

Identification and Chemical Bond Characterization of Wood Extractives in Three Species of Eucalyptus Biomass

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Eucalyptus is considered as the important woody medicinal tree besides as the timber tree. *Eucalyptus* bioresources are abundant in south China and have been used as biomedicine for about 400 years. However, only leaves and roots of *Eucalyptus* had been utilized as medicinal resources. In order to further utilize the wood as biomedicine resources and find the high efficiency separation method, the chemical bond characterization of wood extractives in *Eucalyptus* biomass was investigated by FTIR, ¹H-NMR, and ¹³C-NMR. The result showed that: There were the same groups in the three wood extractives P1, P2 and P3. However, they also contained the many different groups. FT-IR spectra showed that wood extractives P3 had C=O of unconjugated ketones, carbonyls and ester groups, and wood extractives P1 had C=O of conjugated ketones, carbonyls and ester groups. The ¹H-NMR spectroscopy revealed that the three wood extractives had different reactivity, there were high reactive H of phenols(intramolecular concluded) or OH in carboxylic acids only in the wood extractives P1, and the wood extractives P1 and P2 had high reactive H of oximes. As could be seen from ¹³C NMR spectrum, there were alkenes in wood extractives P1 and P2, carbon-oxygen double bond (C=O) of unsaturated aldehydes and ketones in wood extractives P1, and the three wood extractives contained the different alkanes and alkynes.

Key words: Eucalyptus, Woody biomedicine, FTIR, NMR, Group characterization

Eucalyptus is one of the oldest native medicinal plants in Oceania, and *eucalyptus* biomass extractives has been used in hospitals since 19th century in England. Especially with the shortage of natural herbaceous plant resources, the researches on woody medicine become more and more important. And *Eucalyptus* bioresources are very abundant in south China and have been used as biomedicine for about 400 years. The researches on *Eucalyptus* biomass for biomedicine become more and more. The eucalyptone was isolated from the leaves of *E. globulus* by spectroscopic methods (Kenji Osawa *et al.*, 1995). Kenji *et al.* (1996) found that the ethanol extractives

of *E. globulus* leaves possessed antibacterial activity against oral pathogenic microorganisms with MIC values ranging from 0.20µg/mL to 6.25µg/mL. Bong-Sik *et al.* (2000) investigated that 12 compounds isolated from the stem bark of *E. Globulus* had lipid peroxidation inhibitory activity. Cimanga *et al.* (2002) found that the leaves essential oils of *E. tertiornis* and *E. camadulensis* were most active antibacterial among 15 aromatic medicinal plant species. *Eucalyptus* oil was the insect repellent (Fradin *et al.*, 2002). Silva *et al.* (2003) demonstrated the analgesic and anti-inflammatory effects of essential oil extractives of three *Eucalyptus* species. Bachir Raho Ghalem *et al.* (2008) evaluated the excellent inhibitory effect of leaf essential oils of two *Eucalyptus* species on *S. aureus* than that of *E. coli*. Pai Peng *et al.* (2009) analyzed characterization of lipophilic extractives in four species of bamboo culms. And *Eucalyptus*

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leaves were traditionally used to heal wounds and fungal infections (Rahimi-Nasrabadi *et al.*, 2012). Essential oils and extractives of some *Eucalyptus* biomass had wide use in medicine, cosmetic, and so on (Ashour HM, 2008; Sadlon *et al.*, 2010). As the main species of *Eucalyptus* plants, *Eucalyptus urophytis*, *Eucalyptus urophylla* and *Eucalyptus urophylla* were widely planted in south China, and were also considered as the important woody medicinal tree besides as the timber tree, however, only their leaves and roots had been utilized as medicinal resources. What's worse, wood extractives might produce many side effects on wood utilization (Wanxi PENG *et al.*, 2012a; 2013a; QI Hong-chen *et al.*, 2012), and Wanxi PENG *et al.* (2011; 2012b; 2012c; 2013b; Lansheng WANG *et al.*, 2013) studied the molecular characteristics of wood extractives from some *Eucalyptus* biomass. Therefore, the chemical bond characterization of wood extractives in the three *Eucalyptus* biomass was investigated by FTIR, ¹H-NMR, and ¹³C-NMR to further utilize the wood as biomedicine resources and find the high efficiency separation method.

