

Antifungal Activity and Chemical Composition of Neem Seed Extract (*Azadirachta indica* A.)

Lulu Chang,^a Yin Liu,^a Weiwei Zhang,^a Guoqi Xu,^b Lihai Wang,^{b,*} and Wenxia Yuan^{a,*}

The antifungal activities and chemical composition of neem seed extract (*Azadirachta indica*) were investigated. An agar diffusion assay showed that neem seed extract (optimum concentration: 10%) inhibited the growth of *Trametes versicolor* and *Gloeophyllum trabeum*. The minimum inhibitory concentration and minimum fungicidal concentration were 2% and 4%, respectively, against the two fungal species. Scanning electron microscopy revealed severe morphological damage to fungal hyphae, including reduction of spores and hyphal shriveling. Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry analyses identified 17 compounds that represented 80.3% of the total extract, including astaxanthin (34.3%), cinobufagin (16.9%), anodendroside A (6.65%), 16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester (5.09%). Furthermore, *T. versicolor*-infected wood and *G. trabeum*-infected wood treated with 10% neem seed extract displayed mass decreases of $11.4 \pm 5.8\%$ and $25.8 \pm 7.5\%$, respectively, compared with distilled water treatment ($21.6 \pm 4.2\%$ and $46.9 \pm 4.3\%$, respectively). These findings suggest the potential use of neem seed extract for antifungal wood protection.

DOI: 10.15376/biores.17.1.592-603

Keywords: *Neem seed*; *Extract*; *Antifungal activity*; *Chemical composition*

Contact information: a: Basic Experimental Centre for Natural Science, University of Science and Technology, 100083 Beijing, China; b: School of Engineering and Technology, Northeast Forestry University, 150040 Haerbin, China;

* Corresponding authors: lihaiwang@yahoo.com; wxyuan@ustb.edu.cn

INTRODUCTION

The quality of wood products is seriously affected by fungi. Approximately 15% of wood production is lost due to fungal decay in China (Ma *et al.* 2015). Wood rot fungi such as *Trametes versicolor* and *Gloeophyllum trabeum* can degrade lignin, cellulose, and hemicellulose, drastically reducing the quality and changing the mechanical properties of wood (Blanchette 1991; Fabbri *et al.* 1997; Machado 2013; Meyer and Brischke 2015). Therefore, protecting wood from fungal rot is of critical importance.

Chinese white poplar (*Populus tomentosa*) belongs to the section *Populus* (*Leuce*) of the *Populus* genus, and is widely distributed along the Yellow River. Due to its lower contents of fermentation-inhibiting extractives and higher biomass conversion efficiency, it has become one of the most commercially significant tree species in China (Du *et al.* 2014). However, the applications of *P. tomentosa* are limited by its poor decay resistance (Zhao *et al.* 2021). Nowadays, the most widely used wood preservatives, such as chromated copper arsenate, are harmful to the environment and human health (Hastrup *et al.* 2005). Thus, it is necessary to develop an effective, eco-friendly wood preservative.

The effective antifungal components of many natural preservatives are secondary plant metabolites (Xu *et al.* 2013). The use of natural plant extracts as wood fungicides has become a research focus, considering that plant-derived wood preservatives are non-toxic and efficient, with broad-spectrum activity. *Azadirachta indica* A. Juss., known as neem, is an evergreen medicinal plant native to India and Myanmar. Neem extract has been reported to be effective against wood infestation by termites, including *Reticulitermes speratus* Kolbe (Serit *et al.* 1992), and *Coptotermes curvignathus* Hohngren (Sajap *et al.* 2006). Neem extract is also used as an antibacterial agent against *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* (El-Mahmood *et al.* 2013). Furthermore, neem oil shows antifungal effects against *Trametes versicolor* and *Postia placenta* when applied to chir pine wood (*Pinus roxburghii* Sargent) (Dhyani and Tripathi 2006). Mango tree wood (*Mangifera indica*) and rain tree wood (*Albizia saman*) treated with neem extract showed 6 to 7 times higher decay resistance than untreated wood against *Schizophyllum commune* (Islam and Shams 2009). Despite the common use of neem extract as an antimicrobial, few studies have reported on its antifungal activities in *P. tomentosa* wood.

In this study, neem seed extract was tested for antifungal activity against two kinds of fungi that cause severe damage to wood. The morphological changes of the fungi were observed by scanning electron microscope (SEM), and the chemical composition of the extract was elucidated via Fourier transform infrared (FTIR) spectroscopy and gas chromatography-mass spectrometry (GC/MS).

