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Evaluation of the Phytochemical Profiling, Tyrosinase Inhibitory Activity, and Antioxidant Potential of Various Extracts of *Acacia Concinna* (Willd.) DC.

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Abstract

The legume *Acacia concinna* (Willd.) DC. (Fabaceae) is eaten as a food and used for medicinal purposes. This study evaluated the phytochemical profile, total phenolic content and antioxidant and tyrosinase inhibitory activities of leaf, bark, and heartwood extracts of *A. concinna* using different extraction solvents. Crude extracts were prepared by reflux extraction with dichloromethane, ethanol, and deionized water. The dichloromethane extracts of bark and heartwood provided the highest total phenolic content as determined by the Folin-Ciocalteu reaction. Antioxidant activities were assessed using the DPPH and ABTS assays, with the bark ethanolic extract (BE) giving the highest antioxidant activities at IC_{50} values of 3.0 ± 0.03 and 5.0 ± 0.01 µg/mL, respectively. The BE antioxidant capacity was similar to ascorbic acid. Tyrosinase inhibitory activity was determined using an *in vitro* enzymatic spectroscopic model. Highest tyrosinase inhibitory activity was recorded in the heartwood ethanolic extract (HE) with IC_{50} value 0.7634 ± 0.01 mg/mL. GC-MS analysis revealed long-chain fatty acids as the major phytochemicals in BE and HE. BE had excellent antioxidant abilities, whereas HE showed potential tyrosinase inhibitory activity. Long-chain fatty acids and lupeol were the principal phytoconstituents of BE and HE as important bioactive components in *A. concinna* extracts.

Keywords: *Acacia concinna*, Phytochemical profiling, Antioxidant, Tyrosinase inhibitory activity

Introduction

Acacia concinna (Willd.) DC. (Fabaceae), commonly known as "soap pod", is a medicinal plant that grows in the tropical rainforests of Southern Asia. This scandent shrub has branchlets and leaf rachises with prickles, bipinnate compound leaves, and inflorescent yellowish flowers [1]. In Thailand, *A. concinna* is frequently used in religious and spiritual rituals to rinse away bad luck by dipping the pods in holy water as a sign of respect. The pod and bark extracts contain high amounts of saponins that are used in cosmetic products as a natural surfactant [2]. The leaves, bark, and pods are also used in traditional medicine for emetic, antidermatophyte, and antimicrobial properties, and also laxative and dermatophyte treatments [3,4]. The young shoots and leaves are eaten raw or cooked with curry. Phytochemicals are naturally occurring compounds in plants which a great potential for multifunctional pharmacological activities with a high possibility of discovery and development of new pharmaceuticals. The polarity of the solvent utilized influences the phytochemical profile of the extracts [5]. Polyphenols represent the largest category of

phytochemicals and potential as antioxidants and against melanogenesis due to their multiple hydroxyl groups. Phenolics have similar structures to tyrosine which are oxidized by tyrosinase and they can act as substrate analog inhibitors against melanogenesis. Furthermore, tyrosinase is a widespread enzyme found in the tissues of plants, animals, and humans. It catalyzes the oxidation process that converts tyrosinase into melanin, which is then produced as brown pigments on human skin, vegetables, mushrooms, and fruits that turn dark brown. The accumulation of melanin formation causes melisma, age spots, post-inflammatory hyperpigmentation and skin cancer [6]. Hyperpigmentation and skin aging are biological and physiological events caused by gradual damage accumulation from the effects of ultraviolet radiation, pollution, reactive oxygen radicals, smoking, lifestyle and diet [7]. Hyperpigmentation as skin darkening is most often caused by the overproduction and accumulation of melanin pigment. Tyrosinase is an enzyme that catalyzes the conversion of l-tyrosine to melanin and regulates melanogenesis. The role of melanin is to protect the skin from ultraviolet (UV) damage by absorbing UV sunlight and removing reactive oxygen species (ROS). Reactive oxygen species (ROS) in the forms of superoxide anion radical, hydroxyl radical, hydrogen peroxide are generated living organisms as a result of normal cellular metabolism and exogenous factors, these can occur oxidative damage to human cells, causing diseases such as cancer, neurological disorders, atherosclerosis, hypertension, rheumatoid arthritis and cardiovascular disease [8]. Ultraviolet radiation generates reactive oxygen species that enhance the production of tyrosinase and play an important role in the skin aging process [9]. Many natural products have been intensively examined for radical scavenging and tyrosinase inhibition activities to explore the potential of natural resources in the skincare industry [10].

