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Study on inhibition mechanisms of light-induced wood radicals by *Acacia confusa* heartwood extracts

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ABSTRACT

The aim of this study was to investigate the inhibition mechanisms of light-induced wood radicals by *Acacia confusa* heartwood extracts (AcE). Wood radical scavenging analysis was determined by ESR spectroscopy. The results obtained demonstrated that wood radicals could be inhibited through UV absorption of AcE. According to results of AcE photooxidation derivative analyses detected by HPLC–DAD, HPLC–MS/MS and FTIR spectroscopy, *o*-quinones, peroxides and other oxidation derivatives were yielded from flavonols (such as melanoxetin and transilitin) in AcE; okanin (chalcone) might be formed from 7,8,3′,4′-tetrahydroxyflavanone (flavanone); 7,8,3′,4′-tetrahydroxyflavone and 7,3′,4′-trihydroxyflavone (flavones) would transform to flavanone. On the basis of GPC analysis results, proanthocyanidins and derivatives of higher molecular weight might be polymerized from melacacidin (flavan-3,4-diol). Taken together, these results clearly demonstrated that *A. confusa* heartwood extract can absorb UV light and form photooxidation derivatives. Accordingly, wood radicals induced by UV light were inhibited and consequently wood photodegradation was retarded.

1. Introduction

Wood is a photodegradable biomaterial. Compared with the major components of wood, lignin is more sensitive to light. When lignin is irradiated with light, especially UV light, lignin radicals are readily induced. Furthermore, reaction between radicals and active oxygen or other wood components will lead to wood degradation [1,2].

To prevent photodegradation of wood, photostabilizers were commonly used [3–5]. According to their functions, photostabilizers can be classified into five categories: UV absorbers, radical scavengers, excited state quenchers, singlet oxygen quenchers, and antioxidants. Among them, UV absorbers and radical scavengers were more frequently employed. Interestingly, the phenolics from plant secondary metabolites (such as flavonoids or gallic acid derivatives) also have the efficacy to scavenge free radicals [6,7]. However, studies on application of plant phenolics as natural photostabilizers in wood are relatively scarce.

Our previous research [8] found that *Acacia confusa* heartwood extracts (AcE) functioned as photostabilizers to reduce wood photodegradation. Further investigation [9] suggested that the wood photostabilization ability of AcE might come from its radical scavenging activity or antioxidant activity. It is worthy to further explore if AcE can be applied as a photostabilizer. The capability of UV absorption, antioxidant efficacy, excited state quenching efficacy, radical scavenging efficacy and the photostabilization mechanisms also need to be established. This study aimed to investigate the inhibition activity of wood radicals by AcE and discussed the inhibition mechanisms involved.

2. Materials and methods

2.1. Wood radical scavenging analysis

To study the scavenging mechanisms of wood radicals, 1% (w/w) AcE-treated *Cunninghamia lanceolata* sapwood powder (40–60 mesh) was used. The wood radicals, induced by UV light (500 W mercury arc lamp, Oriel 6285), were directly measured in the cavity of the electron spin resonance (ESR) spectrometer (ELEXSYS E-580, Bruker). All specimens were measured at −196 °C. The frequency of microwave was 9 GHz.

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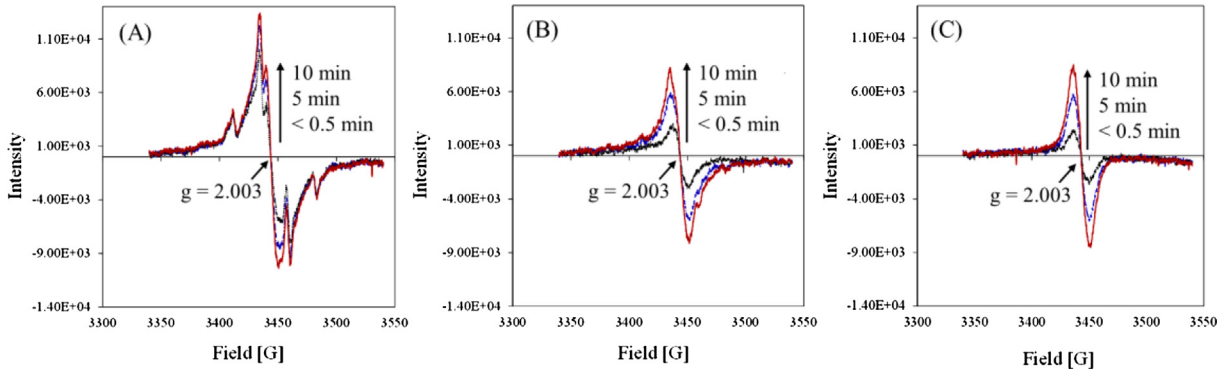

Fig. 1. Variations of free radicals from (A) untreated wood; (B) 1% AcE-treated wood; and (C) AcE.