MATERIALS AND METHODS

Materials and Reagents

The 5-year-old *Eucalyptus urophytis* (*Eucalyptus urophylla* × *Eucalyptus tereticornis*) and *Eucalyptus urophytis* (*Eucalyptus urophylla* × *Eucalyptus camaldulensis*) were collected from Yangjiang Forest Farm, Guangdong province, P. R. China. *Eucalyptus urophylla* were collected from Zengcheng Forest Farm, Guangdong province, P. R. China. Their sample chips were processed from fresh material, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. About 40-60 mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Benzene, methanol, acetic ether and ethanol (chromatographic grade) were prepared for the subsequent experiments. Cotton bag and cotton were all extracted in benzene/ethanol solution for 12 h. The benzene-ethanol solution was mixed according to $V_{\text{ethanol}}/V_{\text{benzene}} = 2$ double.

Methods

Extractives solution preparation

Weighed 50g the above wood powders, and finally parceled by using the cotton bag and

signed, respectively. Extractions were carried out by large-caliber Soxhlet where there was 300mL benzene/ethanol solution, respectively. The extractions were done under the condition of 85°C for 8h. After extraction, the three benzene/ethanol extractives were obtained by evaporation the solvent very slowly under temperature 45°C and vacuum degree 0.07MPa, respectively. Note that P1, P2 and P3 represent the wood extractives of *Eucalyptus urophytis*, *Eucalyptus urophylla* and *Eucalyptus urophytis* biomass, respectively.

FT-IR Analysis

FT-IR spectras of wood extractives were obtained on a FT-IR spectrophotometer (Nicolet 510) using a KBr disc containing 1% finely ground samples.

¹H-NMR Analysis

The ¹H-NMR (Nuclear Magnetic Resonance) spectra were recorded on a Bruker AV III NMR spectrometer at 400.13 MHz using 5 mg of wood extractives in 0.5 ml of CDCl₃ solution. The chemical shifts were calibrated relative to the signals from CDCl₃, used as an internal standard at 7.24 ppm for the ¹H NMR spectra. The acquiring time (AQ) was 3.9 s and relaxation time was 1.0 s. Number of scanning was 128 times.

¹³C-NMR Analysis

¹³C-NMR spectra were obtained on a Bruker spectrometer at 100.6 MHz. The sample (40 mg) was dissolved in 1.0 ml of CDCl₃ solution, The chemical shifts were calibrated relative to the signals from chloroform, used as an internal standard at 77 ppm for the ¹³C-NMR spectra. The spectra were recorded at 25°C after 30000 scans. A 30 pulse flipping angle, a 9.2s pulse width, 1.89 s delay time and 1.36 s acquired time between scans were used. Number of scanning was 15000 times.

RESULTS AND DISCUSSION

Based on the above-mentioned experiments designed, the FT-IR and NMR spectra of the three wood extractives of *Eucalyptus* biomass were obtained. Analyzing the data by computer, open-published papers (Run Cangsun *et al.*, 2001; Schwanninger *et al.*, 2004; Jialong Wen, *et al.* 2010), then their chemical bond characterization were identified.

Group characteristics analyzed by FT-IR

FT-IR spectra of wood extractives P1, P2 and P3 as representations were recorded (Figure 1) in order to further analyze the heterogeneity between the wood extractives. The spectra of the three wood extractives shown some small changes in the peak intensities, indicating that these wood extractives shared the similar mixture of the extractives. A prominent broad band at 3405, 3416 or 3420 cm^{-1} was attributed to the hydroxyl group stretching vibration in alcohol, acid, or phenolic extractives. The very strong methylene and methyl stretching frequencies occurred at 2925/2923 cm^{-1} and 2852/2863 cm^{-1} , respectively. The occurrence of bands at 1595/1608, 1512, and 1329 cm^{-1} were resumed due to the presence of coextracted phenolic substances. The two sharp bands at 14160/1462/1458 cm^{-1} and 1385/1384/1383 cm^{-1} represented the methylene bending vibration and methyl symmetrical bending vibration, respectively. Band at 1271/1270 cm^{-1} was assigned to the G ring plus C=O stretch. The C-O stretching in the aliphatic esters (O-C-O-CHCH₂-) gave the absorption band at 1222/1220/1216 cm^{-1} . Absorption bands at 1124 cm^{-1} in spectrum d indicate C-O or C-C stretching. The absorption peak at 1032/1031/1040 cm^{-1} were assigned to aromatic C-H in plane deformation (G>S), C-O deformation in primary alcohols or C=O stretch (unconjugated). The peak at 877/878 cm^{-1} represented the δ band of cellulose. The absorption peak at 1736 cm^{-1} in P3 had been

