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Phytochemical Constituents with Antimicrobial and Antioxidant Activities from *Xylia xylocarpa* (Roxb.) Taub. Sawdust Extracts

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ABSTRACT

Xylia xylocarpa (Roxb.) Taub. sawdust was extracted with chloroform-methanol 1:1 ratio (v/v) and separated to 4 fractions with hexane, dichloromethane, ethyl acetate and 30% methanol. The crude extract and fractions were tested for antimicrobial activity with *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Aspergillus niger* and wood rot fungi, *Gloeophyllum sepiarium*. The results showed that the concentrations at 50, 100 and 200 mg/mL of crude extract inhibited all tested microorganisms by paper disc diffusion assay. The greatest crude extract inhibition zone was found in *B. subtilis.* with diameters of 17.5±0.28 mm, 20.1±0.42 mm and 25.0±0.40 mm, respectively. Among the four fractions, methanol fraction inhibited *B. subtilis*, *E. coli* and *S. aureus* the highest, followed by ethyl acetate, dichloromethane and hexane fractions. The antioxidant activity of crude extract and fractions were evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay coupled on line to LC-MS/MS for simultaneous activity testing and structure elucidation of active compounds. The results indicate that the antioxidant compounds are in a group of tannins, gallic acid, afzelechin and catechin polymer form. Isolation of these compounds from crude extract and fractions of *X. xylocarpa* (Roxb.) Taub. sawdust is reported for the first time here. These results show potential for adding value to this plant's waste material from the wood industry, for various applications.

Keywords: *Xylia xylocarpa*, sawdust, tannin, antioxidant, antimicrobial, gallic acid

1. INTRODUCTION

Xylia xylocarpa (Roxb.) Taub. is one of the most famous hardwoods in Thailand used for construction. It belongs to the family Leguminosae and subfamily Mimosoideae. The common name is Iron wood and in the Thai language is known as Daeng. This plant is found in tropical rainforest and mixed deciduous forest in the North, Northeast and Southern part of Thailand. Tree height can reach 25-30 m, with straight trunk and low

branching. Bark is creamy brown or red-gray, thin, with peeling in round flakes. The inner bark is pink and the heartwood is reddish brown. Leaves are bipinnate with a single pair of side stalks, 10-22 cm, each compound leaf has 4-5 pairs of opposite leaflets. Leaflets are ovate or elliptic, 3-7 cm wide and 7-20 cm long. Flowers are white or yellow in dense spherical heads, 1-4 cm diameter with 2-5 cm head stalk and bloom in February to March. Fruit are boomerang-shaped, 7-10 cm long, with flat and woody, dry fruit that splits along two sides and ripen in October to December. The seed is oblong until almost round, brown, 1-2 cm long [1, 2]. The wood is extremely hard, sticky and durable. Previously, it was widely used in wood construction for bridges, harbor work, railway crossties, furniture, window frames and doorframes. Nowadays it is used as a forestry conservation plant, as are some other hardwood trees. It has also been grown in reforestation areas for commercial use in Thailand and South-East Asia. The bark, heartwood and flower are used in folk medicine. Bark is used as a digestive tonic and to heal diarrhea. Heartwood is used to treat anemia and diarrhea as well as used to nourish blood. The flower is used as an antipyretic and for heart health [3].