2.2. Analyses of photooxidated *A. confusa* heartwood extracts (AcE)

2.2.1. Sample preparation

To understand the changes in chemical characteristics of AcE after irradiation, methanolic solution (1 mg/mL) of AcE was irradiated by UV light (450 W medium-pressure mercury lamp, Ace glass 7825-34) at 30 ± 2 °C. The irradiation durations were 4, 8, 16, 24 and 48 h, respectively. The irradiated AcE were vacuum-dried and used in the study of AcE photooxidation mechanism.

2.2.2. Photooxidation derivative analysis

2.2.2.1. Functional group analysis. The changes in functional groups of AcE after irradiation were analyzed with Fourier transform infrared spectroscope (FTIR, Varian 3100 Excalibur series, Varian). Irradiated samples were detected in 4 cm^{-1}, 16 scans and 2000–600 cm^{-1} scan range.

2.2.2.2. UV absorption measurement. The UV spectrum of AcE was analyzed with an UV/vis spectroscope (V-550, Jasco), and 20 μg/mL AcE methanolic solution was measured at the wavelength from 210 to 600 nm.

2.2.2.3. Chemical composition analysis. The changes in chemical composition of irradiated AcE were analyzed with Agilent 1200 series high-performance liquid chromatography equipped with a diode array detector (HPLC–DAD) and a Chromolith® performance RP-18e column (100 mm × 4.6 mm i.d., Merck). The mobile phase was solvent A (water/acetic acid, 99.9/0.1, v/v) and solvent B (MeOH, 99.9/0.1, v/v). Elution conditions were 0–5 min of 1% B; 5–10 min of 1–5% B; 10–20 min of 5–8% B; 20–35 min of 8–10% B; 35–40 min of 10–18% B; 40–50 min of 18% B; 50–60 min of 18–25% B; 60–70 min of 25% B; 70–75 min of 25–30% B; 75–80 min of 30–50% B; 80–85 min of 50–100% B; and 85–90 min of 100% B. The flow rate was 2 mL/min. Quercetin was also used as an internal standard (IS) to calculate the changes in chemical components after irradiation.

Moreover, in order to confirm the chemical structures of each peak in the HPLC profile, the known compounds from AcE were co-injected. HPLC–MS/MS (Thermo UltiMate 3000, equipped with ion trap mass spectrometers) was also employed to identify the chemical composition of irradiated AcE by comparing with the mass spectra of those reference standard compounds [10,11]. The separation method is the same as HPLC–DAD. Electrospray ionization, operating in negative ion mode, was used, and the scanning range is 70–3000 *m/z*.

2.2.2.4. Molecular weight analysis. The changes in molecular weight of AcE were analyzed with gel permeation chromatography (GPC) equipped with an UV detector and a TSK-GEL Alpha-2500 column (300 mm × 7.8 mm i.d., TOSOH). The mobile phase was MeOH-8M Urea (pH = 2) (6/4). The flow rate was 0.3 mL/min, and 1-mg/mL samples were injected (15 μL). The detection wavelength was 230 nm. Monomer (melacacidin), dimer (epimesquitol-(4β→6)-epimesquitol-4α-ol) and trimer (epimesquitol-(4β→6)-epimesquitol-(4β→6)-epimesquitol-4α-ol) were collected from *A. confusa* root and were used as molecular weight standards for determining the molecular weight distribution.

3. Results and discussion

3.1. Effects of *A. confusa* heartwood extracts (AcE) on scavenging of wood radicals

The variations of free radicals from wood after UV irradiation are shown in Fig. 1. As can be seen, abundant free radicals formed in the untreated wood at the initial irradiation period (<0.5 min) (Fig. 1A) and the intensity of formation increased with irradiation duration. Since lignin is a good UV absorber [2], it produces phenoxyl radical (g-factor = 2.003) via UV irradiation [12]. Moreover, except for lignin, cellulose can also absorb 5–20% of UV light [2], thus forming hydroperoxy radicals and formyl radicals [13,14]. In other words, UV-induced radicals are produced from both lignin and cellulose. Hence, when wood absorbs UV light, multiple free radicals will be induced, and a complex signal will be detected by the ESR spectroscopy (Fig. 1A).

