai and Lamphun Province, Thailand, in January 2022. The leaf and stem bark samples were washed thoroughly with distilled water and dried at 45- 50°C for two days to ensure they lost most of their moisture content. Then, the dried samples were powdered with a mean particle size ranging from 10 to 200 μm and stored at room temperature in an airtight dark container for further use.

2.2 Extraction of plant

Dry powders of *M. hortensis* leaf and stem bark (250 grams) were macerated with 1 liter of 95% ethanol for 3 days at room temperature or soaked with distilled water at 45°C in a water bath for 3 hours to maintain the crude extract from contamination. Then, the suspension of plant extracts was filtered through filter paper Whatman No.1 (Whatman, USA). A rotary evaporator concentrated the filtrate to remove the solvent and dried it with a lyophilizer to obtain crude extracts. Next, the dried extract was reconstituted by 10% dimethylsulfoxide (DMSO, Labscan, Ireland) using an aseptic condition for stock preparation of each crude extract as 500 mg/ml and stored in an amber glass bottle at 4°C before investigating further.

2.3 Antibacterial activites

Test organisms such as *Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* and *Proteus mirabilis* used in the experiment were obtained from Department of biology, Faculty of Science and Technology, Chiang Mai Rajabhat University, Chiang Mai, Thailand. The antibacterial activities of the aqueous and ethanol extracts were evaluated by agar disc diffusion and dilution method. After reconstituting *M. hortensis* leaf and stem bark extracts with 10% dimethylsulfoxide (DMSO), the two-fold serial dilution of the extracts was used on the test organisms. Serial dilution of the extract was made using 5 concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml) of crude extract of *M. hortensis* leaf and stem bark were prepared using 10% DMSO. Subsequently, agar discs of 6 mm diameter were filled with 25 µl of clude extract and placed on top of a sterile Mueller Hinton agar plate surface. The plates were incubated at 37° for 24 hours. A disc containing the same volume of DMSO (10%) as the negative control, and Gentamicin (10 mg/ml) was used as the positive control. Three replicates were carried out for each extract against each test organism. The inhibition zone were measured to millimeters (mm). Data were expressed as mean±standard deviation.

The minimum inhibitory concentration (MIC) was determined for each test organism. The MIC of these extracts was performed by broth dilution method with the stocks concentration of 500 mg/ml of *M. hortensis* leaf and stem bark extracts, which were resuspended in 10% DMSO to produce two-fold serial dilutions using 5 concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml) of crude extract of *M. hortensis* leaf and stem bark. The inoculum preparation was performed by broth culture method using Mueller Hinton broth. Each tested bacteria culture is adjusted with sterile Mueller Hinton broth to give a turbidity equivalent to the McFarland 0.5 standard. For the inoculation of tubes, a volume of bacterial suspension equal to the volume of diluted antimicrobial solution of *M. hortensis* leaf and stem bark extract, is added to each tube, were incubated at 35–37 °C for 24 hours. The lowest concentration of an antibacterial agent, under defined *in vitro* conditions, where no turbidity was observed by looking through the paper with black lines, was determined and noted as the MIC value. Finally, The MIC value of the clude extract was confirmed by testing the minimum bactericidal concentration (MBC) value using the spread plate technique. All samples were tested in triplicates.

2.4 Cell cytotoxicity testing (MTT assay)

Cytotoxicity of *M. hortensis* leaf and stem bark extract was investigated on macrophages RAW 264.7 cells. The extracts were serially two-fold diluted with Minimum Essential Medium (MEM) as serial dilution ranging from 0.078 to 10.000 mg/ml. Each extract concentration was added to quadruplet wells onto a 96-well tissue culture plate. After that, the clude extracts at different concentrations were added to each well, and incubation was carried out at 37°C for 48 hours. Then, 15 µl of MTT (5 mg/ml) was added to each well and incubated at 37°C in a 5% CO_2 incubator for 4 hours. Finally, DMSO was added to each well to form an MTT-formazan product. Determination of cell viability was performed using an ELISA reader by measuring the absorbance at 540 nm with reference wavelength at 630 nm.