Much research has demonstrated antimicrobial activity from wood such as Kiam wood (*Cotyleobium lanceotatum*) extract which inhibits growth of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* at the minimum bactericidal concentration (MBC) of 300 mg/L [4]. Isoflavones and flavanones isolated from the stem wood of *Erythrina latissima* have shown antimicrobial activity against *E. coli*, *S. aureus*, *B. subtilis* and *Candida mycoderma* and also exhibit weak radical scavenging properties towards DPPH radicals [5]. Quinones isolated from *Tectona grandis* L. sawdust inhibit growth of *Aspergillus niger*, brown rot fungi (*Gloeophyllum sepiarium*, *Gloeophyllum trabeum*, *Piptoporus betulinus* and *Serpula lacrymans*) and white rot fungi (*Bjerkandera adusta*, *Merulius tremellosus* and *Phlebia brevispora*) [6, 7]. Flavonoids from root wood of *Bolusanthus speciosus* show strong antimicrobial activity against *E.coli*, *B. subtilis*, *S. aureus* and *C. mycoderma* and also show moderate to strong radical scavenging properties against DPPH radical [8]. Wood extracts of *Bersama engleriana* have shown activity against gram-positive and gram-negative bacteria, the two *Candida* species and mycobacteria [9]. Phenolic compounds from *Quercus rubur* and *Castanea sativa* wood are the major contributors to the antioxidant capacity [10]. Some phenolic acids, mainly gallic acid found in Oak wood used in wine ageing, show significant correlation with antioxidant capacity [11]. Taxifolin and total flavonoids from wood sawdust of *Larix gmelinii* (Rupr.) show antioxidant activity and the enzyme incubation-water extraction (EI-WE) method improve antioxidant activity [12]. The antioxidant activity of pure compounds from ethyl acetate extract of Olive (*Olea europaea* L.) wood was shown by measuring the radical scavenging activity against DPPH radical. The 7″S-hydroxyoleuropein, jaspolyanoside, ligustroside 3′-*O*-β-D-glucoside shows higher antioxidant activity than other compounds [13]. Lignin and lignin-related compounds from deciduous and coniferous wood species showed antioxidant efficiency [14]. *Myracrodruon urundeuva* heartwood extract containing phenolic compounds gallic acid, flavonoids, luteolin, cinnamic derivatives, tannins and leucoanthocyanidins [15], has shown antifungal activity (against *Fusarium* sp.), termiticidal and antioxidant activities.

Xylia xylocarpa (Roxb.) Taub. wood extract is an interesting species for investigation of phytochemical constituents and their

biological activities, to promote the planting of this tree for various economic forestry uses, not only for construction. Environmental conservation and waste material management concerns make alternate uses of *X. xylocarpa* (Roxb.) Taub. sawdust from the wood industry an interesting way to add value to this material and improve efficient natural resource use. Experiments reviewed in this article focused on phytochemical constituents with antimicrobial and antioxidant activities from *X. xylocarpa* (Roxb.) Taub. sawdust extract. Showing that the *X. xylocarpa* (Roxb.) Taub. crude extract or fractions display antimicrobial activity, the extract or sawdust powder might be used for surface coating of softwood materials and composite woods with antimicrobial properties. *X. xylocarpa* (Roxb.) Taub. sawdust and extracts derived from wood industry material can add value to waste products through medical applications as well as for improving antimicrobial properties of work surfaces, melamine softwood surface coatings, wood composites, wood flooring, furniture surfaces, particle board and fiberboard in homes, as well as in hospitals and kitchens which have high hygienic requirements [16]. Such natural, environmentally friendly, antimicrobial coatings for household products could provide alternatives to using harmful chemical reagents.

2. MATERIALS AND METHODS

2.1 Plant Extraction and Separation

Xylia xylocarpa (Roxb.) Taub. sawdust derived from a wood processing company (Prasitpornchaloen Co., Ltd.) in Thailand. The plant specimen was identified by the office of the forest herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand and the voucher specimen number of BKF No. 184199 was obtained. Sawdust samples were ground and extracted twice by maceration with chloroform-methanol ($CHCl_3$-MeOH, 1:1v/v) and sonicated for 2 h at room temperature, each time. All organic solvents were purchased from Carlo Erba (Rodano-MI, France). The crude extract was dried under vacuum and partition extraction by hexane (fraction 1), dichloromethane (fraction 2), ethyl acetate (fraction 3) and 30% methanol (fraction 4). The crude extract and all fractions were tested for antimicrobial and antioxidant activities.

2.2 Antimicrobial Assay

Bacillus subtilis ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATTCC 25923, *Saccharomyces cerevisiae* TISTR 5004, *Aspergillus niger* TISTR 3012 and *Gloeophyllum sepiarium* CBS 317.50 were used to test for antimicrobial activity by paper disc diffusion assay [17]. Bacteria were spread on nutrient agar (NA, Himedia, Spain) plates at the concentration of 10^6 CFU/mL. *S. cerevisiae* was spread on yeast malt agar (YM, Himedia, Spain) plates at the concentration of 10^5 CFU/mL. *A. niger* and *G. sepiarium* spore suspensions were spread on potato dextrose agar (PDA, Himedia, Spain) plates at the concentration of 10^5 CFU/mL. The sawdust extract and fractions were dissolved in dimethyl sulfoxide (DMSO) [18] to final concentrations of 50, 100 and 200 mg/mL, respectively. DMSO was used as a negative control while amoxicillin and ketoconazol were used as bacteria and fungi positive controls at concentration of 10 and 50 mg/mL, respectively. Sterile filter paper discs (Whatman No. 42, Maidstone, England) 5 mm in diameter were loaded with 20 µL of crude sawdust extract or fractions. Paper discs were dried and pressed on the surface of microbial-inoculated plates. The inhibition zone diameters were evaluated after incubating plates for 1 to 7 days, depending

on the microbial species. The assays were performed in 4 replicates.

