

Evaluation of Anti-Fungal Activities of Environmentally Friendly Wood Preservative from Thermal-Induced Lignified Twigs

Xinqi Gao[†], Yafang Lei[†], Teng Sun, Yuanze Ma, Hao Guan, Li Yan^{*}

Forestry College, Northwest A & F University, Yangling, Shaanxi, 712100, China

^{*} Corresponding authors. E-mail address:

liliyan@nwsuaf.edu.cn (Li Yan).

[†] Xinqi Gao and Yafang Lei contributed equally as first authors to this work.

Table S1. Colony Diameter of Decay Fungi on PDA Medium with LTP-A220.

Fungus	Extract Conc	Colony Diameter (mm) ^a			
		4-day	6-day	8-day	14-day
<i>T. versicolor</i>	Blank control	45.87±0.71	56.00	56.00	56.00
	0 mg/mL	41.65±0.64	56.00	56.00	56.00
	2 mg/mL	10.61±0.28	18.47±1.17	29.64±0.78	56.00
	4 mg/mL	4.50	4.50	4.50	4.50
	6 mg/mL	4.50	4.50	4.50	4.50
	8 mg/mL	4.50	4.50	4.50	4.50
<i>G. trabeum</i>	Blank control	25.62±0.66	42.01±2.19	56.00	56.00
	0 mg/mL	23.26±0.52	41.38±1.27	56.00	56.00
	2 mg/mL	8.59±0.37	12.84±0.27	19.33±0.32	42.20±4.20
	4 mg/mL	5.61±0.09	5.55±0.08	5.64±0.15	6.26±0.22
	6 mg/mL	4.50	4.50	4.50	4.50
	8 mg/mL	4.50	4.50	4.50	4.50

Note a: The initial diameter of the discs inoculated onto the culture medium is 4.5 mm, and the maximum inner diameter of the Petri dishes is 56 mm.

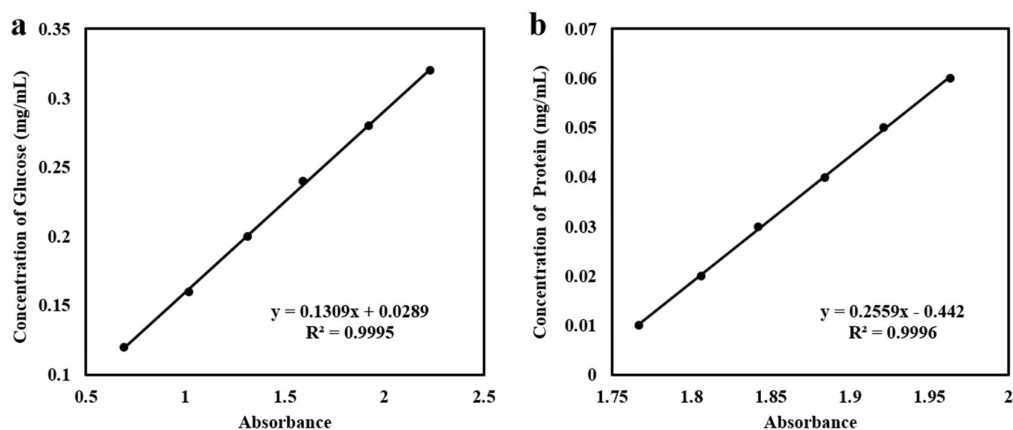


Figure S1 Standard curves for glucose (a) and protein (b).

