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# MOLECULAR CHARACTERISTICS OF PHARMACOLOGY WOODY EXTRACTS OF *EUCALYPTUS CAMALDULENSIS* BIOMASS

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## ABSTRACT

As one of dominant tree species of *Eucalyptus* plants, *Eucalyptus camaldulensis* is deemed as the renewable bioresources, however, the process properties of *E. camaldulensis* wood are further influenced by biomass extracts. Therefore, the molecular and leaching characteristics of *E. camaldulensis* woody extracts was studied and discussed to reuse the resources. The result shown that: (1) Among methanol, acetic ether and benzene/alcohol extraction, the optimal extraction time to reach the largest Lr of woody extracts was 12, 6 and 12 h, respectively; during the different sequential extractions, benzene/alcohol-methanol-acetic ether extraction were the best extracting method which Lr was up to 15.94 %. (2) The wood methanol extracts contained 53 components including γ-sitosterol (31.16), palmitic acid (14.53); the acetic ether extracts contained 78 components including γ-sitosterol (16.05), ethyl propionate (9.12); the benzene/alcohol extracts contained 54 components including cyclohexylbenzene (35.55), 3-methylcyclopentanebenzene (4.4 %). The methanol extracts and acetic ether extracts had a main retention time of 20-30 min, and the benzene/alcohol extracts were 10-20 min. Thus the woody extracts of *E. camaldulensis* had huge potential in biomedical resources.

KEYWORDS: Pharmacology woody extracts, *Eucalyptus camaldulensis* biomass, GC-MS.

## INTRODUCTION

*Eucalyptus* was one of the oldest native woody medicinal plants in Australia, and eucalyptus oil had been applied in hospitals to clean urinary catheters since 19th century in England. Especially, with the development of modern biomedicine and natural healthful products industry, *Eucalyptus* biomedicine was explored more and more. 12 compounds with lipid peroxidation

inhibitory activity were isolated from the stem bark of *E. globulus* (Yun et al. 2000). The most active antibacterial essential oils were those of the leaves of *E. terticornis* and E. camadulensis from 15 aromatic medicinal plant species (Cimanga et al. 2002). 1.8-cineol (eucalyptol) played an important role in the pathogenesis of inflammatory diseases (Juergens et al. 2003). E. coli. Essential oils and extracts of some *Eucalyptus* species had wide use in pharmaceutical, cosmetic and food preparations (Ashour 2008; Rahimi-Nasrabadi et al. 2012). In a word, *Eucalyptus* leaves had been used to heal wounds and fungal infections, suggesting wide applications in cosmetic, pharmaceutical, and food preparations.

Many compounds isolated from woody plants were of great potential as medicines for disease control or as stock for synthesizing useful analogues in industry (Chang et al. 2000). *Eucalyptus* was medicinally used in many purposes. As one of vital cultivars of *Eucalyptus* plants, the *Eucalyptus camaldulensis* biomass was suitable for pulping and wood industry. *E. camaldulensis* was also deemed as the important woody medicinal tree and bioresources. But only leaves and roots of *E. camaldulensis* had been utilized as medicinal resources. Moreover, woody extracts consisted of a large variety of low molecular mass compounds which were small quantities of wood (Bergelina and Holmboma 2008; Peng et al. 2013). And woody extracts could have the many side effects on wood pulping and processing (Bergelina and Holmboma 2008; Hongchen et al. 2012). To further utilize *E. camaldulensis* wood as biomedical resources, the molecular and leaching characteristics of woody extracts were detected and analyzed by optimized extracting techniques.

## MATERIAL AND METHODS

### Materials

One 18-year-old *E. camaldulensis*, which was planted in the Forest Farm of Central South University of Forestry and Technology, China, was collected. The fresh wood was crashed into chips, and dried to absolute dry with rotary evaporator in 55˚C and -0.01 MPa. About 40 mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Acetic ether, methanol, benzene and ethanol were all chromatographic grade as the subsequent experiments. Cotton, cotton bag and quantitative filter paper were treated by benzene/ethanol extraction for 12 h. and $V_{ethanol}/V_{benzene} = 2$.