Compared with ESR results of untreated wood, fewer radicals formed in 1% AcE-treated wood (Fig. 1B) were observed, indicating that AcE could inhibit the formation of wood radicals induced by UV light. Additionally, it could be also observed that the line shapes of ESR signals between wood radicals (Fig. 1A) and AcE radicals (Fig. 1C) were different. The line shapes of wood radicals have several peaks owing to the mixture of radicals from lignin (phenoxyl radicals) and cellulose (hydroperoxy radicals and formyl radicals). Owing to abundant flavonoids in AcE [15], the radicals formed from AcE were phenoxyl radicals. Hence, the line shapes of

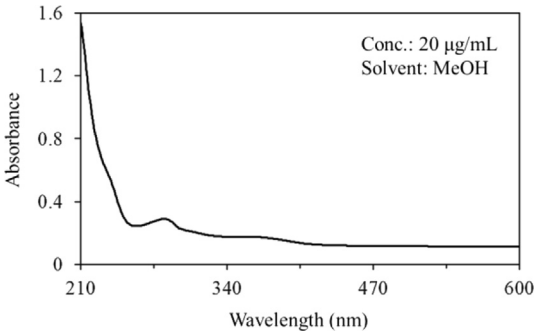

Fig. 2. UV/vis absorption spectrum of AcE.

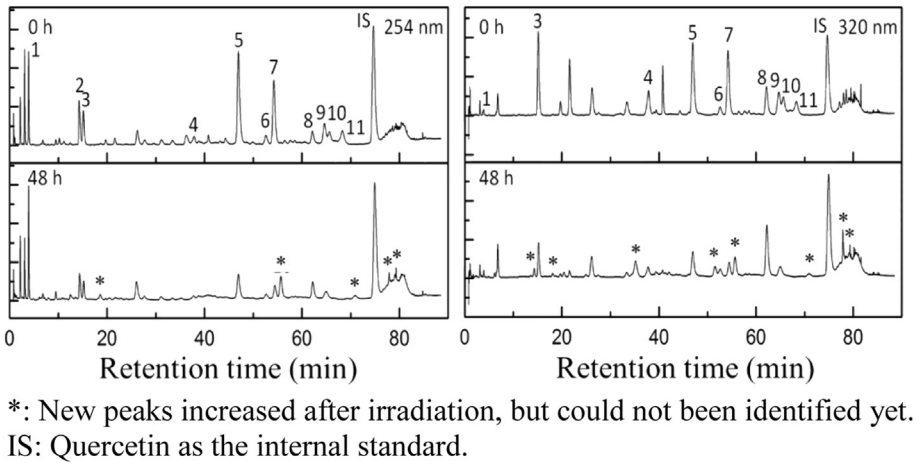

Fig. 3. HPLC profiles of irradiated AcE.

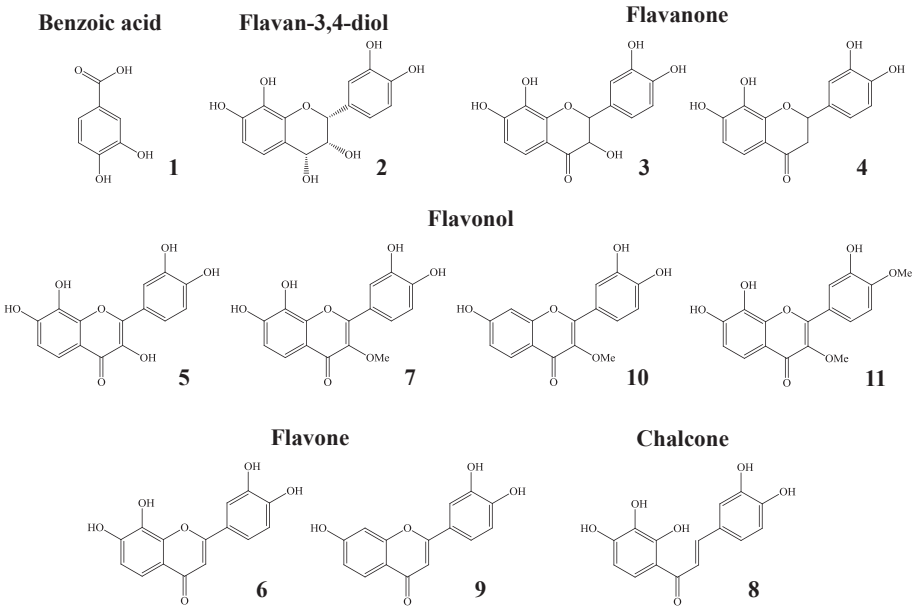

Fig. 4. Extractives in AcE [10,11].