Equations S1–S3, relevant calculations of enzyme activities

The amount of enzyme required to catalyze 1 μ mol substrate per unit time is 1 enzyme activity unit. According to this definition, the activities of the enzymes associated with wood degradation in this study were calculated by the following formula:

$$IU = n \frac{\Delta C_m \cdot V \cdot 10^3}{M \cdot t \cdot V_e} \quad (S1)$$

$$\Delta C_m = C_I - \frac{C_0}{n_I} - C_S \quad (S2)$$

where,

n - the dilution of the crude enzyme solution;

n_I - the dilution factors of the crude enzyme solution after the second dilution with buffer were 2 for endoglucanase and exoglucanase, and 1.5 for β-glucosidase and xylanase, respectively;

V - the volume of the reaction system, mL;

M - the relative molecular weight of glucose, 180.2 g/mol;

t - the reaction time, 60 min;

V_e - the volume of crude enzyme solution in the reaction system, mL;

C_I - the glucose concentration after the reaction, mg/mL;

C₀ - the residual glucose concentration in the diluted crude enzyme solution, mg/mL;

C_s - the glucose concentration introduced by the substrate, mg/mL;

C_I, C₀, and C_s are calculated using the respective glucose standard curves. The C_s value for cellulase is zero.

$$IU = \frac{\Delta OD \cdot V \cdot 10^3}{b \cdot \varepsilon \cdot t \cdot V_e} \quad (3)$$

Where,

ΔOD - absorbance change value;

V - volume of the reaction system, mL;

b - diameter of the cupola, 1 cm;

ε - molar absorption coefficient, manganese peroxidase 49600, laccase 36000, [(mol/L) cm⁻¹];

t - reaction time, min;

V_e - the volume of crude enzyme solution in the reaction system, mL.