2.3 Compound Identification of Active Constituents

Liquid chromatography-tandem mass spectrometer (LC-MS/MS), coupled on line with DPPH assay, was used to separate and elucidate structures and to perform tests for antioxidant activity in the same run. Peak identification was performed by comparison of the retention time, mass spectra and fragmentation patterns with reference compounds and published data. The identification of these compounds was reconfirmed with mass and retention time using extracted-ion chromatograms (EIC) and MS/MS in negative mode.

2.4 HPLC Coupled On-line to ESI-MS and DPPH Assay, for Antioxidant Activity

The high performance liquid chromatography (HPLC) was coupled on-line to Electrospray ionization mass spectrometry (ESI-MS) and a continuous flow DPPH assay [19]. HPLC 1100 series system (Agilent Technologies, Palo Alto, CA) was coupled to a PE SCIEX API 4000 (Applied Biosystem, Foster City, CA) equipped with an electrospray ionization interface. The chromatographic separation was achieved with a phenomenex Gemini column (5 µm, 250 × 4.6 mm i.d.) (Phenomenex, Torrance, CA) protected with an ODS C18 guard column, operated at 25 °C. The mobile phase consisted of 1% (v/v) formic acid and methanol in gradient elution within 55 min.

Mass spectra were recorded within 55 min. The injection volume was 5 µL. The flow rate was set to 600 µL/min. The Analyst 1.3.2 software was used for data acquisition and processing. The continuous flow system for antioxidant activity detection, consisted of an HPLC pump, LC20AD prominence (Shimadzu, Kyoto, Japan), home-made knitted reaction coil PEEK tubing with an inner diameter of 180 µm and a total reaction coil volume of 100 µL. The flow of 0.1mM DPPH was set to 200 µL/min and induced bleaching was detected as a negative peak at 515 nm using the UV-VIS detector (SPD 20AV, Shimadzu, Kyoto, Japan). The LC solution software was used for data acquisition and processing. The polarity of the signal output was reversed in order to obtain positive signals. The system was operated at 25 °C. For the characterization of antioxidant peaks, the fragment ions from their corresponding parent ions in negative mode were used for identification and structural confirmation.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The crude extract and fractions of *X. xylocarpa* (Roxb.) Taub. at concentrations of 50-200 mg/mL showed various degrees of microorganism growth inhibition against *B. subtilis*, *E. coli*, *S. aureus*, *S. cerevisiae*, *A. niger* and *G. sepiarium* by paper disc diffusion assay. The strongest growth inhibition was found against *B. subtilis* followed by *E. coli*, *S. aureus*, *S. cerevisiae*, *A. niger* and *G. sepiarium*. The inhibition zone diameter of *B. subtilis* by *X. xylocarpa* (Roxb.) Taub. crude extract at concentrations of 50, 100 and 200 mg/mL were 17.5 ± 0.28, 20.1 ± 0.42 and 25.0 ± 0.40 mm, respectively. Amoxicillin at concentration 10 mg/mL was used as reference and the inhibition zone diameter was 21.6 ± 0.12 mm. The inhibition zone diameter of *E. coli* by *X. xylocarpa* (Roxb.) Taub. crude extract at concentrations of 50, 100 and 200 mg/mL were 8.7 ± 0.62, 10.5 ± 0.20 and 11.1 ± 0.37 mm, respectively. The inhibition zone diameter of *E. coli* by amoxicillin (used as

reference) at concentration 10 mg/mL was 14.6 ± 0.24.mm. Interestingly, crude extract showed inhibition zones against *S. cerevisiae*, at concentrations of 50, 100 and 200 mg/mL, of 7.8 ± 0.31, 8.5 ± 0.28 and 8.8 ± 0.12 mm, respectively, while the inhibition zone of *S. cerevisiae* by ketoconazol (used as reference) at concentration 50 mg/mL was only 7.5 ± 0.20 mm (Table 1).