2.5 Antiinflammatory activity

The macrophage cell line, RAW 264.7, was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, UK) containing 4 mM L-glutamine, antibiotics (1% penicillin/streptomycin), and 10% fetal bovine serum at 37°C in 5% CO_2. RAW 264.7 cells (3×10^4 cells/well) were exposed to the indicated concentrations of extracts for 24 h. Then, an additional 24 hours of incubation with lipopolysaccharide (LPS) (1 ug/ml) was performed. After that, each culture supernatant was mixed with the Griess reagent to determine the nitric oxide (NO) production by RAW 264.7 cells. The mixture's optical density at 540 nm was determined using a spectrophotometer microplate.

2.6 GC-MS analysis

GC-MS was performed using a GC-TurboMatrix Headspace HS instrument. Gas chromatograph fitted with Initial temp 60°C for 5 min, ramp 7°C/min to 250°C, hold 5 min, InjBauto=250°C and volume 0 µL. An electron ionization system with an ionization energy of 70 eV was used for GC-MS detection. Helium was used as the carrier gas at a 1 ml/min flow rate. The injector and transfer line temperatures were set at 250 °C, respectively. The column temperature was initially kept at 10 °C for 1 minute and was then gradually

increased to 200 °C at a rate of 7 °C/min; finally, it was raised to 250 °C at a rate of 1 °C/min. The crude extract's relative amount of individual bioactive compounds is expressed as a percentage peak area close to the total peak area.

3. Results and discussion

This study evaluated the aqueous and ethanol extract of *M. hortensis* leaf and stem bark against antibacterial and antiinflammatory activities. Additionally, this research has elucidated bioactive substance's results in the effective *M. hortensis* extract by GC-MS profiling. These analyses revealed the presence of natural bioactive compounds related to various biological activities.

3.1 Crude extracts and antibacterial activities

This study depicted the occurrence of crude extracts against gram-positive tested bacteria. The agar disc diffusion susceptibility test of *M. hortensis* leaf and stem bark extracts evaluated an inhibitory effect on 9 tested bacteria strains. Their zone of inhibition against most of the bacterial microbes at 500 mg/ml concentrations was very effective (Table 1). There were 4 gram-positive bacteria (*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Bacillus cereus*) were found high inhibited by ethanol extract of *M. hortensis* stem bark (Table 1). In addition, The MIC and MBC of ethanol extract of *M. hortensis* stem bark were the highest value at 500 mg/ml against gram-positive bacteria such as *E. faecalis*, *E. faecium*, and *B. cereus* (Table 2). The gram-positive bacteria, *E. faecalis*, and *E. faecium*, causes urinary tract infection [15]. On the other hand, *B. cereus* causes crucial foodborne [16]. These results indicated the ethanol extract of *M. hortensis* stem bark had a broad spectrum of antibacterial activity. This result, correlated with essential oil extracted from *M. hortensis* flowers, showed an inhibitory effect on gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* [16]. In addition, the prior reports of polar extract as an aqueous alcohol fraction of *M. hortensis* leaf have broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, yeasts such as *Candida albicans* and *Saccharomyces cerevisiae*, and an actinomycete strain *Nocardia* sp. [17].

Table 1. The inhibition zone diameters of *M. hortensis* leaf and stem bark extracts against tested bacteria

Bacteria	Gram	Diameter of inhibition zone (mm)				Gentamicin (10 mg/ml)
		Aqueous extract (500 mg/ml)		Ethanol extract (500 mg/ml)		
		Leaf	Stem bark	Leaf	Stem bark	
Enterococcus faecalis	+	nz	nz	nz	14.4 ± 0.8	19.5 ± 0.7
Enterococcus faecium	+	nz	nz	8.4 ± 0.7	11.5 ± 1.1	18.3 ± 1.0
Staphylococcus aureus	+	nz	7.2 ± 1.3	7.4 ± 0.5	10.7 ± 1.0	22.5 ± 0.8
Bacillus cereus	+	6.5 ± 1.0	7.3 ± 0.9	7.8 ± 0.8	11.8 ± 0.9	20.6 ± 0.9
Escherichia coli	-	nz	nz	nz	nz	23.1 ± 0.7
Pseudomonas aeruginosa	-	nz	nz	nz	8.8 ± 0.5	18.5 ± 1.2
Klebsiella pneumoniae	-	nz	nz	nz	nz	20.4 ± 0.6
Acinetobacter baumannii	-	nz	nz	nz	8.6 ± 1.2	18.6 ± 0.8
Proteus mirabilis	-	nz	nz	nz	nz	19.5 ± 1.0