Previous studies reported that various parts of *Acacia* species showed potential as skin brighteners. Extracts of *Acacia nilotica* (pods, bark) and *A. seyal* var. *seyal* (wood) demonstrated comparable tyrosinase inhibitory activity to standard kojic acid [11]. A hydroethanolic extract from *A. concinna* pods showed good *in vitro* antioxidant and tyrosinase inhibitory activities [1]. However, a detailed biological assessment of the beneficial properties of other *A. concinna* parts has not been conducted. This study examined extracts from the leaves, bark and heartwood of *A. concinna* using solvents with different polarities. Phytochemical screening, total phenolic content, and antioxidant and tyrosinase inhibitory activities were evaluated. GC-MS analysis was employed to characterize the phytochemical substances in the biological extracts.

Materials and methods

Plant materials and extract preparation

The leaves, bark, and heartwood of *A. concinna* were collected in March 2022 from cultivation areas in Muang district, Khon Kaen Province, Thailand. Plant specimens were identified using keys to genus and species and compared with type specimens. The voucher specimen (BK 069328) was deposited in the Bangkok Herbarium, Bangkok, Thailand. All plant materials were dried in a hot air oven at 60 °C for a minimum of 24 h until the moisture content was reduced to no more than 10 %. They were then blended into a fine powder for optimal extraction. Plant powder samples (10 g) were extracted using a Soxhlet apparatus for 30 min at a temperature of 60 - 80 °C, with 300 mL of dichloromethane, ethanol, and deionized water. Each liquid part was filtrated through Whatman filter paper No.1 and the extracting solvents were removed using a vacuum rotary evaporator and then lyophilized. The obtained crude extracts of *A. concinna* were named leaf dichloromethane (LD), leaf ethanol (LE), leaf water (LW), bark dichloromethane (BD), bark ethanol (BE), bark water (BW), heartwood dichloromethane (HD), heartwood ethanol (HE), and heartwood water (HW) extracts. All extracts were kept at –20 °C until used.

Determination of total phenolic content

Total phenolic content was analyzed based on the Folin-Ciocalteu colorimetric method [12] using gallic acid as a standard. Aliquots of 0.5 mg crude extract were dissolved in methanol (0.5 mL), mixed with 2.5 mL of Folin-Ciocalteu solution and incubated at room temperature for 5 min. Then, 2 mL of Na_2CO_3 solution was added to the reaction mixture, mixed well, and the volume was adjusted to 5 mL with distilled water. The mixture was incubated at room temperature for 2 h and the absorbance was measured at 760 nm. The total phenolic content of each crude extract was calculated using the standard curve of gallic acid ($y = 7.134x + 0.467$, $R^2 = 0.999$) and expressed as mg of gallic acid equivalent (GAE) per g extract.

Determination of radical scavenging activity

1) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was determined by the method [13]. Sample solutions were prepared by dissolving the crude extract in methanol at a concentration of 100 µg/mL. Twenty microliters of sample solution were mixed with 180 µL of DPPH solution in a 96-well plate. The reaction mixtures were then incubated in the dark at room temperature for 20 min, and the absorbance was measured by a microplate reader (SPECTRO Star Nano, BMG LabTech) at 517 nm wavelength. Methanol was used as the control and ascorbic acid was used as a standard. Absorbance values of the control (Ac) and samples (As) were obtained. The percentage radical scavenging of each sample was calculated by the following equation:

Percentage radical scavenging = [(Ac - As)/Ac]×100.