assigned to C=O stretch in unconjugated ketones, carbonyls and in ester groups. However, the 1665 cm^{-1} band appeared in P1 was a C=O stretch in conjugated ketones, carbonyls and in ester groups. The absorption peak at 1425 cm^{-1} in P1 had been assigned to aromatic skeletal vibrations combined with C-H in plane deformation, or CH₂ scissoring. The peak at 920 cm^{-1} in P3 was associated with C-H₃ bending vibration of C-CH₂-CH₃. The peak at 831/836 cm^{-1} in P1 and P3 was associated with C-Cl stretching vibration and C-H in C=C-H bending vibration. The peak at 772/774 cm^{-1} in P1 and P3 was associated with C-H₂ in C-CH₂-C bending vibration. The peak at 700 cm^{-1} in P1 was attributed to C-H bending vibration of -CH=CH- or benzene ring.

Group characteristics analyzed by ¹H-NMR

To obtain further hydrogen bonds information on the three wood extractives, ¹H-NMR spectroscopy were performed. Figure 2 demonstrates the ¹H-NMR spectrum of wood extractives P1, P2 and P3. As could be seen from Figure 2, the three wood extractives had many heterogeneity. The most intense signal, occurring at approximate 1.26 ppm, was attributed to the methylene aliphatic protons, while that centered at about 0.87 ppm was attributed to methyl protons. protons on carbons adjacent to an carboxylic acid group (CH₂-COOH), adjacent to carbonyl in esters (CH₂-C=O), and adjacent to alcohols (CHOH) or ethers (CH-O-C) occurred at ~2.3, ~2.0, and 3.5-4.5