### Experiment methods

*Single extraction*

Weighed 24 pieces of wood powders, each was about 2 g (0.1 mg accuracy) and finally parceled by using the quantitative filter paper and tied by using cotton thread, and signed. Extraction was carried out in 150 ml acetone by the Foss method. Samples were removed at different times (3, 6, 9, 12 h), Solvents were methanol, acetic ether and benzene/ethanol solution ($V_{ethanol}/V_{benzene}=2$), respectively. Methanol extraction, acetic ether extraction and benzene/ethanol extraction were done under the condition of 75, 85 and 95˚C, respectively. Parallel sample number was 2. After extraction, samples were baked to be absolute dried and weighed. Finally, the leaching rate of wood extractives was calculated (Peng et al. 2013; Li et al. 2014).

### Sequential extraction

Based on Tab. 2, Weighed 18 pieces of wood powders, each was 10 g (1.0 mg accuracy), and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Extraction was carried out by large-caliber Soxhlet according to different orders combined by methanol,

Benzene/alcohol and acetic ether. Parallel sample number was 2. After extraction, samples were baked to be absolute dried under 105˚C and weighed. Finally, the leaching rate of extractives was calculated (Peng et al. 2013; Li et al. 2014).

## GC/MS condition

Each 1.0 mg wood extractives of *E. camaldulensis* was analyzed by online linked GC/MS (gas chromatograph/ mass spectrometer), respectively. The GC/MS analysis was carried out on a GC/MS-QP2010 (Shimadzu Corp., Japan), which was linked to a mass selective detector. An elastic fused silica capillary column DB-5 (30 m×0.25 mm) was used. The carrier gas was helium and the injection port temperature was 250˚C (Peng et al. 2013; Li et al. 2014). For benzene-alcohol extracts, the split injection ratio was 3:1, the GC column temperature was programmed as follows: 20˚C.min$^{-1}$ from 50 to 90˚C, 2˚C.min$^{-1}$ from 90 to 200˚C, 12˚C.min$^{-1}$ from 200 to 300˚C. For methanol extracts and acetic ether extracts, the split injection ratio was 5:1, the GC column temperature was programmed as follows: 8˚C.min$^{-1}$ from 50 to 200˚C, 5˚C.min$^{-1}$ from 200 to 300˚C.

# RESULTS

The leaching rate (Lr) trend of *E. camaldulensis* woody extracts in different solvents was described in Tab. 1. Basing on the respective optimal extraction time, different sequential extractions on *E. camaldulensis* wood were carried out by combining methanol, benzene /alcohol and acetic ether, which showed different Lr (Tab. 2). During benzene/alcohol-methanol-acetic ether sequential extraction, three solvent extracts (methanol, acetic ether, benzene/alcohol) were obtained respectively. The total ion chromatograms of three solvent extracts by GC/MS were shown in Fig. 1, respectively. Relative content of each component was counted by area normalization. Analyzing the MS data, the NIST standard MS map by computer, open-published books and papers, then components and their contents were identified (Peng et al. 2013; Li et al. 2014).

*Tab. 1: Lrs of each single extraction (%).*

| Extraction time (h) | Methanol extraction | Acetic ether extraction | Benzene-alcohol extraction |
|---|---|---|---|
| 3 | 5.77 | 6.49 | 7.96 |
| 6 | 8.42 | 7.22 | 7.77 |
| 9 | 8.72 | 6.94 | 8.05 |
| 12 | 8.81 | 6.96 | 8.12 |

*Tab. 2: Lrs of sequential extractions (%).*

| Solvent of sequential extraction | Stage of extraction | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Methanol- Acetic ether- Benzene/alcohol | 3.19 | 4.33 | 4.89 |
| Benzene/alcohol- Methanol- Acetic ether | 3.20 | 6.29 | 6.45 |
| Acetic ether- Benzene/alcohol - Methanol | 2.75 | 4.38 | 5.78 |

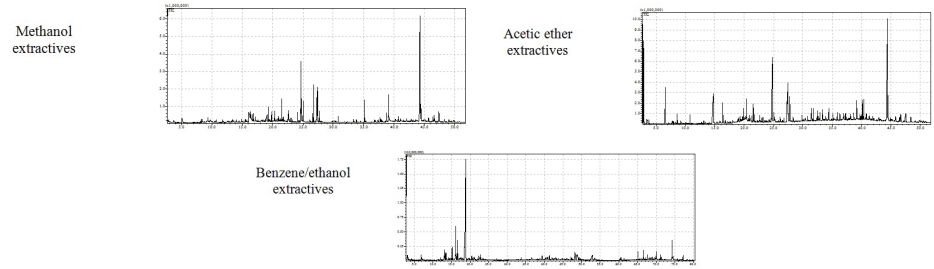


*Fig. 1: Total ion chromatogram of woody extracts from E. camaldulensis by GC/MS-.*