2) 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The ABTS radical scavenging activities of the samples were investigated following the modified method [14]. Sample solutions were prepared in the same way as for the DPPH assay. ABTS·cation radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate. A mixture solution was stored in the dark at room temperature for 12 - 16 h before use. The ABTS solution was diluted with 95 % ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, 280 µL of ABTS working solution were mixed with 20 µL of sample solution in a 96-well plate. The absorbance was measured by a microplate reader (SPECTRO Star Nano, BMG LabTech) at 734 nm. Methanol was used as the control and ascorbic acid was used as a standard. Absorbance values of the control (Ac) and samples (As) were obtained. The percentage radical scavenging of each sample was calculated by the following equation:

Percentage radical scavenging = [(Ac - As)/Ac]×100.

Extracts that possessed higher radical scavenging activity than 50 % by DPPH or ABTS assays were further evaluated for IC_{50} values.

Evaluation of tyrosinase inhibition activity

The enzymatic assay to determine mushroom tyrosinase activity was determined by the method [15]. Each crude extract was dissolved in 10 % DMSO at a concentration of 5 mg/mL. Forty microliters of sample solution were mixed with 80 µL of sodium phosphate buffer (0.1 M, pH 6.8) and 40 µL of 31 units/mL tyrosinase enzyme (dissolved in sodium phosphate buffer) in a 96-well plate. The reaction mixtures were incubated at room temperature for 10 min before mixing with 40 µL of 2.5 mM L-DOPA and left to stand at room temperature for 10 min. A microplate reader (SPECTRO Star Nano, BMG LabTech) was used to

measure the absorbance of the solutions at a wavelength of 475 nm, with kojic acid used as a standard. The percentage inhibitions of tyrosinase activity were calculated using the following equation:

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

where A = absorbance of the blank, B = absorbance of the control, C = absorbance of the extract in the presence of tyrosinase, and D = absorbance of the extract in the absence of tyrosinase.

Gas chromatography-mass spectrometry (GC-MS) analysis

The protocol of GC-MS analysis was adapted from a previous study [16]. High-purity helium was used as the carrier gas with a constant flow rate of 1 mL/min. The injector was set at 250 °C and performed under split mode with a split ratio of 10:1 (in 1 µL). A fused silica capillary Hewlett Packard HP-5 (5 % phenyl methyl siloxane) column (30 m×0.25 mm i.d., 0.25 µm film thickness) was used for the GC-MS analysis, detected by a GC-MS-QP 2010 Ultra (Shimadzu). The initial oven temperature was maintained at 70 °C for 3 min before increasing at a rate of 5 °C/min to 280 °C and maintained for 10 min. The thermostat on the transfer line heater was set to 280 °C. The mass scanning range was set from 50 - 550 Mahasarakham University in full scan. Chemical compounds in the extracts were identified by computer matching of the retention times and mass spectral data against samples in the National Institute of Standards and Technology (NIST).

Statistical analysis

All results were produced in triplicate and expressed as mean and standard deviation (SD). Pearson's correlation coefficient was calculated using Microsoft Excel 2021. One-way analysis of variance (ANOVA) and post-hoc Tukey tests were used in the analyses, with data analyzed using the Statistical Package for the Social Sciences (SPSS). A value of $p < 0.05$ was regarded as significant.

Results and discussion

A Soxhlet extraction was used to prepare the crude extracts from *A. concinna* leaves, bark, and heartwood using dichloromethane, ethanol, and water solvents (**Figure 1**). The percentage yield (% yield) was calculated using the dry weights of the crude extracts. (**Table 1**).

Table 1 Percentage yield of plant extracts.