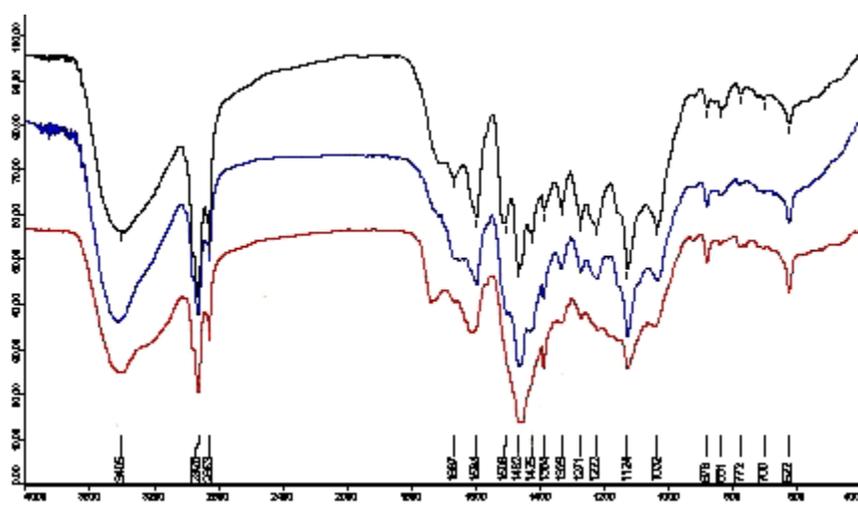


Figure 1 FT-IR spectra of wood extractives P1, P2 and P3

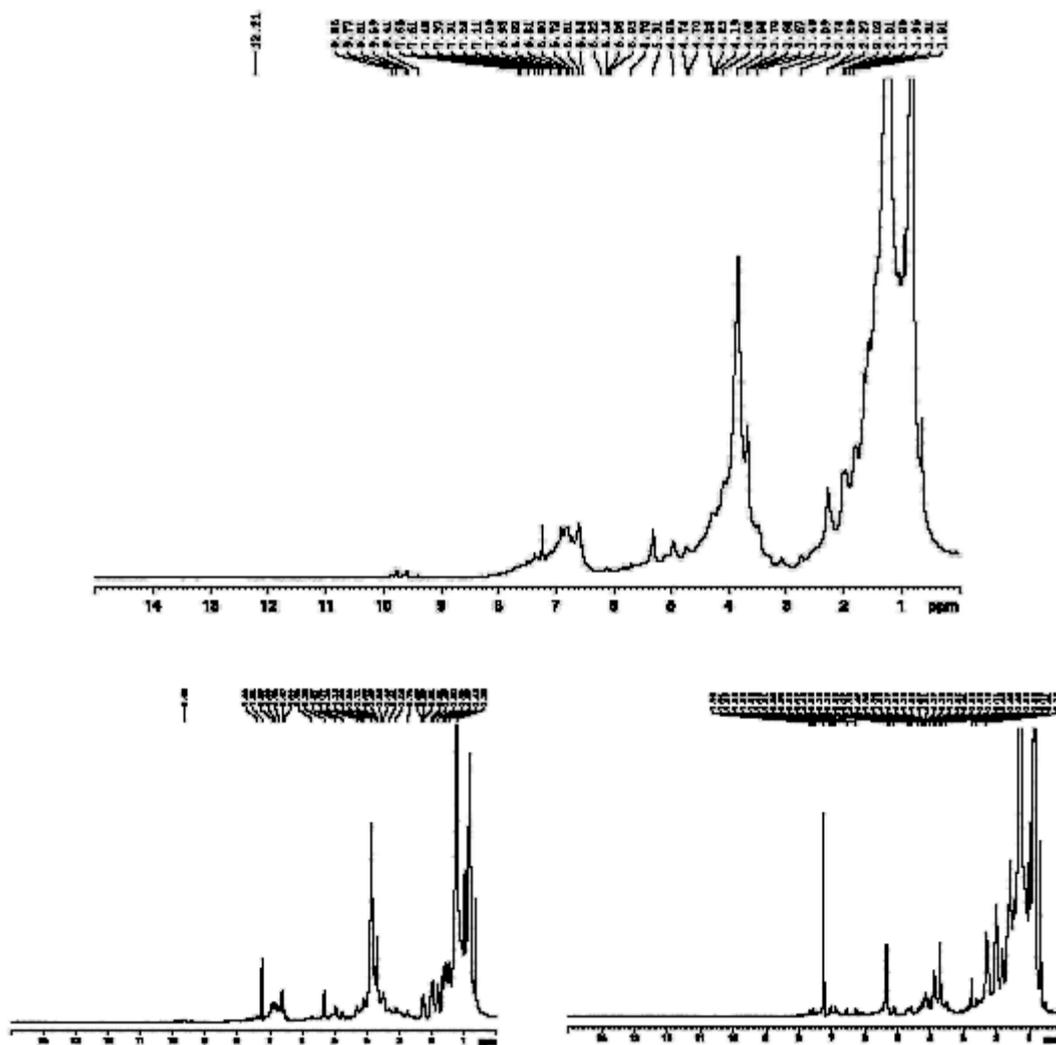


Fig. 2. $^1\text{H-NMR}$ spectra of wood extractives P1, P2 and P3

ppm, respectively. The protons on carbon adjacent to alkene exhibited the peaks at 5.1–5.5 ppm. The small signal at about 4.17–9.99 ppm represented the chemical shifts for protons on carbons adjacent to pyridines and its derivatives. The spectrum demonstrates peak at 7.24 ppm was residual chloroform present in CDCl_3 . In addition, there were different number and chemical shift of the peak for wood extractives P1, P2 and P3, resulting that the three wood extractives had different types and quantity of the groups.