Data are reported as Mean ± SD; nz = No inhibition zone

3.2 Cell cytotoxicity analysis

The cytotoxicity testing was widely used in *in vitro* toxicology studies. In the present study, the cytotoxic effect of ethanol extract of *M. hortensis* leaf and stem bark against macrophage RAW264.7 cell line were determined by MTT assay. The results elucidated that ethanol extracts of *M. hortensis* leaf and stem bark at concentrations of ≤0.625 mg/ml and aqueous extracts of *M. hortensis* and stem bark at concentrations of ≤5 mg/ml had cell viability of more than 80% (Table3). Then, *M. hortensis* extracts are poor cytotoxic agents against the macrophage RAW264.7 cell line. This research showed that the corresponding cytotoxic analysis, the

aqueous and ethanol extract of *M. hortensis*, were not cytotoxicity of the RKO colon cancer cell line, was assessed by MTT reduction assay [18].

In this research, a comparison of vehicle control, in which dimethyl sulfoxide (DMSO) was used as the solvent for the ethanol extracted, and DI (Deionized water) was used as the solvent for the aqueous extract. The results revealed that cell viability was 97.04% and 103.39%, respectively. Therefore, these extract concentrations were used to analyze the activity of inflammatory mediators through the secretion of nitric oxide by stimulating RAW264.7 cell cultures.

Table 2. The MIC and MBC (mg/ml) of *M. hortensis* leaf and stem bark extracts against tested bacteria

Bacteria	Concentration (mg/ml)							
	Aqueous extract				Ethanol extract			
	Leaf		Stem bark		Leaf		Stem bark	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Enterococcus faecalis	nd	nd	nd	nd	nd	nd	500	500
Enterococcus faecium	nd	nd	nd	nd	>500	nd	500	500
Staphylococcus aureus	nd	nd	>500	nd	>500	nd	>500	nd
Bacillus cereus	nd	nd	>500	nd	>500	nd	500	500
Pseudomonas aeruginosa	nd	nd	nd	nd	nd	nd	>500	nd
Acinetobacter baumannii	nd	nd	nd	nd	nd	nd	>500	nd

nd = Not detected

Table 3. The cytotoxicity analysis of *M. hortensis* leaf and stem bark extracts in macrophage RAW264.7 cell line by MTT assay

Tested concentration (mg/ml)	Cell viability (%)[1]			
	Aqueous extract		Ethanol extract	
	Leaf	Stem bark	Leaf	Stem bark
Cell Control (CC)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Vehicle control (VC)	103.39 ± 1.65	103.39 ± 1.65	97.04 ± 2.16	97.04 ± 2.16
0.078	88.34 ± 1.60	108.38 ± 1.48	104.64 ± 6.38	103.28 ± 9.82
0.156	94.74 ± 3.29	94.73 ± 3.46	106.11 ± 2.15	102.28 ± 9.48
0.312	96.82 ± 9.76	86.51 ± 6.63	109.38 ± 2.66	104.38 ± 7.68
0.625	92.90 ± 3.64	98.05 ± 9.43	107.50 ± 7.95	104.60 ± 7.09
1.250	108.74 ± 4.03	104.66 ± 1.94	68.15 ± 5.98	2.45 ± 0.12
2.500	104.40 ± 5.47	104.10 ± 7.08	4.90 ± 2.20	6.07 ± 1.32
5.000	106.21 ± 5.15	102.89 ± 6.97	10.71 ± 4.67	8.96 ± 1.66
10.000	78.80 ± 2.03	10.77 ± 4.71	18.95 ± 2.46	7.30 ± 1.68