Part	Percentage yield		
	Dichloromethane	**Ethanol**	**Water**
Leaf	4.26 ± 0.24	5.81 ± 0.28	11.53 ± 0.24
Bark	4.18 ± 0.13	5.54 ± 0.15	10.23 ± 0.98
Heartwood	3.37 ± 0.23	4.35 ± 0.16	10.22 ± 0.09

Figure 1 Leaf (A) Bark and Heartwood (B) of *A. concinna*.
LD; Leaf Dichloromethane, LE; Leaf Ethanol, LW; Leaf Water
BD; Bark Dichloromethane, BE; Bark Ethanol, BW; Bark Water
HD; Heartwood Dichloromethane, HE; Heartwood Ethanol, HW; Heartwood Water

Radical scavenging activity

All extract solutions were evaluated for their radical scavenging activities at a concentration of 100 µg/mL. Extracts presenting higher than 50 % inhibition of radical scavenging activities by DPPH or ABTS assays were further investigated for their IC_{50} values. *A. concinna* extracts recorded more effective scavenging performance by the ABTS radical than the DPPH radical (**Figure 2**). Four extracts; LE, LW, BE, and BW showed potential as antioxidants from their IC_{50} values. The IC_{50} value of standard ascorbic acid by DPPH and ABTS assays of 4.4 ± 0.02 and 6.0 ± 0.04 µg/mL (**Figure 2(A)**). BW exhibited moderate antioxidant properties, with IC_{50} values by DPPH and ABTS assays of 18 ± 0.01 and 23 ± 0.02 µg/mL, respectively. BE showed the most potent antioxidant activities, with IC_{50} values examined by DPPH and ABTS assays of 3.0 ± 0.03 and 5.0 ± 0.01 µg/mL, respectively. Based on the IC_{50} data from the DPPH and ABTS testing models, the antioxidant power of BE was considered similar to ascorbic acid. The bark of *A. concinna* was proposed as a highly potential source to discover novel natural antioxidative agents.

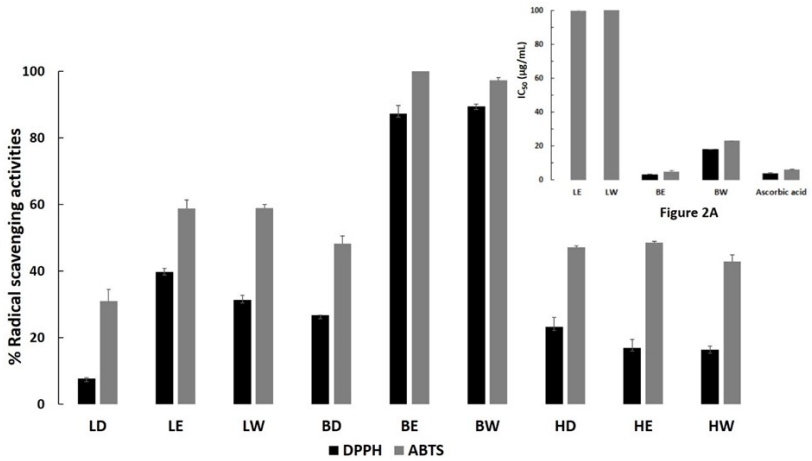

Figure 2 DPPH and ABTS radical scavenging activities of *A. concinna* extracts. The insert **Figure 2(A)** shows IC_{50} values (µg/mL) of the potential antioxidant extracts compared to standard ascorbic acid.
LD; Leaf Dichloromethane, LE; Leaf Ethanol, LW; Leaf Water
BD; Bark Dichloromethane, BE; Bark Ethanol, BW; Bark Water
HD; Heartwood Dichloromethane, HE; Heartwood Ethanol, HW; Heartwood Water

A quantitative analysis of polyphenolics was also carried out. Results revealed that total phenolic content of *A. concinna* extracts was in the range of 12.4 - 203.0 mg GAE/ g crude extracts. The dichloromethane solvent provided the highest total phenolic content compared to ethanol and water. BD and HD recorded significantly highest total phenolic content as 203.0 ± 19.64 and 192.0 ± 6.0 mg GAE/g extract, respectively ($p \geq 0.05$), while all aqueous extracts presented small amounts of phenolics as 12.4 - 42.0 mg GAE/g extracts (**Figure 3**).