The $^1\text{H-NMR}$ spectroscopy showed that the three wood extractives had high reactivity. The peak at ~5.5, 4–8, 7.4–10.2 ppm represent the chemical shifts

for active protons on carbons adjacent to alcohols (CHOH), RAr-OH, and oximes, respectively. There were high reactive H of phenols (intramolecular concluded) or OH in carboxylic acids only in the wood extractives P1. And the wood extractives P1 and P2 had high reactive H of oximes.

Group characteristics analyzed by $^{13}\text{C-NMR}$ Figure 3 showed the $^{13}\text{C NMR}$ spectrum of the wood extractives P1, P2 and P3. Among the $^{13}\text{C NMR}$ spectrum, there were many similarities. The peak at 177 ppm arisen from carbonyl (C=O) of carboxylic acids, carboxylic acid esters and carboxylic acid anhydrides. The peak at 110–160 ppm represent the carbon atoms from unsaturated compounds

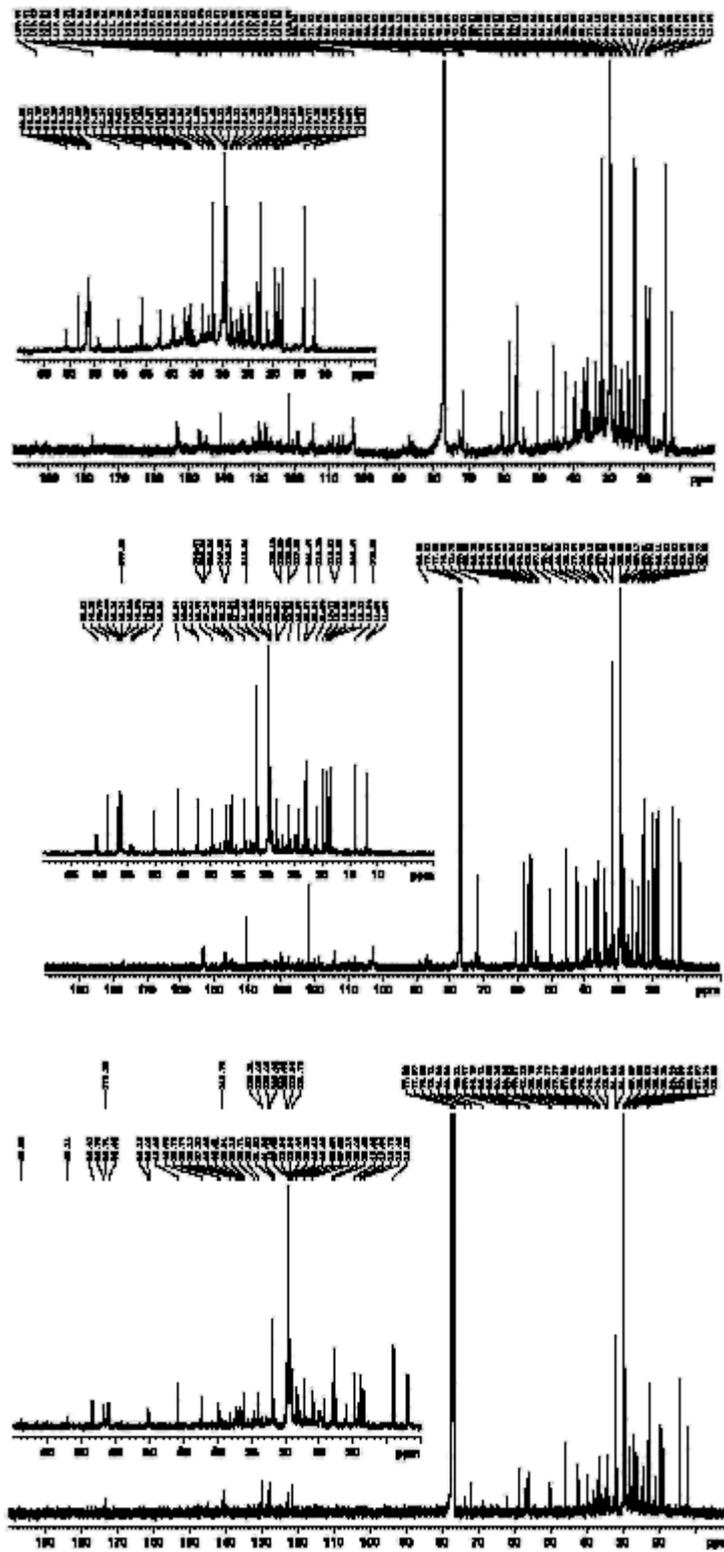


Fig. 3. ¹³C-NMR spectra of wood extracts P1, P2 and P3

such as olefin or coextracted phenolic substances. The peak at 140.7-147.5ppm, 130.0-134.5ppm, 120.3-129.7ppm were unsaturated carbon doublebonds ($-\text{CH}=\text{CH}-$) in heteroaryl ring and olefin, fatty acids or fatty acid esters. The characteristic signals observed in the spectra were the strong resonances (144, 130, 126 ppm), expressed C- α , C-2/C-6, and C-1 of coextracted phenolic substances, respectively. However, only in wood extractives P1 and P2, the peak at 114.2-118.9 ppm were C-3/C-5 and C- β of coextracted phenolic substances, and there were the strong peaks at 152.9-155.5ppm. The peak at 103.0-109.1ppm were substituted alkenes in wood extractives P1 and P2.. All signals at 0.0 to 50.0 ppm could be attributed to carbon atoms in a single bond of alkanes. And all signals at 50.0 to 87.0 ppm were assigned to $\text{C}=\text{C}$ in a single bond of alkynes. There were some difference in the ^{13}C NMR spectrum of the wood extractives P1, P2 and P3. The peak at 193.5ppm was carbon-oxygen double bond ($\text{C}=\text{O}$) of unsaturated aldehydes and ketones in wood extractives P1, implying that there were no unsaturated aldehydes and ketones in wood extractives P2 and P3, or that the unsaturated aldehydes and ketones of wood extractives P2 and P3 had been oxidized. The peaks were relatively close, however the chemical shifts of the peak at 0-87ppm were different, meaning that the wood extractives P1, P2 and P3 contained the different alkanes and alkynes.