EXPERIMENTAL

Materials

Neem seeds were collected in Kunming (Yunnan, China) during July of 2019. The seeds were washed and inside air-dried for one week. The wood fungi under investigation, *Trametes versicolor* and *Gloeophyllum trabeum*, were obtained from the Chinese Strain Preservation Center (Beijing, China). The fungi were preserved in potato dextrose agar (PDA) and stored at 4 °C. The experimental tree (*P. tomentosa*) grew in the Yichun area in Heilongjiang province. Sapwood samples (20 mm × 20 mm × 10 mm) were dried to constant weight at 105 °C.

Preparation of Neem Seed Extract

The neem seeds were ground to 20-mesh. A total of 20 g of neem powder was mixed with 280 mL of 60% v/v ethanol solution (Chang *et al.* 2018). The mixture was stirred in a water bath at 50 °C for 90 min. The residual ethanol was evaporated using a vacuum rotary evaporator (RE52AA; Huanyu, Zhejiang, China). The extracts were stored at 4 °C for further use.

Agar Diffusion Assay

Petri dishes containing 15 mL of PDA were used for the antifungal activity assay, conducted on solid media using the disk diffusion method (Bajpai *et al.* 2008). The PDA solution was prepared using sterile distilled water, serially diluted, and added at final concentrations of 0.5%, 1%, 5%, 10%, and 15% to 5-mm diameter holes punched in the agar. The plates were incubated at 26 °C for 5 to 7 days. The inhibition zone were measured using a vernier caliper.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MICs of the neem seed extract against *T. versicolor* and *G. trabeum* were determined using the twofold dilution method (Fernández *et al.* 2000). A small amount of mycelium was mixed with sterile distilled water in test tubes. The turbidimetric method was used to obtain a homogeneous spore suspension of 1×10^6 CFU/mL using McFarland standards (Liu *et al.* 2021). The neem seed extract was dissolved in sterile distilled water and diluted; the extract was added to potato dextrose broth (PDB) to obtain final concentrations of 4%, 2%, 1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0313%, and 0.0156%. Next, 1 mL of each fungal suspension was cultured in a test tube in PDB for 24 to 72 h at 26 °C. The blank controls were test tubes containing only PDB, while the negative controls were test tubes inoculated with the fungal spore suspension. According to the Clinical and Laboratory Standards Institute (CLSI 2012), the MIC is indicated by the test tube without observable fungal growth (precipitation or surface growth or internal turbidity).

The minimum fungicidal concentration (MFC) was determined using the drug-containing medium method. Extract solutions of different concentrations were prepared according to the concentration gradient: MIC+2%, MIC+1%, and MIC. Next, 1 mL of neem seed extract was mixed with 9 mL of melted PDA in the dish. And the 5-mm fungal cakes were cut from the dishes and inoculated on the drug-containing dish and incubated at 26 °C for 3 to 5 days. The concentration at which fungal growth was suppressed in the plate represented the MBC.

Effect of Neem Seed Extract on Hyphal Morphology

Mycelial plugs from the periphery of the fungal colonies were fixed in 2.5% glutaraldehyde (pH 7.4) and cultured at 4 °C for 12 h. The mycelia were washed with 0.1 M phosphate buffer (pH 7.4) for 5 min. The samples were dehydrated in ethanol solutions (once in 30, 50, 70, 80, 90, and 95% v/v ethanol, and three times in 100% ethanol for 20 min each) and dried at 25 °C for 24 h (Chen *et al.* 2017). The samples were sputter-coated with gold, and subsequently observed using a SEM (FEI Quanta-200; FEI Company, Hillsboro, OR, USA).

FTIR Analysis of Extract

Neem seed extract was analyzed using a Nicolet iS10 instrument (Thermo Fisher Scientific, Madison, WI, USA). A tablet was prepared by mixing 10 mg KBr and 300 mg neem seed extract, grinding the mixture to a powder, and compressing the powder under 200 bars of pressure. The FTIR spectra of the tablet were analyzed using Spectrum One software (ver. 5.0.1, Sacramento, CA, USA) (Sun *et al.* 2011).

GC/MS Analysis of Extract

The chemical composition of neem seed extract was analyzed by GC/MS (HP6890GC/5973MSD; Agilent Technologies, Santa Clara, CA, USA) fitted with a HP-5 capillary column (30 m \times 0.32 mm \times 0.2 μ m film thickness). The initial oven temperature was 190 °C, which was increased by 5 °C/min to 250 °C, held isothermal for 10 min. Helium was the carrier gas. Electron ionization mass spectra were collected at 70 eV ionization voltages from m/z 10 to 350 in full scan mode. The National Institute of Standards and Technology spectrum library and manual analysis were used for serial retrieval and the obtained mass spectrograms. The peak area normalization method was used to obtain the relative content of each component.