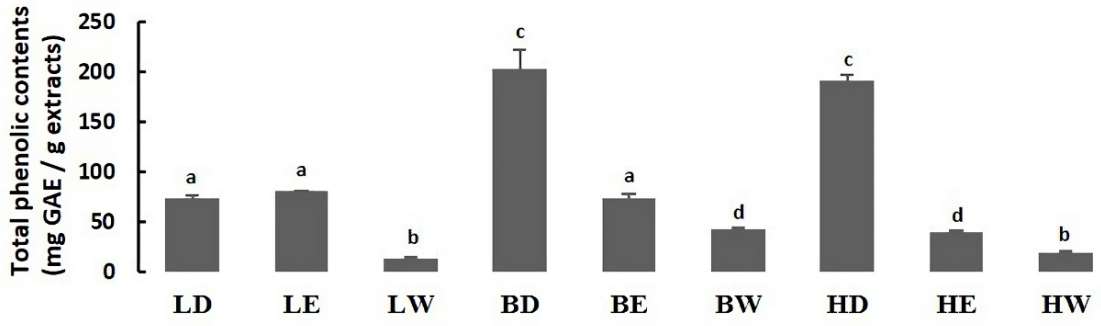

Figure 3 Total phenolic content of *A. concinna* extracts.
LD; Leaf Dichloromethane, LE; Leaf Ethanol, LW; Leaf Water
BD; Bark Dichloromethane, BE; Bark Ethanol, BW; Bark Water
HD; Heartwood Dichloromethane, HE; Heartwood Ethanol, HW; Heartwood Water

Results were expressed as mean ± SD. and evaluated using ANOVA together with post-hoc Tukey tests. Letters on the bar graph represent significant differences ($p < 0.05$).

Tyrosinase inhibitory activity

The tyrosinase inhibitory activity of *A. concinna* extracts was evaluated using an *in vitro* enzymatic assay. At a concentration of 200 µg/mL, all samples inhibited tyrosinase activity, ranging from 34 to 73 % (**Table 2**). Extracts presenting higher than 50 % inhibition of tyrosinase inhibitory activity was further investigated for their IC_{50} values. Extracts from the heartwood showed higher enzyme inhibition activity than extracts from the leaves and bark. Kojic acid was used as a standard tyrosinase inhibitor with an IC_{50} value of 0.4273 ± 0.01 mg/mL. Among the 9 extracts, HE and HW showed the highest tyrosinase inhibitory activities, with IC_{50} values 0.7634 ± 0.01 and 0.7991 ± 0.02 mg/mL, respectively and half the value of kojic acid.

Table 2 The tyrosinase inhibitory activity of *A. concinna* extracts.

Solvent	Plant/part	% Inhibitory	IC_{50}
Dichloromethane	Bark	34.01361 ± 0.08^{d}	
	Heartwood	48.29932 ± 0.04^{c}	
	Leaf	44.55782 ± 0.01^{c}	
Ethanol	Bark	48.57955 ± 0.01^{c}	
	Heartwood	73.01136 ± 0.02^{b}	0.7634 ± 0.01
	Leaf	29.59184 ± 0.02^{e}	

Solvent	Plant/part	% Inhibitory	IC_{50}
Water	Bark	38.09524 ± 0.01[d]	0.7991 ± 0.02
	Heartwood	71.42857 ± 0.02[b]	
	Leaf	41.19318 ± 0.03[c]	
	Kojic (standard)	81.36020 ± 0.02[a]	0.4273 ± 0.01

GC-MS analysis of *A. concinna* ethanolic extracts

Radical scavenging properties of the crude extracts derived from plants are usually correlated with total phenolic content. In this study, BE, LE, and HE ethanolic extracts of *A. concinna* demonstrated high antioxidant and tyrosinase inhibitory activities. Various fatty acids also recorded significant antioxidant and antimelanogenic effects [17]. GC-MS was applied to characterize the phytochemical profiles are shown in **Table 3** and **Figure 4**.

Table 3 Phytochemical profiles of *A. concinna* extracts determined by GC-MS.