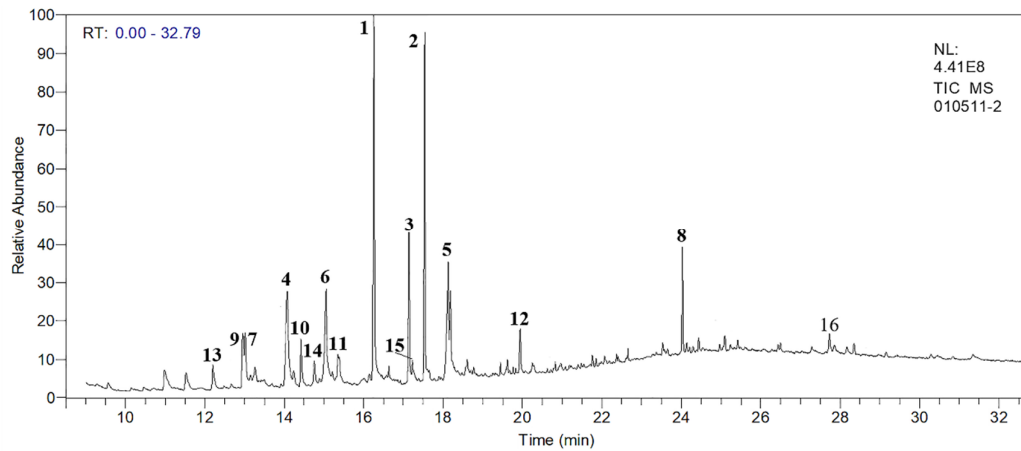
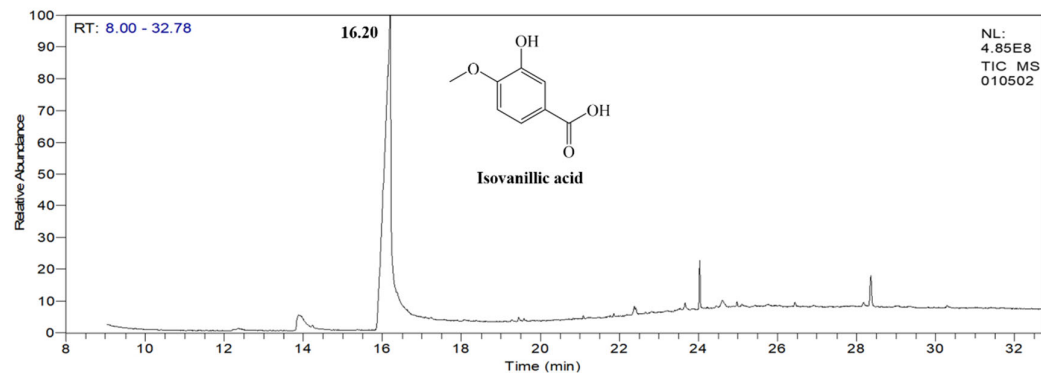
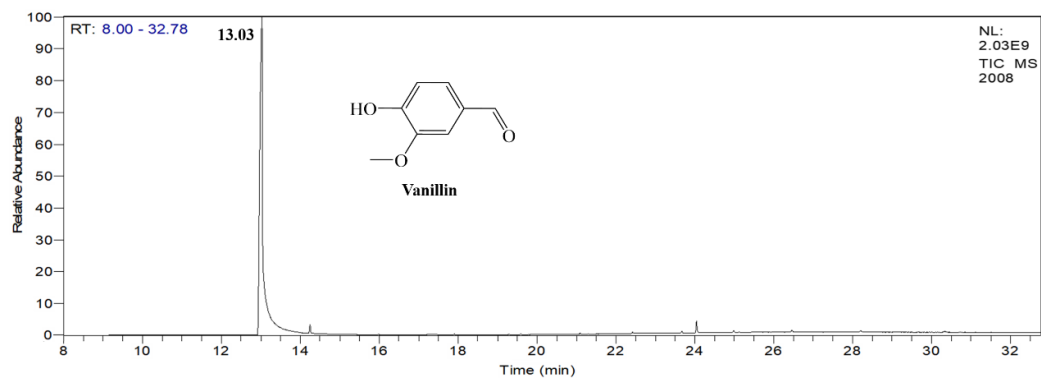
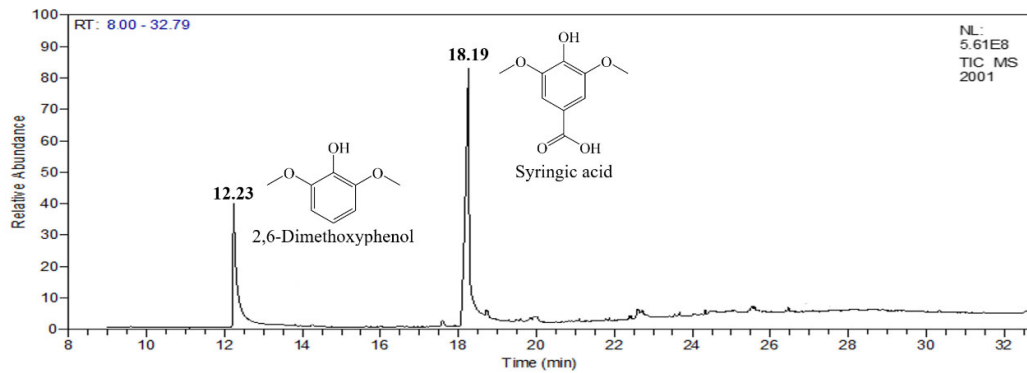
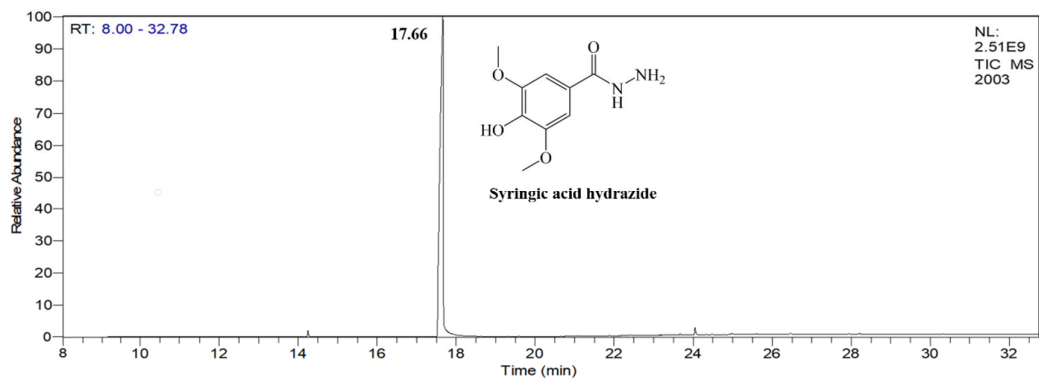
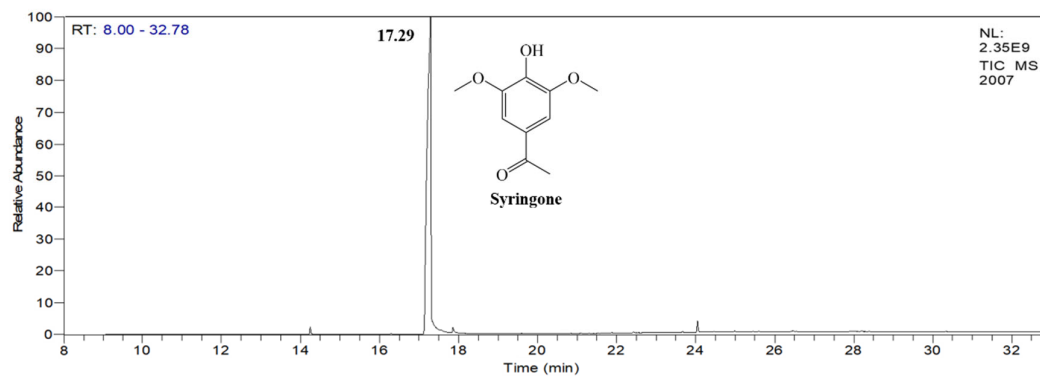
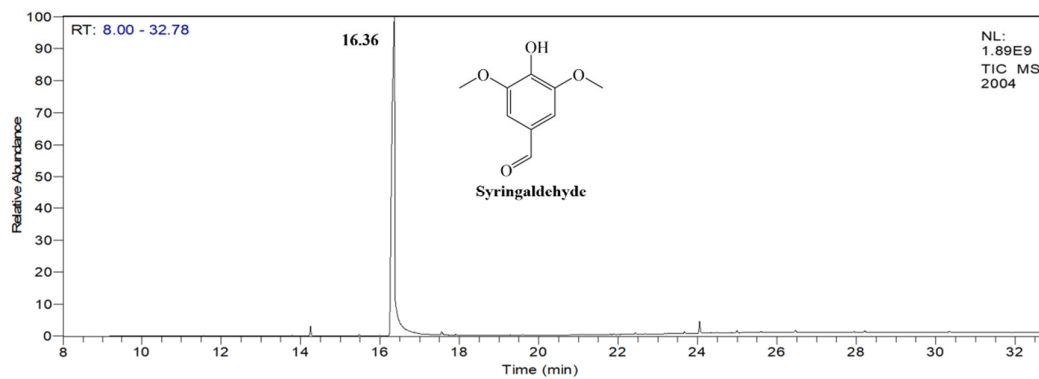