Wood Decay Test

Wood impregnation with neem seed extract

Samples of *P. tomentosa* were treated with neem seed extract at a relative vacuum of -0.09 MPa for 30 min, after which the vacuum was released. The blocks were removed from the treatment solution, and wiped lightly to clean the surfaces. The samples were weighed (to the nearest 0.01 g) to determine the degree of treatment solution retention in each sample. The drug loading rate was calculated using the following formula,

$$R = (m_2 - m_1)c \times 10/V(\text{kg}\cdot\text{m}^{-3}) \quad (1)$$

where m_1 , m_2 , c , and V represent the sample weight before and after treatment, the mass of preservatives solution in 100 g of the treatment solution, and the block volume, respectively. After the blocks were dried to constant weight at 45 °C, they were sterilized for 30 min, and subsequently placed in a 500-mL flask for the decay test.

Decay test

The treated wood samples were tested for decay resistance according to Chinese Standard GB/T 13942.1-2009 (2009). White-rot fungi (*T. versicolor*) and brown-rot fungi (*G. trabeum*) were cultured in 250-mL glass flasks. The wood blocks were inoculated in bottles containing active cultures of either *T. versicolor* or *G. trabeum*. Nine replicate experiments were performed for each treatment. The bottles were incubated for 12 weeks at 26 °C and 75% relative humidity. After incubation, the test samples were removed from the culture flasks and dried at 45 °C to constant weight, and then weighed to the nearest 0.01 g. The mass loss of each block was calculated using the following formula,

$$\text{Mass loss (\%)} = [(m_3 - m_4)/m_3] \times 100 \quad (2)$$

where m_3 and m_4 represent dry mass before and after the test, respectively.

RESULTS AND DISCUSSION

Antifungal Activity of Neem Seed Extract

The neem seed extract displayed moderate to high antifungal activity against both fungi. The extract at 1% concentration exhibited weak inhibitory effects on the growth of *T. versicolor* (inhibition zone: 8.31 ± 1.0 mm) and *G. trabeum* (inhibition zone: 8.44 ± 0.5 mm) (Table 1).

Table 1. Means of Inhibition Zones (mm) of Neem Seed Extract against Growth of *Trametes versicolor* and *Gloeophyllum trabeum*

Fungi	Concentration (%)					
	Control	0.5%	1%	5%	10%	15%
<i>Trametes versicolor</i>	N.D.	N.D.	8.31 ± 1.0	11.54 ± 2.0	14.31 ± 2.5	14.33 ± 0.5
<i>Gloeophyllum trabeum</i>	N.D.	N.D.	8.44 ± 0.5	13.96 ± 1.3	15.91 ± 0.9	15.78 ± 2.8

N.D. = Not Detected

The largest inhibition zones on the agar plate of *T. versicolor* and *G. trabeum* measured 14.33 ± 0.5 mm and 15.91 ± 0.9 mm, respectively. Notably, the extract at 10% concentration showed strong inhibitory effects against both types of fungi, while the inhibition zones decreased when using a higher concentration of extract against *G. trabeum*. Therefore, neem seed extract at 10% concentration was used in the follow-up wood decay test.

Determination of MIC and MBC

The MIC and MBC were used to evaluate the sensitivity of different fungi to the neem seed extract. The neem seed extract had an inhibitory effect on both fungi (Table 1), but there was no obvious inhibitory effect on either type of fungus when the concentration was under 1%. In particular, *T. versicolor* and *G. trabeum* were substantially inhibited by neem seed extract at 2% concentration and 4% concentration, respectively, with no mycelial growth observed at these concentrations (Table 2). Therefore, *G. trabeum* was more sensitive than *T. versicolor* to the neem seed extract *in vitro*.