1 = Analysis was performed in triplicated

3.3 Effect of *M. hortensis* leaf and stem bark crude extracts in LPS-stimulated RAW264.7 cells.

This study determined the effects of aqueous and ethanol extracts of *M. hortensis* leaf and stem bark on the production and secretion of inflammatory mediators. The secretion of nitric oxide from RAW264.7 cell culture was stimulated by lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 at a 1 μg/ml concentration. The results indicated that the ethanol extract of *M. hortensis* stem bark at a concentration of 0.625 mg/ml had the highest adequate nitric oxide secretion as 100 % inhibition (Table 4). The efficiency of nitric oxide inhibitory activity was decreased with the reduced concentration of extracts (Table 4). Interestingly, The ethanol extract of *M. hortensis* stem bark and leaf extracts were the most effective in inhibiting nitric oxide secretion, with IC_{50} values of 0.25 and 0.41 mg/ml, respectively. (table 5). The activated macrophage RAW264.7 cells can produce increased expression of inflammatory mediators such as nitric oxide (NO). Then, LPS-stimulation, RAW264.7 cells could generate NO production, which plays an important role in inflammatory response. Therefore, in this research, the ethanol extract of *M. hortensis* stem bark showed an antiinflammatory effect by attenuating NO generation.

Table 4. The effect of *M. hortensis* leaf and stem bark crude extracts on nitric oxide (NO) production in lipopolysaccharides (LPS)-stimulated RAW264.7 cell

Tested concentration (mg/ml)	Inhibition (%)[1] Aqueous extract		Tested concentration (mg/ml)	Inhibition (%)[1] Ethanol extract	
	Leaf	Stem bark		Leaf	Stem bark
0.312	3.63 ± 2.75	2.25 ± 2.52	0.039	10.28 ± 5.87	0.00
0.625	9.74 ± 2.30	15.14 ± 0.94	0.078	12.77 ± 4.61	0.00
1.250	22.53 ± 2.41	47.93 ± 2.88	0.156	24.81 ± 1.86	11.11 ± 5.21
2.500	55.74 ± 4.98	89.22 ± 2.86	0.312	35.40 ± 4.95	74.90 ± 1.90
5.000	94.90 ± 2.50	97.85 ± 0.84	0.625	74.490 ± 3.11	100.44 ± 0.89

1 = Analysis was performed in triplicated

Table 5. The IC_{50} value of *M. hortensis* leaf and stem bark crude extracts on nitric oxide (NO) production in lipopolysaccharides (LPS)-stimulated RAW264.7 cells

The crude extract		IC_{50} (mg/ml)
Leaf	Aqueous extract	2.58
	Ethanol extract	0.41
Stem bark	Aqueous extract	1.46
	Ethanol extract	0.25

3.4 Gas Chromatography-Mass Spectrophotometry Analysis (GC- MS analysis)

GC–MS is a rapid and accurate technique to identify the phytochemical constituents that could contribute to the medicinal quality of the plant. The natural compounds were confirmed based on the peak area, retention time, and molecular formula. In this present study, the GC-MS technique analyzed the highest antibacterial and antiinflammatory activity as the ethanol extract of *M. hortensis* stem bark. The chromatogram of this extract confirms the presence of various substances with different retention times (RT), as shown in 18 peaks in Figure 1. The GC-MS profiling of this effective extract revealed eighteen compounds (Table 6). The most separated peak was identified to be 9,12-Octadecadienoic acid, ethyl ester (12.356%) followed by trans-13-Octadecenoic acid (9.284%) and oleic acid (5.956%), respectively (Table 7).