## DISCUSSION

### Single extraction of *E. camaldulensis* woody extracts

It was observed that during methanol extraction, the Lr of woody extracts increased, and reached the maximum (8.81 %) when extraction time was 12 h. During acetic ether extraction, the Lr of woody extracts first increased and then decreased, and reached the maximum (7.22 %) when extraction time was 6 h. During benzene/alcohol extraction, the Lr of woody extracts first decreased, and then increased, and reached the maximum (8.12 %) when extraction time was 12 h. The result was not fully in accordance with the theoretical time-adding increase of Lr during Soxhlet extraction. The reason was that during solvent extractions, extracts could leach out from wood to increase Lr, but solvent could react with wood to decrease Lr. Especially, alcohol and acetic ether could react with –COOH and –OH of wood to form ether bond or ester bond, which would decrease the Lr. And the optimal extraction time of methanol extraction, acetic ether extraction, and benzene/alcohol extraction were 12, 6, and 12 h, respectively.

### Sequential extraction of *E. camaldulensis* woody extracts

The statistical results showed that the Lr of *E. camaldulensis* woody extracts by methanol-acetic ether-benzene/alcohol sequential extraction was 12.41, 15.94 by benzene/alcohol-methanol-acetic ether, and 12.91 % by acetic ether-benzene/alcohol-methanol. Tab. 2 also showed that both the three sequential extractions displayed gradually increased Lr of woody extracts, which were larger than that of any single extraction. During the sequential extraction, benzene/alcohol-methanol-acetic ether extraction was the optimum extraction mode for the Lr was 15.94 %.

### Molecular characteristics of *E. camaldulensis* woody extracts

According to GC/MS result, 53 components were identified from woody methanol extracts of *E. camaldulensis*. The result showed that the main components were γ-sitosterol (31.16), palmitic acid (14.53), trans-9-octadecenoic acid (5.79), 9,12-trans octadecadienoic methyl (ester) (5.18), phenol (4.79), (z)-9-octadecenoic acid methylester (4.56), 3,5-dime-thoxy-4-hydroxycinnamic acid (3.38), vanillic acid (2.1), 1-(-3-methoxy-4-hydroxyphenyl)-1-hydroxy-2-propanone (1.98), 4-hydroxy-3,5-dimethoxy acetylbenzene (1.95), 4-hydroxy-3,5-dimethoxybenzaldehyde (1.83), di-n-butylphthalate (1.71), syringic acid (1.61), stearic acid (1.6), methyl linoleate (1.26), sitosterone (1.23), 8.beta.h-cedran-8-ol (1.18), methyl hexadecanoate (1.18), oleamide (1.06), α-cedrene (0.84 %), and so on. 78 components were identified from woody acetic ether extracts of

*E. camaldulensis* according to GC/MS result. The result showed that the main components were γ-sitosterol (16.05), ethyl propionate (9.12), palmitic acid (8.76), isosorbide (6.05), 9,12-methyl linoleate (3.68), trans-9-octadecenoic acid (3.52), 4-hydroxy-3,5-dimethoxy benzaldehyde (1.82), 2-butoxyethanol (1.73), stearic acid (1.58), vanillin (1.08), diisooctyl phthalate (0.72), 2,4-bis (1-phenylethyl) phenol (0.68), friedelin (0.56), cedrol (0.55 %), and so on.

54 components were identified from woody benzene/ethanol extracts of *E. camaldulensis* according to GC/MS result. The result showed that the main components were cyclohexylbenzene (35.55), 3-methylcyclopentanebenzene (4.4), γ-sitosterol (3.24), acetal (1.26), 1-methylheptyl-benzene (0.89), 4-(3-hydroxy-1-propenyl)-2-methoxyphenol (0.67), diisobutyl phthalate (0.55), 1-methylphenylpentane (0.54), diisooctyl phthalate (0.54), phenol (0.53), 1-ethyl butylbenzene (0.48), bis (2-ethylhexyl) adipate (0.45), 1,2,3-trimethyl-1 h-indene (0.44), 4-hydroxy-3,5-dimethoxybenzaldehyde? (0.41), trans-9-octadecenoic acid (0.4), cedarwood oil (0.39 %), and so on.

## Distribution properties of E. camaldulensis woody extracts

The richest components of methanol extracts were γ-sitosterol (31.16), palmitic acid (14.53), trans-9-octadecenoic acid (5.79), etc; and there were 23 kinds of water-soluble compounds (52.91 of the total peak area), 12 kinds of acids (28.18), 9 kinds of resin compounds (15.03), 5 kinds of hydrocarbons (2.18), 4 other substances (1.7 %).