Table 2. Effects of Neem Seed Extract at Different Concentrations against Mycelial Growth of *Trametes versicolor* and *Gloeophyllum trabeum*

Fungus	Extract Concentration (%)									
	4	2	1	0.5	0.25	0.0125	0.0625	0.0313	0.0156	0
<i>Trametes versicolor</i>	—	—	+	+	++	++	++	++	++	++
<i>Gloeophyllum trabeum</i>	—	++	++	++	++	++	++	++	++	++

*: —: No mycelial growth; +: Few mycelia (growing colonies <50%); ++: Lots of mycelia (growing colonies >50%)

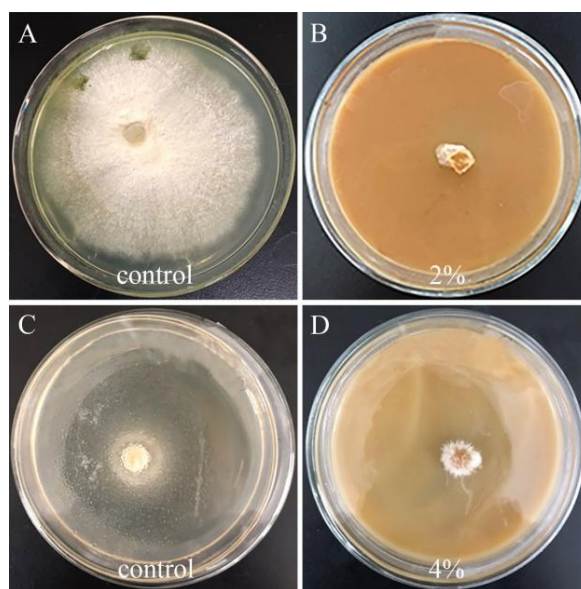


Fig. 1. Determination of minimum fungicidal concentration of neem seed extract (*Azadirachta indica*) against *Trametes versicolor* and *Gloeophyllum trabeum*. (A) control of *T. versicolor*; (B) *T. versicolor* treated with 2% extract; (C) control of *G. trabeum*; (D) *G. trabeum* treated with 4% extract)

The drug-containing medium method was used to evaluate the MBC. The neem seed extract completely inhibited the mycelial growth of *T. versicolor* and *G. trabeum* at 2% and 4% concentration, respectively, whereas the mycelia grew well in the control medium (Fig. 1). The *G. trabeum* control medium was covered with mycelia and spores. There were no mycelia and spores observed in the medium except for the PDA derived from the original medium. The mycelia of *G. trabeum* appeared long and discontinuous, and the spores stopped growing. Therefore, the MBC of neem seed extract against *T. versicolor* and *G. trabeum* were 2% and 4%, respectively.

Effects of Neem Seed Extract on Hyphal Morphology

The hyphal morphologies of *T. versicolor* and *G. trabeum* before and after treatment with neem seed extract were observed by SEM. In both cases, the appearance and morphology of the fungi changed after treatment with neem seed extract. The control mycelia of both fungi were thick, elongated, continuous, intact, and smooth. The spores of *T. versicolor* treated with extract were reduced remarkably, and the mycelia were thinner than control mycelia (Fig. 2 A, B). After exposure to the neem seed extract, the surface of *G. trabeum* mycelia appeared rough (Fig. 2 C, D). This could be explained by the damage caused to fungal cells by the extract, resulting in leakage of the cytoplasm and shrinkage of the hyphae (Soylu *et al.* 2010).