CONCLUSION

There were the same groups in the three wood extractives P1, P2 and P3. However, they also contained the many different groups. FT-IR spectra showed that wood extractives P3 had $\text{C}=\text{O}$ of unconjugated ketones, carbonyls and ester groups, wood extractives P1 had $\text{C}=\text{O}$ of conjugated ketones, carbonyls and ester groups. And there were C-Cl stretching vibration and C-H in C=C-H bending vibration, C-H₂ in C-CH₂-C bending vibration in wood extractives P1 and P3. The ^1H -NMR spectroscopy showed that the three wood extractives had high reactivity. There were high reactive H of phenols (intramolecular concluded) or OH in carboxylic acids only in the wood extractives P1. And the wood extractives P1 and P2 had high reactive H of oximes. Based on the ^{13}C NMR spectrum there were alkenes in wood extractives P1 and P2. wood extractives P1 had carbon-oxygen double bond ($\text{C}=\text{O}$) of unsaturated

aldehydes and ketones. The chemical shifts of the peak at 0-87ppm were different, meaning that the wood extractives P1, P2 and P3 contained the different alkanes and alkynes.

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REFERENCES

1. Ashour HM. Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. *Cancer Biol Ther.* , 2008; **7**(3): 399-403
2. Bachir Raho G, Benali M., Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and Pharmacology*, 2008; **2**(10):211-215
3. Bong-Sik Y, In-Kyoung L, Jong-Pyung K, Sung-Hyun Ch, Gyu-Seop Sh and Ick-Dong Y., Lipid peroxidation inhibitory activity of some constituents isolated from the stem bark of *Eucalyptus globulus*. *Archives of Pharmacal Research*, 2000; **23**(2): 147-150
4. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ., Main Navigation Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic. *Journal of Ethnopharmacology*, 2002; **79**(2): 213-220
5. Kenji O, Hideyuki Y, Hiroshi M, Koichi T, Hideji I., Eucalyptone from *Eucalyptus globulus*. *Phytochemistry*, 1995; **40**(1):183-184
6. Kenji O, Hideyuki Y, Hiroshi M, Koichi T, Hideji I. Macrocarpals H, I, and J from the Leaves of *Eucalyptus globulus*. *J. Nat. Prod.*, 1996; **59**(9):823-827
7. Fradin MS, Day JF., Comparative efficacy of insect repellents against mosquito bites. *New England J. Med.* 2002; **347**: 13-18
8. Jia-Long Wen, Zhaojun Sun, Yong-Chang Sun,