Table 1. Antimicrobial activities of *Xylia xylocarpa* (Roxb.) Taub. sawdust crude extract against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Gloeophyllum sepiarium*.

Crude extract (mg/mL)	Inhibition zone diameters [1] (mm)					
	B. subtilis	*E.coli*	*S. aureus*	*S. cerevisiae*	*A. niger*	*G. sepiarium*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
50	17.5 ± 0.28	8.7 ± 0.62	6.2 ± 0.14	7.8 ± 0.31	6.3 ± 0.23	6.0 ± 0.20
100	20.1 ± 0.42	10.5 ± 0.20	7.3 ± 0.23	8.5 ± 0.28	6.7 ± 0.14	7.6 ± 0.37
200	25.0 ± 0.40	11.1 ± 0.37	8.6 ± 0.23	8.8 ± 0.12	7.2 ± 0.25	9.3 ± 0.23
Amoxicillin 10	21.6 ± 0.12	14.6 ± 0.23	19.2 ± 0.25	-	-	-
Ketoconazol 50	-	-	-	7.5 ± 0.20	16.0 ± 0.00	14.0 ± 0.00

[1] mean ± standard error of 4 replications

The strongest growth inhibition of all fractions partitioned from crude extract was found against *B. subtilis,* followed by *E. coli* and *S. aureus.* The methanol fraction showed stronger bacteria growth inhibition than hexane, dichloromethane and ethyl acetate fractions. The inhibition zone diameter of *B. subtilis* by methanol fraction at concentrations of 50, 100 and 200 mg/mL were 14.7 ± 0.25, 16.7 ± 0.25 and 22.8 ± 1.19, respectively. Amoxicillin at concentration 10 mg/mL was used as reference and the inhibition zone diameter was 21.6 ± 0.12 mm (Table 2).

Table 2. Antimicrobial activities of *Xylia xylocarpa* (Roxb.) Taub. fractions against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*.

Plant extract (mg/mL)	Inhibition zone diameters [1] (mm)		
	B. subtilis	*E.coli*	*S. aureus*
n-Hexane fraction			
0	0 ± 0.00	0 ± 0.00	0 ± 0.00
50	6.3 ± 0.55	52 ± 0.14	0 ± 0.00
100	7.0 ± 0.20	5.8 ± 0.12	0 ± 0.00
200	10.1 ± 1.43	7.1 ± 0.12	0 ± 0.00
Dichloromethane fraction			
50	7.8 ± 0.42	6.1 ± 0.12	0 ± 0.00
100	8.8 ± 0.42	6.8 ± 0.23	0 ± 0.00
200	10.6 ± 0.68	8.0 ± 0.54	0 ± 0.00
Ethyl acetate fraction			
50	11.5 ± 0.45	10.5 ± 0.20	0 ± 0.00
100	15.2 ± 0.77	12.0 ± 0.00	0 ± 0.00
200	18.3 ± 0.42	13.0 ± 0.00	0 ± 0.00
Methanol fraction			
50	14.7 ± 0.25	11.6 ± 0.12	6.0 ± 0.00
100	16.7 ± 0.25	13.0 ± 0.20	6.5 ± 0.20
200	22.8 ± 1.19	14.3 ± 0.23	7.0 ± 0.40
Amoxicillin			
10	21.6 ± 0.12	14.6 ± 0.23	19.2 ± 0.25

[1] mean ± standard error of 4 replications

Antibacterial and antifungal activities of wood extracts were also found in other plants. It has been reported that water extract of Kiam wood (*Cotyleobium lanceotatum*) also inhibits growth of *E.coli* and *S. aureus* [4]. The stem wood of *Erythrina latissima* and the root wood of *Bersama engleriana* show antimicrobial activity against *E.coli, B. subtilis* and *S. aureus* [5, 8], and antifungal activity of $CHCl_3$-MeOH extract of *Tectona grandis* L. sawdust has been reported by our group [6, 7]. The active compounds from *Calocedrus macrolepis* var.*formosana* (Florin) heartwood also show antifungal activity [20]. The crude methanolic extracts of stem heartwood of *Euroschinus papuanus* exhibit a broad-spectrum antifungal activity [21].

3.2 Phytochemical Constituents

The yield of *X. xylocarpa* (Roxb.) Taub. $CHCl_3$-MeOH (1:1v/v) crude extract was 9.87% dry w/w, while hexane, dichloromethane, ethyl acetate and methanol fraction was 25.18, 16.45, 29.90 and 16.81% dry w/w from the crude extract, respectively.

