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Development of Mokusaku Oil Obtained by Pyrolysis of Wood

Toshihiro OKABE[1], Yoshihiko INAMORI[2], Yasuhiro MORITA[3], Kyoharu FUKUDA[4], Tadashi ARII[5], Eiko ANZAI[6], Touru FUKUI[6]

[1]Aomori Industrial Research Center, 4-11-6 Daini-tonyamachi, Aomori 030-01113, Japan
Fax: 017-739-9613, E-mail: okabe@aomori-tech.go.jp

[2]Osaka University of Pharmaceutical Science, 4-20-1 Nasahara, Takatsuki-shi, Osaka 569-1094, Japan, (Adviser of Koyo Fine Chemical Corp.)

[3]Osaka Organic Chemical Industry Ltd., 18-8 Katayama-cho, Kashiwara-shi, Osaka 569-0020, Japan

[4]Tokyo University of Agriculture and Technology, 3-5-8, Saiwai Chou Fuchu-shi, Tokyo 183-8509, Japan

[5]Rigaku Corporation, 3-9-12 Matsubara,Akishima, Tokyo 196-8666, Japan.

[6]Byotaiseir Laboratory, 47-11 Kumanomachi, itabashiku, Tokyo 173-0025, Japan

In case of heating of wood under inactive gas atmosphere, the pyrolysis is completely finished at 450-500°C. Wood-vinegar liquors are dark brown liquid obtained by the pyrolysis of wood at 180～300°C. When contents of formaldehyde in wood-vinegar liquors, which is discussed as one of social problems in an application of specification pesticide was investigated by trap method of TG-GCMS using Hinoki (*Chamaecyparis obtusa*) in Kiso district, its peak was found at retention times of 2.1 minute on the spectrogram. One kilogram of mokusaku oils is obtained by vaccum distillation of five kilogram of tars extracted from wood. When wood tars, which were extracted from wood distilled together with wood vinegar liquors, were further distilled, mokusaku oils containing 72% of phenol components were obtained. The components of mokusaku oils are almost equal to those of creosote. As mokusaku oils contain a large quantity of phenolic components as compared with wood vinegar liquors, they are considered to have strong microbicidal and insecticidal activities.

Key words: wood vinegar liquors, wood tars, mokusaku oil, phenolic components

1. INTRODUCTION

So far, creosote which belongs to coal tar system, has widely been used as the reinforcemental agents of antimicrobial, deodorizer and rubber etc. However, the use of creosote was recently prohibited, because these compounds are in danger of inclusion of carcinogens [1, 2].

Coal tar system creosote oils are the mixtures of chemicals produced in the fine process of coal tar. The annual output of creosote oils in the year 2002 A.D., are about seventy-seven ten thousand ton. Ten percent of their outputs are used as preservative of wood, while remaining ninety percent of yield is used as raw materials of the reinforcemental agents of rubber by the second processing.

Creosote published in the Japanese Pharmacopoeia is mainly refined from wood [3, 4]. Raw materials and chemical components of creosote of Japanese Pharmcopoeia is different in comparison with those of coal tar system creosote. As 3,4-benzpyrene, which is doubtful of carcinogens and strong stimulator was found to be contained in coal tar system creosote, safety of this compound is discussed as serious social problems at present [5, 6].

In European Union, sale of coal tar system creosote oil and wood treated with this oil for the consuming public is entirely prohibited in the year 2001 A.D. In the year 2003 A.D., sale of coal tar system creosote is also prohibited,irrespective of contents of 3,4 -benzpyrene.

In our country, the revision of "Public building construction standard specification" is revised by the Ministry of Land, Infrastructure and Transport in the year 2003 A.D. and the articles of "Coal tar system creosote are omitted from wood antiseptic" was added in this law.

Like European Union, in our country, sale of coal creosote is prohibited,irrespective of contents of 3,4-benzpyrene. From the year 1998 A.D. considering that 3,4-benzyprene is doubtful of endocrine-disrupting chemical (environmental hormone), their investigations have been carried out in Ministry of the Environment. Because formaldehyde was further detected in wood vinegar liquors obtained by dry distillation of wood, its uniformity of the quality is required at present.