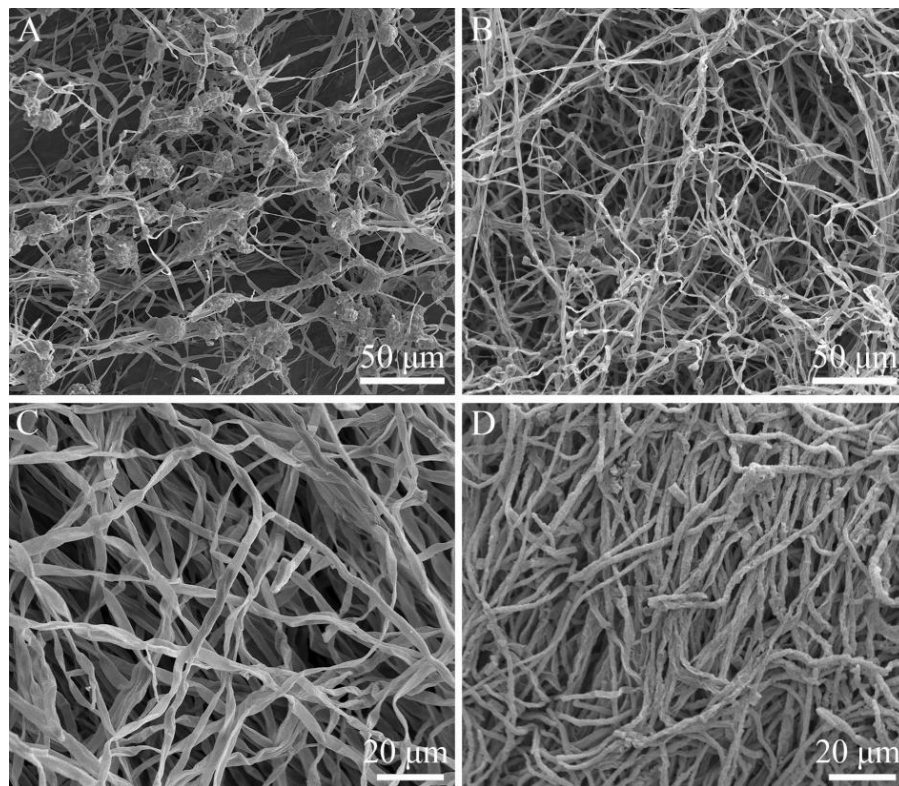


Fig. 2. Scanning electron microscope images of the hyphae of (A) *Trametes versicolor* (control); (B) *T. versicolor* treated with 2% neem seed extract; (C) control of *G. trabeum*; and (D) *G. trabeum* treated with 2% neem seed extract

Antifungal Properties of Wood Treated with Neem Seed Extract

Poplar samples that were impregnated with neem seed extract at 10% concentration and distilled water exhibited resistance to fungal attack (Table 3). The retention of neem

seed extract by the wood samples amounted to $75.12 \pm 2.1 \text{ kg}\cdot\text{m}^3$ (Table 3). The average weight losses of the poplar blocks after exposure to *T. versicolor* and *G. trabeum* were $25.8 \pm 7.5\%$ and $46.9 \pm 3.4\%$, respectively. Thus, the natural poplar wood belonged to the “moderately resistant class” (GB/T 13942.1, Chinese, 2009). After exposure of poplar wood samples to *T. versicolor* and *G. trabeum*, the average weight losses of poplar blocks treated with neem seed extract were $11.4 \pm 5.8\%$ and $21.6 \pm 4.2\%$, respectively; these values conformed to the “resistant class” (GB/T 13942.1, Chinese, 2009). The result showed that treatment with neem seed extract had inhibitory effects against both types of fungal growth in poplar wood. Furthermore, after treatment with neem seed extract, the weight loss of the *T. versicolor*-infected wood and *G. trabeum*-infected wood decreased by $14.4 \pm 1.7\%$ and $25.3\% \pm 0.8\%$, respectively, compared with untreated wood. These results indicate that the neem seed extract exerted a stronger inhibitory effect against *G. trabeum*.

Table 3. Average Percentage of Weight Loss of Treated vs. Untreated Poplar after Exposure to *Trametes versicolor* and *Gloeophyllum trabeum* for 12 Weeks

Agents	Retention (kg·m ³)	Weight loss ratio (<i>T. versicolor</i> , %)	Weight loss ratio (<i>G. trabeum</i> , %)
Neem seed extract	75.12 ± 2.1	11.4 ± 5.8	21.6 ± 4.2
Distilled water	0	25.8 ± 7.5	46.9 ± 3.4

FTIR Analysis of Neem Seed Extract

The composition of neem seed extract, including its functional groups, was investigated using FTIR spectroscopy. As shown in Table 4 and Fig. 3, neem seed extract displayed absorbance peaks at 3240, 2920, 2860, 1730, 1590, 1390, 1260, 1050, 992, and 529 cm⁻¹. The absorption bands indicate that the extract contained alkane, alkyl, alkene, alcohol, ester, carboxylic acid, aldehyde, ketone, and phenol groups. Many plant extracts contain various substances with antibacterial activity, such as terpenoids and phenolic compounds (Bouftira *et al.* 2010). These reactive groups may contain carbonyls (—C=O) and hydroxyls (—OH). However, FTIR can only provide qualitative data on the extract composition; the identification of specific substances requires other methods.

Table 4. Fourier Transform Infrared Spectroscopy Analysis of Neem Seed Extract

Functional Group	Compound Type	Frequency Range (cm ⁻¹)	Appearance	Wavenumber (cm ⁻¹)
O-H	Alcohols, phenol	3200–3500	stretch, broad	3240
C-H	Alkanes	2960–2850	strong, stretch	2920
	Alkyl	2870–1380	medium, weak	1390, 1590
	Alkenes	675–1000	-	992
C-O	Alcohols, ester, carboxylic acid	1000–1260	strong, stretch	1260
C=O	Aldehyde, ketone	1680–1750	strong	1730
XH	Alcohols, ester	1015–1300	strong	1050
*Data according to Sun (2011)				