No.	Retention time	Compounds	% Relative peak areas		
			BE	HE	LE
1	20.770	Nonanoic acid, 9-oxo-, ethyl ester	0.24		
2	30.855	n-Hexadecanoic acid	0.08		
3	30.944	Ethyl 9-hexadecenoate	0.13		
4	31.461	Hexadecanoic acid ethyl ester	23.80		0.29
5	33.291	Ethyl 15-methyl-hexadecanoate	0.20		
6	34.593	trans,trans-9,12-Octadecadienoic acid, propyl	0.10		
7	34.720	(E)-9-Octadecenoic acid, ethyl ester	33.35		
8	35.133	Hexadecanamide	6.82	4.40	
9	35.142	Octadecanoic acid, 17-methyl-, methyl ester	0.22		
10	37.527	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7	0.46		
11	37.675	cis-9-Hexadecenal	0.42		
12	37.806	Linoleic acid ethyl ester	1.51		
13	37.899	Ethyl stearate, mono 9-epoxy	4.63		
14	38.282	(Z)-9-Octadecenamide	18.53	91.77	99.71
15	38.627	Eicosanoic acid, ethyl ester	4.53		
16	39.173	2-Butyl-3-methyl-5-(2-methylprop-2-enyl) cyclohexane	3.04		
17	40.409	Undec-10-ynoic acid, undecyl ester	0.27		
18	40.746	Ethyl stearate, 9,12-diepoxy	0.31		
19	41.175	Octadecanoic acid, 9,10-epoxy-, isopropyl	0.24		
20	41.374	Ethyl stearate, mono 9-epoxy	0.12		
21	44.853	Ethyl tetracosanoate	0.24		
22	41.855	Docosanoic acid, ethyl ester	0.76		
23	43.494	Lup-20(29)-en-3-one		1.50	
24	44.569	Lupeol		0.47	
25	46.298	Betulinaldehyde		0.40	
26	53.420	Tetracosamethyl-cyclododecasiloxane		1.46	
		Total	**100**	**100**	**100**