Figure S2. Gas chromatogram of the LTP-N220.





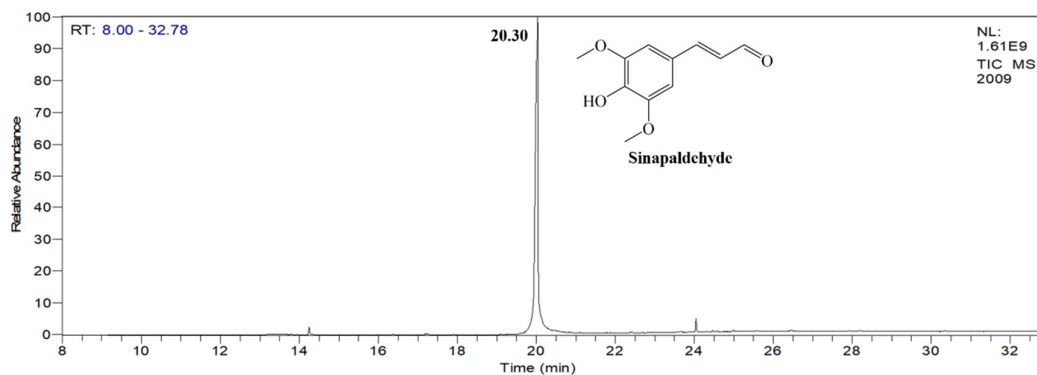


Figure S3. Gas chromatography of standards for some components in LTP-N220.