The compound from separation of *X. xylocarpa* (Roxb.) Taub. sawdust crude extract was achieved using a reversed phase C_{18} column with gradient methanol under acidic conditions as a mobile phase. The eluate was split into two lines, one flowing to the DPPH line for antioxidant activity and the other flowing to ESI-MS. The sample was analyzed in negative and positive ionization modes. In this experiment the negative mode gave a better result than the positive mode so the negative mode was selected. The negative ions of the major active compounds of the crude extract are listed in Table 3 and identification of these compounds is proposed.

Table 3. Compound identification from crude extract ($CHCl_3$-MeOH, 1:1v/v) of *Xylia xylocarpa* (Roxb.) Taub. sawdust using LC-ESI-MS-DPPH assay data in negative ionization. t_R is the retention time of the peaks from the antioxidant activity detector.

Peak no.	t_R (min)	ESI-MS (m/z) MS	MS/MS	Tentative ID
1	15.2	169.4	125.2	gallic acid[1]
2	25.1	561.4	409.2, 289.5, 391.3	(epi)afzelechin-(epi)catechin
3	25.7	544.8	289.3, 409.0, 390.9, 435.4	(epi)afzelechin-(epi)afzelechin
4	26.2	273.6	123.5, 148.9, 163.1	(epi)afzelechin
5	27.2	833.7	681.5, 561.0, 529.2	(epi)afzelechin-(epi)afzelechin-(epi)catechin
6	29.4	817.8	665.8, 561.0, 707.4	(epi)afzelechin-(epi)afzelechin-(epi)afzelechin
7	34.4	583.4	253.5, 430.7, 279.3	unknown 1
8	34.5	723.9	677.1	unknown 2
9	37.7	627.6	313.1	unknown 3

[1]Compared with the standard compounds

The DPPH based antioxidant activity profile (Figure 1 a,c,e) showed that at least nine compounds with antioxidant activity. The total ion current (TIC) output from the ESI-MS in negative mode of crude extract, ethyl acetate fraction and methanol fraction are shown in Figure 1 (b,d,f).

In the negative ionization mode, the mass spectrum data showed fragmentation profiles with m/z 289 and 273 which are correlated to (epi)catechin and (epi)afzelechin, respectively. The m/z of dimers and trimers are shown in Figure 2.

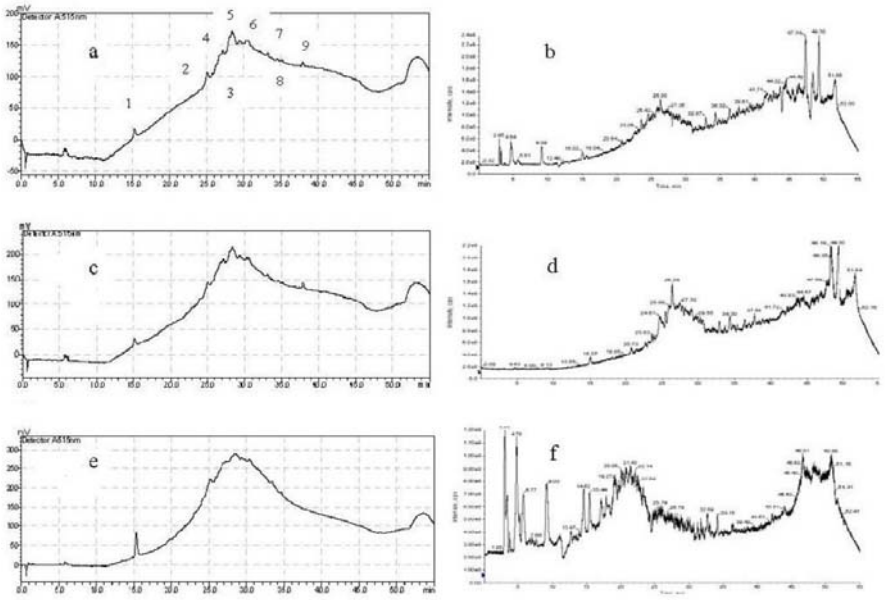

Figure 1. HPLC separation of *Xylia xylocarpa* extract with simultaneous antioxidant activity assay and MS detection. a, c, e. The chromatogram from the antioxidant activity assay detection at 515 nm of crude extract, ethyl acetate and methanol fractions, respectively. b, d, f. The total ion current (TIC) output from the ESI-MS in negative mode fraction of crude extract, ethyl acetate and methanol fractions, respectively. For peak assignments, see Table 3. Conditions are described in the text.