In this work, to obtain mokusaku oil with high safety in terms of specification prevention materials (specification pesticide), the establishment of production process of this oil and its development of utilization field are investigated [7, 8]. The following three experiments were concretely carried out. 1). The development of production process of mokusaku oil and the analysis of chemical components using differential thermo-balance, TG-GCMS and GC-MS were examined, 2) Water-solubility of nanoparticle preparations (water-soluble preparations of mokusaku oil was also tried, and 3) Furthermore, antimicrobial activity of water-soluble preparations of mokusaku oil were investigated.

2. EXPERIMENTAL METHODS

2.1 Comparison with Components of Wood Vinger Liquors, Mokusaku Oil and Creosote by TG-GCMS

Contents of formaldehyde in 1.38 mg of wood of Hinoki (*Chamaecyparis obtusa*) in Kiso district, which was used as sample, was examined by TG-GCMS.

2.2 Production Process of Mokusaku Oil

When pyrolysis of 750 kg of Japanese oak in Aomori Prefecture was proceeding at 400～500°C, 90 m^3 of wood gas, 350 kg of water-soluble wood vinegar liquors and 50 kg of wood tar were obtained. After 50 kg of wood tar was further distilled in vacuum (130°C /3.99kPa), 10 kg of oil of which tar components were removed, were obtained (this oil is hereafter showed as "Mokusaku oil" in this paper) [9, 10].

2.3 Comparison with Components of Mokusaku Oil and Creosote by GC-MS Analysis

Analysis conditions

(1) Sample: Mokusaku oil of Japanese oak and creosote (KISHIDA Chem. Co.)
(2) Injection amounts: 0.5 μL
(3) Column: DE-1701 15 m X ID 0.25 mm 0.25 μm
(4) Column temperature: 60°C /10min → 260 °C (8°C /min)
(5) INJ: 260 °C
(6) Interface: 260 °C
(7) He: 1.0mL/min (SPL 50:1)

After both samples were dissolved in 1wt/wt% diethylether, they were compared by GC-MS analysis.

2.4 Measurement of Particle Diameter of Emulsion

Particle diameter of water-soluble preparations of mokusaku oil was measured by dynamic light scattering-type particle diameter distribution equipment.

2.5 Antimicrobial Activity Test of Mokusaku Oil

Water-soluble preparations of mokusaku oil (particle diameter: 15.7 nm) and 10% mokusaku oil ethanol solutions were prepared. Of two compounds, components of mokusaku oil water-soluble preparations were as follows: namely, weight ratio of mokusaku oil: emulsifying agent: ethanol: water is 10: 20: 10: 60. Antimicrobial activity tests of water-soluble preparations of mokusaku oil (particle diameter: 15.7 nm) and 10% mokusaku oil ethanol solution (particle diameter: 65.9 nm) were investigated under the following conditions.

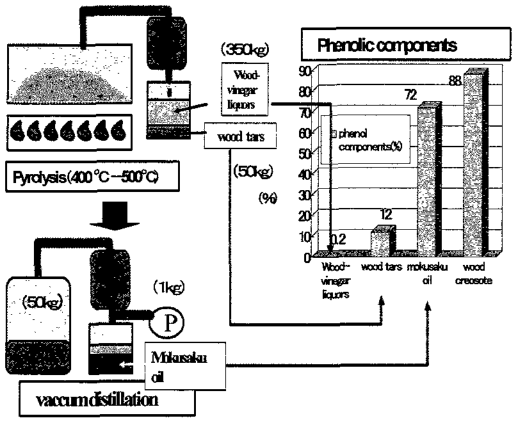

Fig.1 Production method of mokusaku oil and Its phenol components

The microorganisms used were as follows. Bacteria: *Escherichia coli* ATCC 25922, Methicillin-resistant *Staphylococcus aureus* (MRSA: clinical isolate). Fungus: *Aspergillus* sp. (clinical isolate). Inoculation sizes were prepared so as to be final concentration of 10^5 CFU/ml.

Culture condition: *Escherichia coli* and MRSA were cultivated at 37°C for 18 hr, and *Aspergillus* sp. was cultivated at 31°C for 4 days.

Antimicrobial activity test was determined by a standard disk method using above-mentioned three microorganisms as test organisms. In disk method, three tested strains were prepared to be 10^6 CFU/ml. Twenty-five μl of an undiluted preparations of mokusaku oil were sunk into disks. These disks were subsequently put on the plates containing test bacteria and plates were cultivated at 37°C for 18 hr. The same disk was also put on the plate containing *Aspergillus* sp. at 31°C for 4 days.