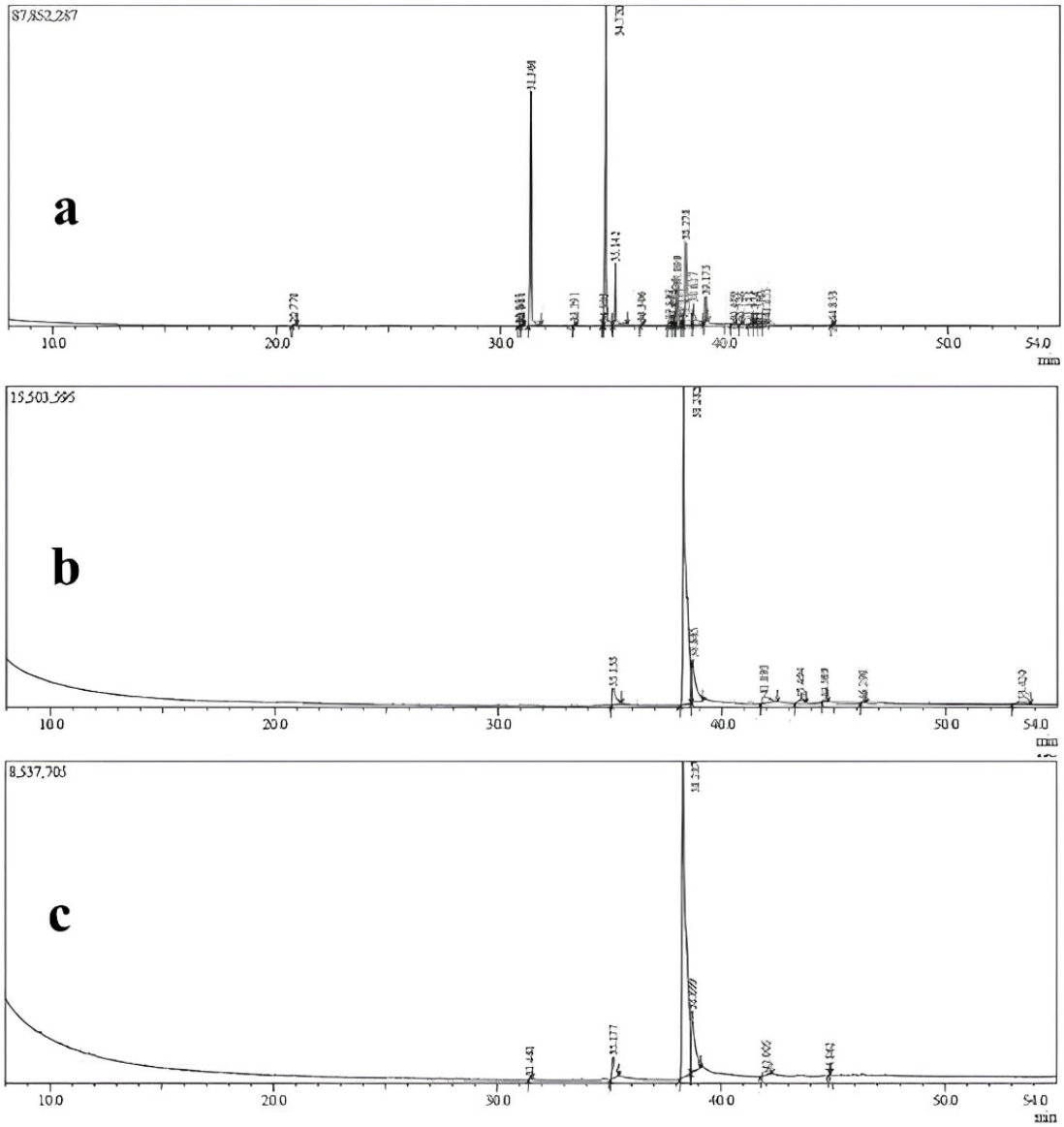

Figure 4 GC-MS chromatogram of *A. concinna* extracts; (a) Bark extract; (b) Heartwood extract; (c) Leaf extract.

Table 3 lists the identified extract compounds by GC-MS analysis. BE contained 22 compounds, with major phytochemicals as long-chain fatty acids including 9-octadecanoic acid ethyl ester, hexadecanoic acid, and hexadecanamide. LE contained 2 compounds as 9-octadecenamide and hexadecanoic acid ethyl ester, while HE contained 6 compounds with 9-octadecenamide a major component at 91.77 % relative peak area. 9-Octadecanoic acid ethyl ester, hexadecanoic acid, and the esterified derivatives of long-chain fatty acids have been reported as excellent antioxidants and inhibitors of tyrosinase activity [17,18]. The heartwood extract contained lupeol and betulinaldehyde, with reported antioxidant, tyrosinase inhibitory activity, antitumor, anti-HIV, anti-inflammatory, antibacterial, and antimalarial effects [19,20]. Lupeol showed significant tyrosinase inhibitory activity and antioxidant activity [21,22]. It has been reported the evaluated the antimicrobial and antioxidant activities of *Acacia* extracts [23]. They reported the presence of lupeol, betulinic acid, and betulinic acid-3-trans-caffeate from the bark of *A. ataxacantha*. Lupeol was

also identified in *A. concinna* as a key factor in tyrosinase inhibition and antioxidant activity. The heartwood extracts also showed high antioxidant and tyrosinase inhibitory activities due to the presence of lupeol.

Conclusions

The evaluation of *A. concinna* extracts demonstrated significant antioxidant properties, particularly in the bark extracts, which showed the highest potency with IC_{50} values comparable to ascorbic acid. The quantitative analysis of polyphenolics in *A. concinna* extracts revealed that dichloromethane was the most effective solvent for extracting phenolics, yielding the highest total phenolic content. Bark extracts exhibit the highest total phenolic content and antioxidant activities compared to heartwood and leaf extracts. Heartwood extracts demonstrate the highest tyrosinase inhibitory activity. Long-chain fatty acids, particularly 9-octadecanoic acid ethyl ester, hexadecanoic acid and lupeol were the principal phytoconstituents of BE and HE as important bioactive components in the *A. concinna* extracts.

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