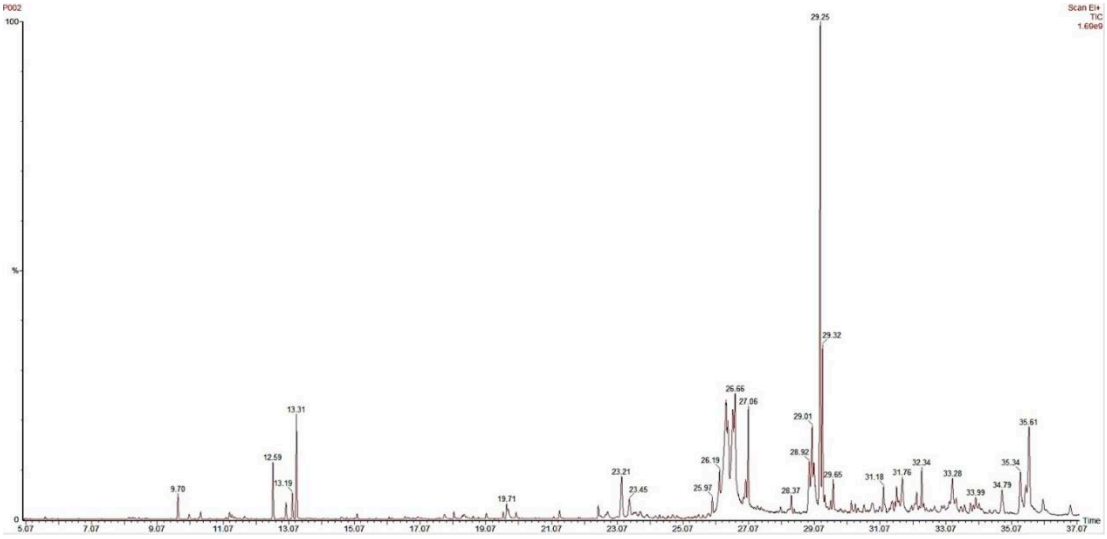

Figure 1. GC-MS chromatogram of the ethanol extract of *M. hortensis* stem bark

Table 6. The GC-MS profiling compounds identified in the ethanol extract of *M. hortensis* stem bark

Number	Retention time (RT)	Name of compound	Molecular formula	Peak Area (%)
1	9.701	Eucalyptol	$C_{10}H_{18}O$	0.699
2	12.592	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	$C_{10}H_{16}O$	1.519
3	13.187	endo-Borneol	$C_{10}H_{18}O$	0.701
4	13.307	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{20}O$	2.586
5	19.710	Linoleyl methyl ketone	$C_{75}H_{134}O$	0.483
6	23.207	9-Octadecene, 1,1-dimethoxy-, (Z)-	$C_{20}H_{40}O_2$	1.846
7	26.190	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	9.284
8	26.588	Oleic Acid	$C_{18}H^{34}O_2$	5.956
9	26.664	Isopropyl palmitate	$C_{19}H_{38}O_2$	4.810
10	27.064	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O$	2.473
11	28.374	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O$	0.457
12	28.920	19,19-Dimethyl-eicosa-8,11-dienoic acid	$C_{22}H_{40}O_2$	2.019
13	29.255	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	12.356
14	29.325	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O$	3.469
15	29.650	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	0.787
16	32.336	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{18}H_{32}O$	1.215
17	33.992	5à-Pregn-16-en-20-one	$C_{21}H_{32}O$	0.582
18	35.608	6-Hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydro-1-phenanthrenemethanol, (1à, 4aá, 10a.alpha)-	$C_{20}H_{28}O_2$	4.037

Table 7. The chemical structure of high components identified in the ethanol extract of *M. hortensis* stem bark by GC-MS analysis

Name of compound	Chemical groups	Retention time (RT)	Chemical structure	Biological activities
9,12-Octadecadienoic acid, ethyl ester	Fatty acid ester	29.255		antimicrobial, hypocholesterolemic, nematicide antiarthritic, hepatoprotective anti androgenic, hypocholesterolemic nematicide, 5-Alpha reductase inhibitor, antihistaminic, anticoronary Insectifuge, antieczemic and antiacne [19]
trans-13-Octadecenoic acid	Unsaturated fatty acids	26.190		antiinflammatory, dermatitigenic, anaemiagenic, insecticides,flavor [20]
Oleic acid	Fatty acid	26.588		antifungal, antiinflammatory, antioxidants [21]

4. Conclusions

Millingtonia hortensis Linn. is a practical traditional medicinal plant that is a rich source of compounds with antibacterial and antiinflammatory activity. This research has been carried out on their bioactive substance and properties from GC-MS profiling to identify the highest potential natural compounds of *M. hortensis* crude extract. The results of this present study evaluated that the ethanol extract of *M. hortensis* stem bark could be used as the highest potential antibacterial and antiinflammatory activity. Further analysis can be extended to assess their efficient phytochemical and pharmacological properties. These effective crude extracts could be examed *in vivo* to confirm their safety and efficacy.

5. Acknowledgements

The authors gratefully thank Science Center CMRU, Faculty of Science and Technology, Chiang Mai Rajabhat University, Chiang Mai, for the accommodation and for providing the necessary laboratory facilities to carry out this work.

Author Contributions: Conceptualization, A.D.; methodology, A.D.; formal analysis, A.D.; investigation, T.S.; resources, S.K. and W.J.; data curation, A.D. and S.K.; writing–original draft preparation, A.D.; writing–review and editing, A.D. and W.J.; visualization, S.K.; supervision, W.J.; project administration, A.D.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

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