radicals from wood and AcE were different. Compared to 1% AcE-treated wood, the line shapes were similar to AcE, indicating that the radicals from 1% AcE-treated wood and AcE were similar. Moreover, compared to the differences in intensities of ESR signals between wood radicals and AcE radicals at <0.5 min irradiation period, the variations could be found obviously that the intensity was much lower in AcE radicals than that in wood radicals. However, compared to 1% AcE-treated wood, the intensity was similar to that of AcE radicals, but different from that of untreated wood. It implied that the free radicals formed in 1% AcE-treated wood were also phenoxyl radicals. Since AcE can also absorb UV light (Fig. 2), it is reasonable to infer that the phenoxyl radicals from 1% AcE-treated wood mainly originated from AcE via UV light absorption. According to these results, it could be concluded that AcE could absorb UV light, thus inhibiting the formation of wood radicals.

3.2. Photooxidation mechanisms of A. confusa heartwood extracts (AcE)

Our previous studies have examined the influence of AcE on wood photostability [8,9] and found that the lignin degradation would be inhibited via primary photooxidation of AcE. Similar to other photostabilizers, AcE may be oxidized after UV irradiation, although it can inhibit wood photodegradation via UV light absorption. To understand the changes in chemical composition of AcE after photooxidation, the irradiated AcE were analyzed and the

Table 1
Changes in relative ratio[a] of chemical compounds after irradiation.

Compound	254 nm		320 nm	
	Control	Irradiated	Control	Irradiated
1	1.00[a]	1.25	1.00	1.18
2	1.00	0.64	N/A	N/A
3	1.00	0.55	1.00	0.33
4	1.00	0.55	1.00	0.32
5	1.00	0.27	1.00	0.27
6	1.00	0.50	1.00	0.48
7	1.00	0.21	1.00	0.17
8	1.00	1.25	1.00	1.41
9	1.00	0.45	1.00	0.48
10	1.00	N/A	1.00	N/A
11	1.00	N/A	1.00	N/A

[a] Relative ratios were calculated from $I_{compound}/I_{Quercetin}$.