The richest components of acetic ether extracts were γ-sitosterol (16.05), ethyl propionate (9.12), palmitic acid (8.76), etc; and there were 24 kinds of water-soluble compounds (31.10), 12 kinds of acids (16.11), 11 kinds of resin compounds (14.95), 27 kinds of hydrocarbons (5.32), 4 other substances (0.36 %).

The richest components of benzene/ethanol extracts were cyclohexylbenzene (35.55), 3-methylcyclopentanebenzene (4.4), γ-sitosterol (3.24), etc; and there were 28 kinds of water-soluble compounds (49.29), 4 kinds of acids (0.71), 5 kinds of resin compounds (1.91), 14 kinds of hydrocarbons (1.23), 4 other substances (0.86 %).

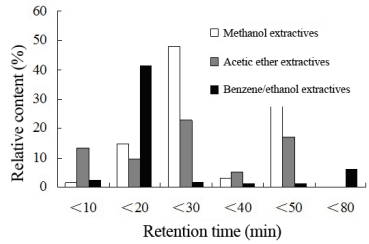


*Fig. 2: Distribution characteristic of E. camaldulensis woody extracts.*

The retention time of each solvent extracts of *E. camaldulensis* wood showed a particular rule. The methanol extracts of *E. camaldulensis* wood had a main retention time between 20-30 min, which accounts for 48.06 % of the total relative content; and the methanol extracts between 40-50 min also accounts for 32.94 % of the total relative content. The acetic ether extracts had a main retention time between 20-30 min, the relative content was 22.72 %. The benzene/alcohol extracts had a main retention time between 10-20 min, which accounts for 41.51 % of the total relative content, but the other retention time displayed a wide distribution of components and their relative contents were very few (Fig. 2).

### Properties of *E. camaldulensis* woody extracts

There were many biomedicine components in the woody extracts of *E. camaldulensis* biomass. Because of its officinal value, cedrol was a kind of sesquiterpenoids with mildly fragrant smell (better) and would bring calmness effect (Shuo 2004). Vitamin E, which was benefit to human body, had amazing cosmetic results and auxiliary therapy effect on vitiligo, were widely used in food, medicine, cosmetics and feed industry (Xiaoyan and Tiankui 2000). Stigmast-4-en-3-one was a natural medicine which had hypoglycemic effect (Fathaiya et al. 1995). Cedarwood oil could be used extensively to cure various health problems like kidney disorders, arthritis, skin and respiratory problems, and best suited for cosmetics and perfumes. More important, squalene, which could protect liver, resist fatigue and strengthen the body's resistance, and improve human immunity, was considered as important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries (Kim and Karadeniz 2012). Especially, γ-sitosterol, the rich ingredient of the three extracts from *E. camaldulensis* wood, could reduce serum cholesterol and had effect on atherosclerotic lesion development (Balamurugan et al. 2011). According to the relative content of biomedicine components, the methanol and acetic ester extracts of *E. camaldulensis* wood was suitable for the extraction of γ-sitosterol.

What's more, the others from woody extracts of *E. camaldulensis* biomass also contained many valuable components. For example, 2-butoxyethanol was mainly used for fiber, painting, drying paint, varnish, enamel and paint stripping solvents, also used for fiber wetting agent, pesticide scattered, and resin plasticizers synthetic and organic intermediates (Miyazawa et al. 2003). Cyclohexylbenzene was a fine chemical raw materials and pharmaceutical intermediates. In addition, the most components of the three extracts were used to grow bioenergy (Lansheng et al. 2013). Generally, the extracts of *E. camaldulensis* biomass were not only fine bioenergy but also high value-added chemical raw materials.

## CONCLUSIONS

During single extraction, among three extracting solvents including methanol, acetic ether and benzene/alcohol, the optimal extracting time to reach the largest Lr of *E. camaldulensis* woody extracts was 12, 6 and 12 h, respectively. During the sequential extraction, each sequential extraction displayed gradually increased Lr, whose total leaching volume was larger than that of single extraction; benzene/alcohol-methanol-acetic ether displayed the best extracting effect whose Lr was up to 15.94 %.