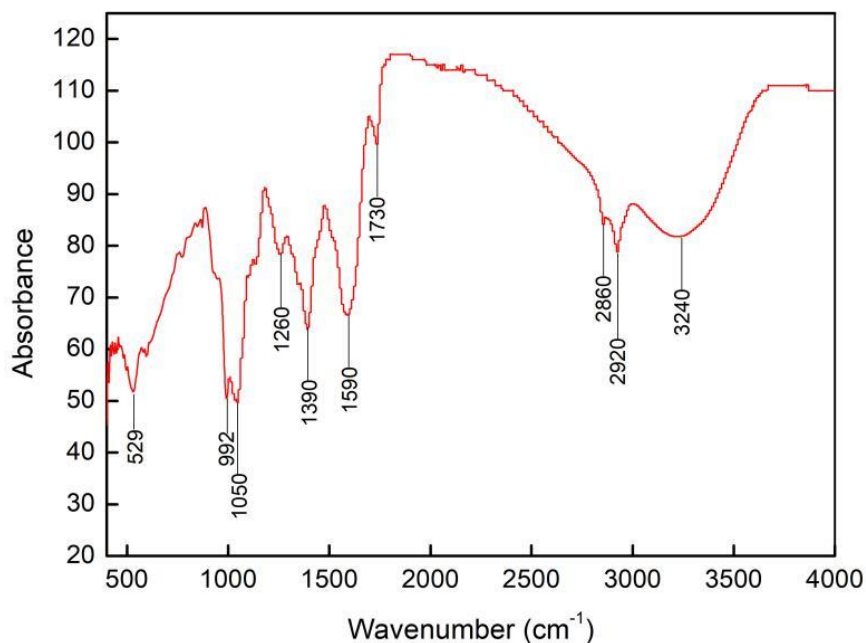


Fig. 3. Fourier transform infrared spectroscopy analysis of neem seed extract

GC/MS Analysis of Neem Seed Extract

The chemical composition of neem seed extract was determined by GC/MS (Table 5). A total of 17 compounds were isolated from the ethanolic extract, accounting for 80.31% of the total peak area, among which terpenoids, fatty acids, and sulfides were the most abundant.

Table 5. Composition of Neem Seed Extracts and the Relative Contents of 17 Components

No.	Component	Retention Time (min)	Relative Content (%)
1	Diallyl trisulfide	10.44	0.35
2	g-Hydroxy-isoeugenol	15.72	0.32
3	Hexadecanoic acid	17.35	0.22
4	Oleic acid	18.96	0.78
5	Squalene	24.58	0.09
6	Dipropyl trisulfide	28.73	0.03
7	Marinobufagin	29.86	4.77
8	Astaxanthin	31.29	34.32
9	Pittosapogenin	31.48	1.02
10	Stigmast-5-en-3-ol	32.48	0.49
11	Withaferin	33.43	1.97
12	Cinobufagin	36.67	16.88
13	9(11),12-Dien-28-oic acid, 3-(acetyloxy)-, methyl ester	38.64	1.10
14	16-Acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester	40.23	5.09
15	Khivorin	44.16	0.91
16	8,12-Di-O-acetylingol	52.72	5.32
17	Anodendroside A	54.07	6.65

The main components identified in neem seed extract were astaxanthin (34.32%), cinobufagin (16.88%), anodendroside A (6.65%), and 16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester (5.09%).

Astaxanthin has an inhibitory effect on bacteria including *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Nath and Ravi 2013). Cinobufagin and anodendroside A display strong antioxidant effects in addition to inhibiting the growth of cancer cells (Qi *et al.* 2011). Diallyl trisulfide has antiparasitic activities on pathogenic protozoa including *Entamoeba histolytica* and *Giardia lamblia* (Lun *et al.* 1994). The neem seed extract contains various antifungal components.

CONCLUSIONS

1. Neem seed extract showed inhibitory effects against both *T. versicolor* and *G. trabeum*. In a disk diffusion assay, the largest inhibition zones on the agar plate of *T. versicolor* and *G. trabeum* were 14.33 ± 0.5 mm and 15.91 ± 0.9 mm, respectively. Notably, neem seed extract at 10% concentration showed strong inhibitory effects against both fungi.
2. The two tested fungi were sensitive to neem seed extract, and their MIC and MFC values were both 2% and 4% against *T. versicolor* and *G. trabeum*, respectively.
3. The resistance of *P. tomentosa* wood treated with neem seed extract against decay caused by *T. versicolor* and *G. trabeum* was higher than that of untreated wood, such that the application of neem seed extract raised the wood samples to the “resistant class” level.
4. The extract had obvious effects on fungal morphology. For instance, the spores were decreased in number, and the mycelia became rough.
5. The absorption bands indicated that the extract contains alkane, alkyl, alkenes, alcohol, ester, carboxylic acid, aldehyde, ketone, and phenol groups. A total of 17 compounds were isolated from neem seed extract including astaxanthin (34.32%), cinobufagin (16.88%), anodendroside A (6.65%), and 16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester (5.09%).