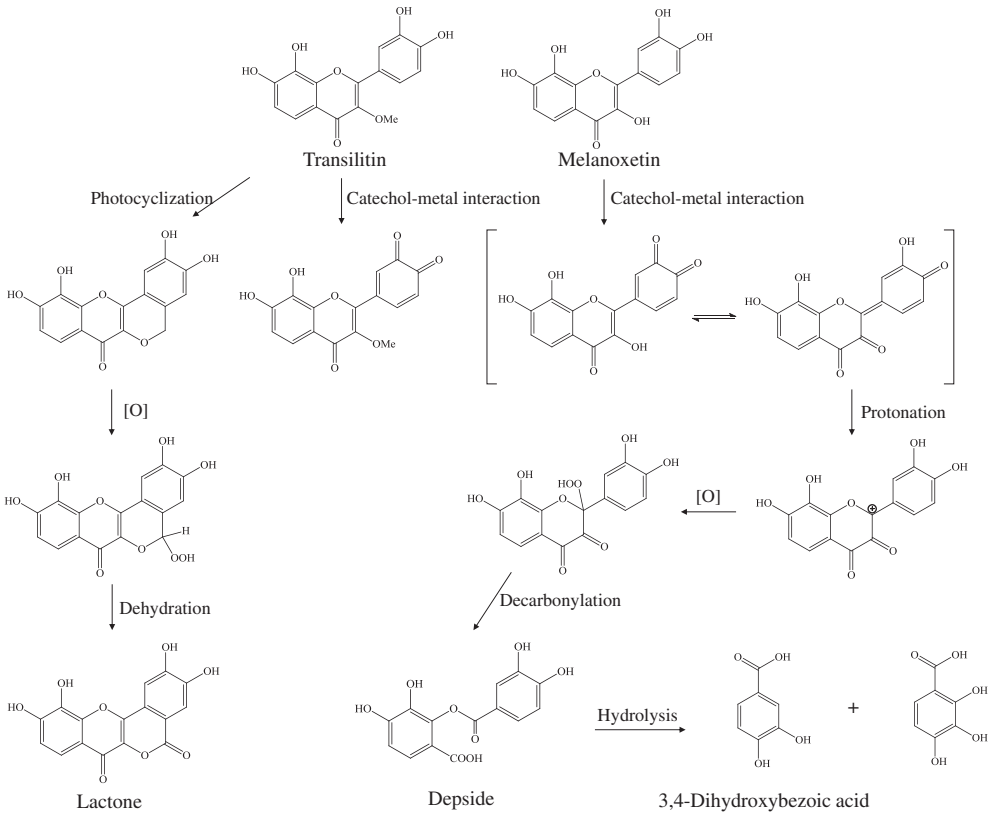

Fig. 5. Proposed photooxidation mechanisms of melanoxetin and transilitin.

HPLC profiles are shown in Fig. 3. The absorption peaks from **1** to **11** were identified via co-injection of known compounds from AcE [10,11] and LC–MS analysis. These 11 compounds identified are: 3,4-dihydroxybenzoic acid (**1**), melacacidin (**2**), 3,7,8,3′,4′-pentahydroxydihydroflavanone (**3**), 7,8,3′,4′-tetrahydroxyflavanone (**4**), melanoxetin (**5**), 7,8,3′,4′-tetrahydroxyflavone (**6**), transilitin (**7**), okanin (**8**), 7,3′,4′-trihydroxyflavone (**9**), 7,3′,4′-trihydroxy-3-methoxyflanove (**10**) and 7,8,3′-trihydroxy-3,4′-dimethoxyflavone (**11**) (Fig. 4).

Further analysis of the changes in relative ratio of each peak after irradiation (Table 1) reveals that the relative ratios of compounds **2**, **3**, **4**, **5**, **6**, **7**, **9**, **10** and **11** decreased. Among them, compounds **5** (melanoxetin) and **7** (transilitin), both of which are flavonols, showed more significant decrease. Some studies investigated the photooxidation of flavonols [16–18] and reported that flavonols would be oxidized to B-ring *o*-quinone intermediates, peroxides and other oxidation derivatives via catechol–metal interaction after UV irradiation if there are 3′-OH and 4′-OH groups in the B-ring. Hence, when the flavonols from AcE, such as melanoxetin and transilitin, were exposed to UV light, they would transform into B-ring *o*-quinone intermediates (Fig. 5). In addition, both depside and benzoic derivatives (*i.e.*, 3,4-dihydroxybenzoic acid) would be formed from B-ring *o*-quinone intermediates if there is 3-OH substitution in the C-ring, such as melanoxetin. However, if there is 3-OMe group substitution in the C-ring, lactone derivatives might be partially formed via photocyclization [19,20]. Therefore, flavonols from AcE, such as transilitin, substituting 3-OMe, would be oxidized and yielded lactone

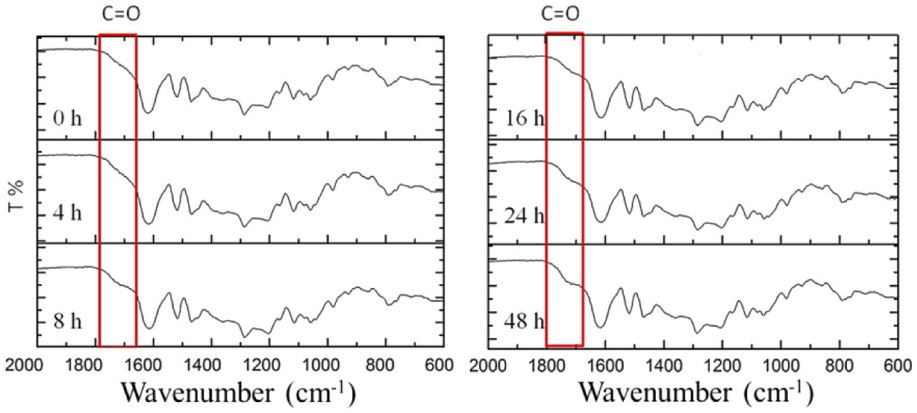

Fig. 6. FTIR spectra of irradiated AcE.