Table S2. Reducing sugar content of LTP prepared with air and nitrogen as heating medium at 180, 200 and 220°C

Temperature/°C	Content of Reducing Sugar/%	
	AIR	Nitrogen
180	1.11 ± 0.014	3.26 ± 0.020
200	1.59 ± 0.005	4.68 ± 0.077
220	2.44 ± 0.105	4.98 ± 0.047

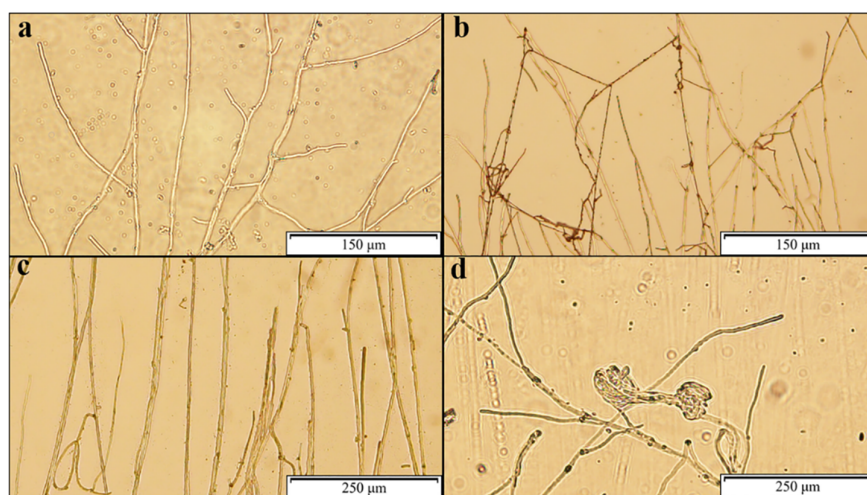


Figure S4. The mycelium of normally growing *T. versicolor* (a) and *G. trabeum* (c), as well as the mycelium of *T. versicolor* (b) and *G. trabeum* (d) affected by LTP-N220.

Table S3. Average fungal colony diameter of decay fungi on PDA or media with varying compositions.

Fungus	Ingredient	Colony Diameter (mm) ^a		
		7 days	14 days	21 days
<i>T. versicolor</i>	Control group	56.00	56.00	56.00
	Syringaldehyde	4.50	4.50	4.50
	Syringic acid hydrazide	4.50	4.50	4.50
	Syringone	4.50	4.50	4.50
	Syringic acid	4.50	15.12±2.09	32.20±2.68
	Isovanillic acid	44.30±1.5	56.00	56.00
	Vanillin	4.50	4.50	4.50
	Methyl vanillate	4.50	4.50	4.50
	Vanillone	4.50	4.50	4.50
	Sinapaldehyde	31.87±1.23	56.00	56.00
	2,6-Dimethoxyphenol	44.20±0.032	56.00	56.00
	Coniferyl aldehyde	28.43±0.53	39.47±2.78	56.00
<i>G. trabeum</i>	Control group	33.44±0.35	56.00	56.00
	Syringaldehyde	4.50	4.50	4.50
	Syringic acid hydrazide	4.50	4.50	4.50
	Syringone	4.50	4.50	4.50
	Syringic acid	22.21±1.58	37.48±1.48	46.53±3.52
	Isovanillic acid	20.23±2.00	45.18±1.42	50.36±1.09
	Vanillin	4.50	4.50	4.50
	Methyl vanillate	4.50	4.50	4.50
	Vanillone	4.50	4.50	4.50
	Sinapaldehyde	4.50	4.50	4.50
	2,6-Dimethoxyphenol	4.50	4.50	4.50
	Coniferyl aldehyde	4.50	4.50	4.50