ACKNOWLEDGMENTS

The authors are grateful for the support of the Fundamental Research Funds for the Central Universities, Grant No. FRF-TP-20-019A1.

REFERENCES CITED

- Bouftira, I., Abdelly, C., and Sfar, S. (2010). “Characterization of cosmetic cream with *Mesembryanthemum crystallinum* plant extract: Influence of formulation composition on physical stability and anti-oxidant activity,” *International Journal of Cosmetic Science* 30(6), 443-452. DOI: 10.1111/j.1468-2494.2008.00469.x

- Blanchette, R. A. (1991). "Delignification by wood-decay fungi," *Annual Review of Phytopathology* 29(1), 381-403. DOI: 10.1146/annurev.py.29.090191.002121
- Bo, W. U., Zhang, Q., and Li J. Z. (2009). "Research status and development trend of wood-plastics composites," *Materials Review*. DOI: 10.1007/s10965-008-9216-0
- Bajpai, V. K., Shukla, S., and Kang, S. C. (2008). "Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L.," *Bioresource Technology* 99(18), 8903-8908. DOI:10.1016/j.biortech.2008.04.060
- Chang, L. L., Xu, G. Q., and Wang, L. H. (2018). "Preparation and antifungal activities of microcapsules of neem extract used in *Populus tomentosa* deteriorated by three mold fungi," *BioResources* 13(4), 8373-8384. DOI: 10.15376/biores.13.4.8373-8384
- Chen, S. H., Ng, S. L., Cheow, Y. L., and Ting, A. S. Y. (2017). "A novel study based on adaptive metal tolerance behavior in fungi and SEM-EDX analysis," *Journal of Hazardous Materials* 334(15), 132-141. DOI:10.1016/j.jhazmat.2017.04.004
- GB/T (2009). "Method for laboratory test of natural decay resistance of woods, (13942.1-2009)," Chinese Standard, Beijing, China.
- CLSI (2012). "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard (9th Ed.)," Clinical and Laboratory Standards Institute, Wayne, USA.
- Du, Q., Xu, B., Gong, C., Yang, X.-H., Pan, W., Tian, J.-X., Li, B.-L., and Zhang, D.-Q. (2014). "Variation in growth, leaf, and wood property traits of Chinese white poplar (*Populus tomentosa*), a major industrial tree species in Northern China," *Canadian Journal of Forest Research* 44(4), 326-339. DOI:10.1139/cjfr-2013-0416
- Dhyani, S., and Tripathi, S. (2006). "Protection of hard and softwood through neem leaves extracts and oil: A direction towards development of eco-friendly wood preservatives," IRG/WP/06-30394. The International Research Group on Wood Protection, Stockholm.
- EI-Mahmood, A. M., Ogbonna, O. B., and Raji, M. (2013). "The antibacterial activity of *Azadirachta indica* (neem) seeds extracts against bacterial pathogens associated with eye and ear infections," *Journal of Medicinal Plants Research* 4(14). DOI:10.5897/JMPR09.169
- Fabbri, A. A., Ricelli, A., Brasini, S., and Fanelli, C. (1997). "Effect of different antifungals on the control of paper biodeterioration caused by fungi," *International Biodeterioration and Biodegradation* 57, 61-65. DOI: 10.1016/S0964-8305(97)00001-2
- Fernández, H., Mansilla, M., and González, V. (2000). "Antimicrobial susceptibility of *Campylobacter jejuni* subsp. *jejuni* assessed by E-test and double dilution agar method in Southern Chile," *Memórias Do Instituto Oswaldo Cruz*, 95(2), 247-249. DOI: 10.1590/S0074-02762000000200020
- Hastrup, A., Iii, F. G., Clausen, C. A., and Bo, J. (2005). "Tolerance of *Serpula lacrymans* to copper-based wood preservatives," *International Biodeterioration and Biodegradation* 56(3), 173-177. DOI:10.1016/j.ibiod.2005.06.008
- Islam, M. M., and Shams, M. I. (2009). "Protective antifungal effect of neem (*Azadirachta indica*) extracts on mango (*Mangifera indica*) and rain tree (*Albizia saman*) wood," *International Biodeterioration and Biodegradation* 63(2), 241-243. DOI:10.1016/j.ibiod.2008.07.010