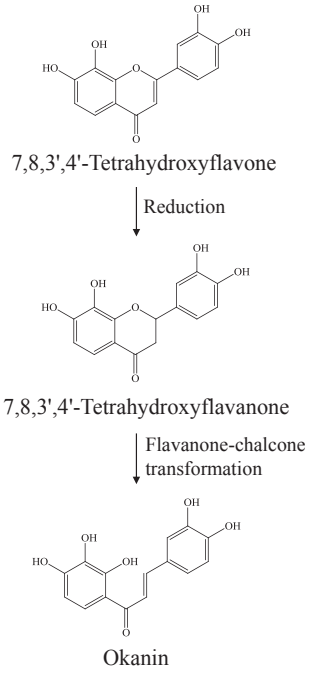

Fig. 7. Proposed mechanism of okanin formation.

derivatives. Consequently, the contents of flavonols from AcE decreased after UV irradiation.

Moreover, the FTIR results (Fig. 6) showed that the absorption peak at 1720 cm^{-1}, indicating of stretching of the carbonyl group, increased with irradiation duration. Since *o*-quinones, peroxides and other oxidation derivatives would be yielded from the flavonols in AcE, increase in carbonyl groups can be attributed to flavonol photooxidation.

In addition, the relative ratio of compound **4** (7,8,3′,4′-tetrahydroxyflavanone) decreased, whereas that of compound **8** (okanin) increased after UV irradiation. According to prior research [21], chalcone can be derived from flavanone via flavanone–chalcone transformation by UV irradiation. Hence, it could be understood that the increased ratio of okanin (chalcone) might be yielded from 7,8,3′,4′-tetrahydroxyflavanone (flavanone) via flavanone–chalcone transformation (Fig. 7). In addition, Chen and coworkers [22] demonstrated that flavone would transform into flavanone via reduction. Hence, the flavones from AcE (7,8,3′,4′-tetrahydroxyflavone and 7,3′,4′-trihydroxyflavone) would transform into flavanones, then undergo further degradation reaction (Fig. 7), such as flavanone–chalcone transformation.

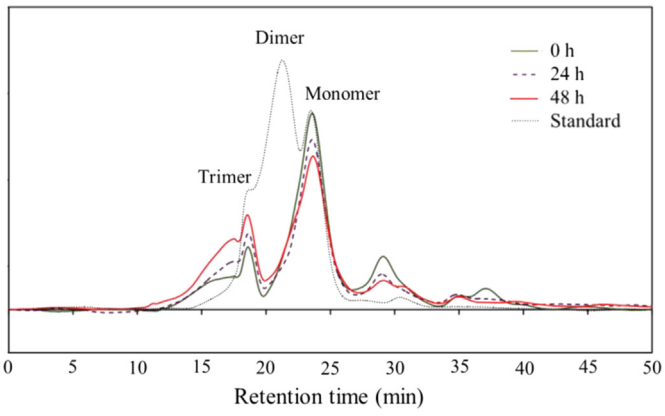

Fig. 8. Changes in molecular weight of irradiated AcE.

Comparing the changes in molecular weight (Fig. 8) shows that the intensity of monomer decreased while that of trimer and oligomer (degree of polymerization > 3) increased, indicating the occurrence of polymerization reactions after irradiation. Terrier and coworkers [23] reported that the proanthocyanidin could be polymerized from flavan-3,4-diol through dehydrogenation and protonation. It is likely that the derivatives of higher molecular weight originated from the flavan-3,4-diol of AcE, such as melacacidin, which warrants further investigation.

4. Conclusions

This study investigated the inhibition mechanism of wood radicals by AcE. The variations in wood radicals from 1% AcE-treated wood demonstrated that AcE inhibited the formation of wood radicals via UV light absorption. Analyzing the changes in chemical composition and the functional groups from AcE after UV irradiation reveals that melanoxetin, transilitin, 7,3′,4′-trihydroxy-3-methoxyflanove and 7,8,3′-trihydroxy-3,4′-dimethoxyflavone, *i.e.*, flavonols, were oxidized to *o*-quinones, peroxides and other oxidation derivatives; okanin (chalcone) was derived from 7,8,3′,4′-tetrahydroxyflavanone (flavanone) via flavanone–chalcone transformation; 7,8,3′,4′-tetrahydroxyflavone and 7,3′,4′-trihydroxyflavone, which are flavones, transformed into flavanones via reduction. The changes in molecular weight of AcE observed indicated that derivatives of higher molecular weight might be yielded from melacacidin (flavan-3,4-diol). It could be concluded that the AcE could absorb UV light, followed by yielding photooxidation derivatives, meanwhile, the wood radicals formed were reduced and consequently wood photodegradation was inhibited.

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