- Shao-Ni Sun, Feng Xu, and Run-Cang Sun., Structural Characterization of Alkali-Extractable Lignin Fractions from Bamboo. *Journal of Biobased Materials and Bioenergy*, 2010; **4**: 1-18
9. Lansheng WANG, Wanxi PENG, Zhi LIN, Yueping TAN, Liwen SHI., Analysis on Molecular Characteristics of Wood Extractives from Eucalyptus urophydis Biomass. *Przegł'd Elektrotechniczny*, in pressed
 10. Pai Peng, Fang-Xia Yang, Feng Xu, Jian-Min Fang, Rahimi-Nasrabadi M, Ahmadi F and Batooli H., Essential oil composition of *Eucalyptus procera* Dehnh. leaves from central Iran[J]. *Natural Product Research*, 2012; **26**(7):637-642
 11. Run-Cang Sun, and Gwynn Lloyd Jones., Identification and Characterization of Lipophilic Extractives in Four Species of Bamboo Culms. *Journal of Biobased Materials and Bioenergy*, 2009; **3**: 1-9
 12. Run Cang Sun, X.F. Sun., Identification and quantitation of lipophilic extractives from wheat straw. *Industrial Crops and Products*, 2001; **14**: 51-64
 13. QI Hong-chen, PENG Wan-xi, WU Yi-qiang, WU Shu-bin, XU Gan-jun., Effects of Alkaline Extraction on Micro /Nano Particles of Eucalyptus camaldulensis Biology. *Journal of Computational and Theoretical Nanoscience*, 2012; **9**: 1-4
 14. Sadlon AE, Lamson DW., Immune-modifying and antimicrobial effects of *Eucalyptus* oil and simple inhalation devices. *Altern Med Rev.*, 2010; **15**(1): 33-47
 15. Schwanninger M., Rodrigues J.C., Pereira H., Hinterstoisser B., Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vibrational Spectroscopy*, 2004; **36**: 23-40
 16. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ., Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. *J Ethnopharmacol*, 2003; **89**: 277-283.
 17. Wanxi Peng, Lansheng Wang, Fengjuan Wu, Qiu Xu, 3-(4-Bromophenyl)-4-(4-hydroxyanilino) furan-2(5H)-one. *Acta Crystallographica Section E-Structure Reports Online*, 2011; **67**(9): O2329-U206
 18. Wanxi PENG, Lansheng WANG, Qiu XU, Qingding WU, Shilong XIANG., TD-GC-MS Analysis on Thermal Release Behavior of Poplar Composite Biomaterial under High Temperature. *Journal of Computational and Theoretical Nanoscience*, 2012a; **9**: 1-3
 19. Wanxi Peng, Fengjuan Wu, Lansheng Wang and Qiu Xu., Crystal structure of 3-(4-bromophenyl)-4-(4-chlorophenylamino) furan-2(5H)-one, C₁₆H₁₁BrClNO₂. *Z. Kristallogr. NCS*, 2012b; **227**: 61-62
 20. Wan-Xi Peng, Cui Le., Crystal structure of 3-(3-bromophenyl)-4-(3,5-dichlorophenylamino) furan-2(5H)-one, C₁₆H₁₀BrCl₂NO₂. *Z. Kristallogr. NCS*, 2012c; **227**: 267-268
 21. Wanxi PENG, Hongchen QI., Study on Digestion Kinetics Model of Wood extractives from Eucalyptus camaldulensis Biomass. *Przegł'd Elektrotechniczny*, in pressed, 2013a.
 22. Wanxi PENG, Lansheng WANG, Liwen SHI, Yueping TAN., Effect of Lipophilic Extractives on Properties of Eucalyptus camaldulensis Wood. *Journal of Food, Agriculture & Environment*, 11(2), in pressed 2013b.