The woody methanol extracts of *E. camaldulensis* contained 53 components, especially including γ-sitosterol (31.16), palmitic acid (14.53), trans-9-octadecenoic acid (5.79). The woody acetic ether extracts contained 78 components, especially including γ-sitosterol (16.05), ethyl propionate (9.12), palmitic acid (8.76). The woody benzene/alcohol extracts contained 54 components, especially including cyclohexylbenzene (35.55), 3-methylcyclopentanebenzene (4.4), γ-sitosterol (3.24 %). The both of methanol extracts and acetic ether extracts had a main retention time between 20-30 min. The benzene/alcohol extracts had a main retention time between 10-20 min.

The properties analytical result suggested that *E. camaldulensis* woody extracts contained rich components which had huge potential in biological medicine industry, especially including γ-sitosterol, sitosterone, cedarwood oil and squalene which were so rich as to potentially purify the single effective biomedical ingredients.

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## REFERENCES

1. Ashour, H.M., 2008: Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. Cancer Biol. & Ther. 7(3): 399-403.
2. Balamurugan, R., Duraipandiyan, V., Ignacimuthu, S., 2011: Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. Eur. J. Pharm. 667(1-3): 410-418.
3. Bergelina, E., Holmboma, B., 2008: Reactions and distribution of birch extractives in kraft pulp oxygen delignification. J. Wood Chem. Technol. 28: 261-269.
4. Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté J., Pieters L., Vlietinck, A.J., 2002: Main navigation correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic. J. Ethnopharm. 79(2): 213-220.
5. Chang, S.T., Wang, S.Y., Wu, C.L., Shiah, S.G., Kuo, Y.H., Chang, C.J., 2000: Cytotoxicity of extractives from taiwania cryptomerioides heartwood. Phytochemistry 55(3): 227-232.
6. Fathaiya, J., Suhaila, M., Nordin, L., 1995: .Hypoglycaemic effect of stigmast-4-en-3-one, from *Parkia speciosa* empty pods. Food Chem. 54(1): 9-13.
7. Hongchen, Q., Wanxi, P., Yiqiang, W., Wu, S.B., Xu, G.J., 2012 : Effects of alkaline extraction on micro /nano particles of *Eucalyptus camaldulensis*. Biology. J. Comput. Theor. Nanosci. 9(9): 1525-1528.
8. Juergens, U.R., Dethlefsen, U., Steinkamp, G., Gillissen, A., Repges, R., Vetter, H., 2003: Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: A double-blind placebo-controlled trial. Respirat. Med. 97(3): 250-256.
9. Kim, S.K., Karadeniz, F., 2012: Biological importance and applications of squalene and squalane. Adv. Food & Nut. Res. 65: 223-233.
10. Lansheng, W., Wanxi, P., Zhi, L., Minglong, Z., 2013: Molecule characteristics of eucalyptus hemicelluloses for medical microbiology. J. Pure Appl. Microbio. 7(2): 1345-1349.
11. Li, D.L., Peng, W.X., Ge, S.B., Mo, B., Zhang, Z.F., Qin, D.C., 2014: Analysis on active molecules in *Populus nigra* wood extractives by GC-MS. Pak. J. Pharm. Sci. 27(6): 2061-2065.
12. Miyazawa, M., Itsuzaki, Y., Ishikawa, K., Ishisaka, K., 2003: Stereospecific hydroxylation of (+)- cedrol by using human skin microbial flora *Staphylococcus epidermidis*. Nat. Prod. Res. 17(5): 313-317.
13. Peng, W.X., Wang, L.S., Lin, Z., Minglong, Zhang, M., 2013: Identification and chemical bond characterization of wood extractives in three species of *Eucalyptus* biomass. J. Pure Appl. Microbio 7(2): 67-73.

14. Rahimi-Nasrabadi, M., Ahmadi, F., Batooli, H., 2012: Essential oil composition of *Eucalyptus procera* Dehnh. leaves from central Iran. Nat. Prod. Res. 26(7): 637-64.
15. Shuo, X.E., 2004: Valuation of sedative effect and mechanism of inhaled cedrol with the method of the behavioral pharmacology. For. Med.: Herbal 19(6): 256.
16. Xiaoyan, S., Tiankui, Y., 2000: Function and application of the natural vitamin E. China Oils & Fats 25(6): 45-47.
17. Yun, B.S., Lee, I.K., Kim, J.P., Chung, S.H,, Shim, G.S., Yoo, I.D., 2000: Lipid peroxidation inhibitory activity of some constituents isolated from the stem bark of *Eucalyptus globulus*. Arch. Pharm. Res. 23(2): 147-150.

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