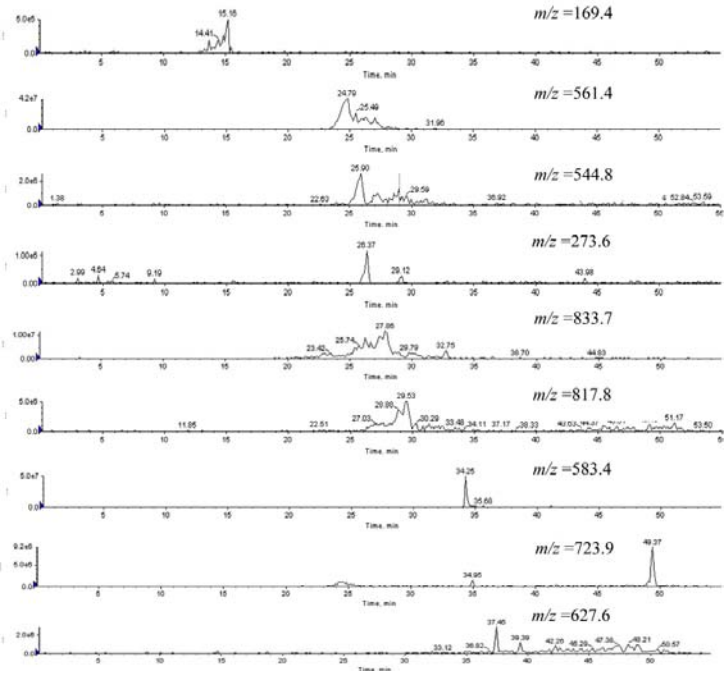

Figure 2. HPLC-ESI-MS/MS of the sample crude extract from *Xylia xylocarpa* (Roxb.) Taub. Extract ion chromatogram (EIC) of each identified phenolic compound acquired in negative mode $[M-H]^-$.

Peak 1 (t_R =15.2 min) presented with a $[M-H]^-$ at m/z 169.4, and a fragment ion at m/z 125.2 $[M-H-CO_2]^-$. This compound was identified as gallic acid, with retention time and mass data that fit with the authenticated compound. Peak 2 (t_R =25.1 min) showed a $[M-H]^-$ at m/z 561.4, and a fragment ion at m/z 409.2 (RDA rearrange, 152 amu), 289.5 (epi-catechin), and 391.3. This compound was identified as (epi)afzelechin-(epi)catechin [22, 23]. Peak 3 (t_R =25.7 min) showed a $[M-H]^-$ at m/z 544.8 and MS/MS fragmentation at m/z 289.3, 409.0, 390.9, and 435.4. This compound was tentatively identified as (epi)afzelechin-(epi)afzelechin. Peak 4 (t_R=26.2 min) showed a $[M-H]^-$ at m/z 273.6 and a fragmentation ion at m/z 123.5, 148.9, and 163.1. This compound was proposed as (epi)afzelechin. Peak 5 (t_R =27.2 min) showed a $[M-H]^-$ at m/z 833.7 and fragmentation at m/z of 681.5, 561.0, 529.2. This compound was identified as (epi)afzelechin-(epi)afzelechin-(epi)catechin. Peak 6 (t_R = 29.4 min) showed m/z of 817.8$[M-H]^-$ and fragmentation at m/z 665.8, 561.0, and 707.4. This compound was tentatively identified as (epi)afzelechin-(epi)afzelechin-(epi)afzelechin [22]. Peak 7 (t_R=34.4 min) showed a $[M-H]^-$ at m/z 583.4, and a fragment ion at m/z 253.5, 430.7, and 279.3. Peak 8 (t_R = 34.5 min) showed a $[M-H]^-$ at m/z 723.9 and fragmentation at m/z 677.1. Peak 9 (t_R = 37.7 min) showed a $[M-H]^-$ at m/z 627.6 and fragmentation at m/z 313.1. These three compounds need more data for identification. The extracted-ion chromatograms (EIC) of each compound are shown in Figure 2. The structure elucidation is presented in Table 3, and chemical structures of proposed compounds are shown in Figure 3.