- Liu, Y., Jin, L., Wang, C., Sheng, J., and Song, Y. (2021). "Thymol-functionalized hollow mesoporous silica spheres nanoparticles: preparation, characterization and bactericidal activity," *Bulletin of Materials Science* 44(2), 1-7. DOI: 10.1007/s12034-021-02425-2
- Lun, Z. R., Burri, C., Menzinger, M., and Kaminsky, R. (1994). "Antiparasitic activity of diallyl trisulfide (dasuansu) on human and animal pathogenic protozoa (*Trypanosoma* sp. *Entamoeba histolytica* and *Giardia lamblia*) in vitro," *Annales de la Société Belge de Médecine Tropicale* 74(1), 51-59. DOI: 10.1016/0001-706X(94)90066-3
- Ma, X. X., Jiang, M. L., and Wang, J. Y. (2015). "The climate change effects on boundaries of wood decay and termite hazard zones in China," *Scientia Silvae Sinicae* 51(11), 83-90. DOI:10.11707/j.1001-7488.20151111.
- Meyer, L., and Rischke, B. C. (2015). "Fungal decay at different moisture levels of selected European-grown wood species," *International Biodeterioration and Biodegradation* 103, 23-29. DOI: 10.1016/j.ibiod.2015.04.009
- Serit, M., Ishida, M., Nakata, K., Kim, M., and Takahashi, S. (1992). "Antifeeding potency of neem (*Azadirachta indica*) extractives and limonoids against termite (*Reticulitermes speratus*)," *Journal of Pesticide Science* 17(4), 267-273. DOI:10.1584/jpestics.17.4_267
- Sun, M., Ma, X. X., Yao, Q. X., Wang, R.-C., Ma, Y.-X., Feng, G., Shang, J.-X., Xu, L., and Yang, Y.-H. (2011). "GC-MS and TG-FTIR study of petroleum ether extract and residue from low temperature coal tar," *Energy and Fuels* 25, 1140-1145. DOI: 10.1021/ef101610z
- Machado, G. d., Cookson, L. J., Christoforo, A. L., Polito, W. L., da Silva, M. R., Calil Junior, C., and Lahr, F. A. R. (2013). "Wood preservation based on neem oil: evaluation of fungicidal and termiticidal effectiveness," *Forest Products Journal* 63(5), 202-206. DOI:10.1080/10549811.2016.1236277
- Nath, U. U., and Ravi, R. (2013). "Isolation of astaxanthin from marine yeast and study of its pharmacological activity," *International Current Pharmaceutical Journal* 2(3), 67-69. DOI: 10.3329/icpj.v2i3.13584
- Qi, F., Inagaki, Y., Gao, B., Gui, X., and Xu, H. (2011). "Bufalin and cinobufagin induce apoptosis of human hepatocellular carcinoma cells via fas- and mitochondria-mediated pathways," *Cancer Science* 102(5), 951-958. DOI: 10.1111/j.1349-7006.2011.01900.x
- Sajap, A. S., Lardizabal, M. L. T., Ahmad, F. B., and Sahri, M. H. (2006). "Feeding response of subterranean termite, *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) to *Azadirachta excelsa* (Meliaceae) extractives and its timber," *Sociobiology* 48(2), 447-455. DOI:10.1590/S0085-56262006000100020
- Soylu, E. M., Kurt, S., and Soyly, S. (2010). "In vitro and in vivo antifungal activity of essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*," *International Journal of Food Microbiology* 143(3), 183-189. DOI: 10.1016/j.ijfoodmicro.2010.08.015
- Sun, M., Ma, X. X., Yao, Q. X., and Wang, R. C. (2011). "GC-MS and TG-FTIR study of petroleum ether extract and residue from low temperature coal tar," *Energy and Fuels* 25, 1140-1145. DOI: 10.1021/ef101610z
- Xu, G. Q., Wang, L. H., Liu, J. L., and Wu, J. Z. (2013). "FTIR and XPS analysis of the changes in bamboo chemical structure decayed by white-rot and brown-rot fungi," *Applied Surface* 280, 799-805. DOI: 10.1016/j.apsusc.2013.05.065

Zhao, X., Wang, J., Wang, L., Ren, S. M., Wang, Y. M. (2021). "Preparation and properties of nano-TiO₂-Chinese herbal medicine composite wood," *BioResources* 16(2), 4252-4274. DOI:10.15376/biores.16.2.4252-4274

Article submitted: September 28, 2021; Peer review completed: October 25, 2021;
Revised version received and accepted: November 22, 2021; Published: November 30, 2021.

DOI: 10.15376/biores.17.1.592-603