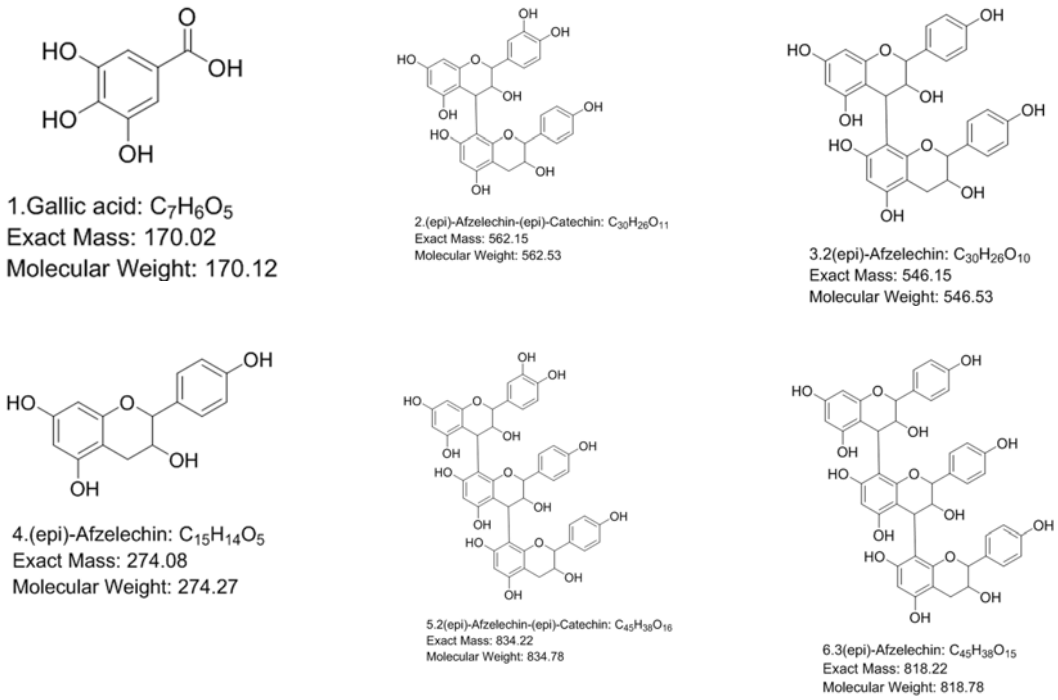

Figure 3. Chemical structure of compounds found in *Xylia xylocarpa* (Roxb.) Taub. sawdust crude extract.

The compounds found in this experiment are gallic acid, tannins in monomer (epi) afzelechin, dimer (epi)afzelechin-(epi) afzelechin and trimer (epi)afzelechin-(epi) afzelechin-(epi) afzelechin form. Afzelechin has been previously reported as having antioxidant activity [24]. Synthesized gallic acid has previously been found to have free radical scavenging ability [25]. Gallic acid and gallic acid methyl ester has been reported for antimicrobial activity against methicillin-resistant *Staphylococcus aureus* [26]. Several tannins have shown antimicrobial activity against *Staphylococcus aureus* [27, 28]. Moreover, tannins extracted from perennial plants such as black jack oak (*Quercus marilandica*), sumac (*Rhus copallina*) and sand plum (*Prunus angustifolia*) have been shown to inhibit growth of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* [29]. Tannins of inner bark extracts of *Stryphnodendron adstringens*, a leguminous tree species, have also shown significant antifungal activity [30].

4. CONCLUSIONS

Xylia xylocarpa (Roxb.) Taub. sawdust from Thailand was macerated with chloroform-methanol 1:1 ratio (v/v) and then partition extracted to 4 fractions with hexane, dichloromethane, ethyl acetate and methanol. All fractions and the crude extract were tested for antimicrobial activity with *B. subtilis*, *E. coli*, *S. aureus*, *S. cerevisiae*, *A. niger* and the wood rot fungi, *G. sepiarium*. The crude extract showed inhibition of bacteria, yeast and fungi at the concentration of 50 mg/mL, while all fractions were active against *B. subtilis* and *E. coli*. The antioxidant compounds found in *X. xylocarpa* (Roxb.) Taub. sawdust are belong to a group of tannins, such as afzelechin and its polymers. At least nine compounds, including gallic acid, afzelechin and catechin, were elucidated by the system of LC-MS/MS coupled on line with DPPH assay. All compounds discussed in this report are presented here for the first time for this plant.

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