3. RESULTS AND DISCUSSION

3.1 Production Method of Mokusaku Oil and Its Chemical Components

Wood vinegar liquors were dark brown liquid obtained after cooling of gas produced by dry distillation of wood and these solutions were widely used as deodorizer for home use and soil conditioner of agriculture and horticulture. However, in wood vinegar liquors, formaldehyde, which is subject to the regulation of the exhaust of volatile organic compounds (VOC), was detected. As 3,4-benzpyrene, which is in danger of carcinogen, has been contained in tar components, attention has been focused on their safety.

Though the quality of wood vinegar liquors is not regular, the acquisition of these liquors of which the quality is stabilized have been desirable.

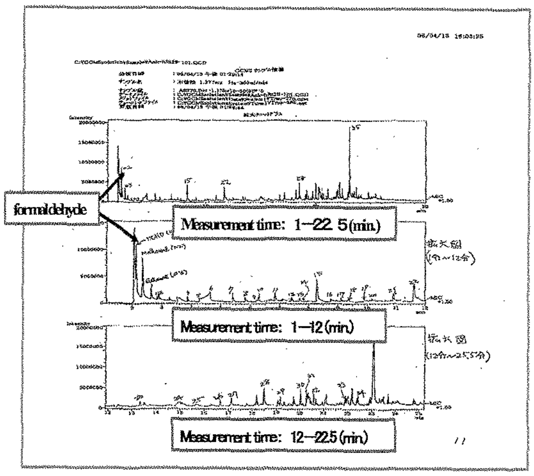

Fig.2 Analysis of wood-vinegar liquors extracted by pyrolysis from Hinoki (*Chamaecyparis obtusa*) in Kiso district

Therefore, to obtain mokusaku oil with high safety, wood tar, which is produced by carbonization of plants or woods, was noticed (Fig. 1). Furthermore, mokusaku oil, in which formaldehyde or 3,4-benzpyrene were not contained, could be obtained by distillation of above-mentioned wood tar *in vacuum* (130/°C about 4 kPa). From the result of measurement of phenol components, it was also found that they have been contained at the rate of 72% in mokusaku oil and their contents were almost equal to those of wood creosote (Creosote listed in Japanese Pharmacopoeia).

3.2 Comparison with Wood-Vinegar Liquors, Mokusaku Oil and Phenol Components by the Measurement of TG-GCMS

Chemical components of mokusaku oil were investigated by trap method of TG-GCMS. At the same time, components of wood-vinegar liquors and creosote (KISHIDA Chem. Co.) listed in Japanese Pharmacopoeia were also compared.

As shown in Fig. 2, in the chemical analysis of wood-vinegar liquors extracted by pyrolysis from Hinoki (*Chamaecyparis obtusa*) in Kiso district, the peak due to formaldehyde was detected at retention time (t_R) of 2.1 minutes.

On the other hand, in the chemical analysis of mokusaku oil and creosote listed in Japanese Pharmacopoeia, the peak due to formaldehyde was not detected. The chemical components of mokusaku oil were found to be almost equal to those of creosote listed in Japanese Pharmacopoeia from the results of the chromatograms of TG-GCMS.

3.3 Comparison with Mokusaku Oil and Creosote by the Measurement of GC-MS

Chemical components of mokusaku oil and creosote listed in Japanese Pharmacopoeia were compared by the measurement of GC-MS and these gas chromatograms were shown in Fig.3. Chemical components of mokusaku oil and creosote listed in Japanese Pharmacopoeia were also summarized in Table 1. Guaicol, phenol, cresol and xylenol was found to be contained at the ratio of 76% in the components of mokusaku oil and these components could be found to be almost equal to those of creosote listed in Japanese Pharmacopoeia. The peaks of numbers 15, 19, 5 and 8 have the same fragment peaks as MS, m/e 138(M^+), 123 (M^+ - CH_3), 95 (123 - CO), 77 (95 - H_2O) and 67 (95 - CO) and from analytical results of these data, this compound was presumed to be methylguaicol, but the position of methyl group attached to benzene ring is not decided at present.

As the peaks of numbers 23, 26, 11 and 12 have the same fragment peaks as MS, m/e 152(M^+) and 137 (M^+ - CH_3), this compound was considered to be ethylguiacol. The peaks of numbers 14, 21, 4 and 10 have the same fragment peaks as MS, m/e 122 (M^+) and 107 (M^+ - CH_3), so this compound was presumed to be xylenol, but the positions of methyl and ethyl groups attached to benzene ring were not detected.

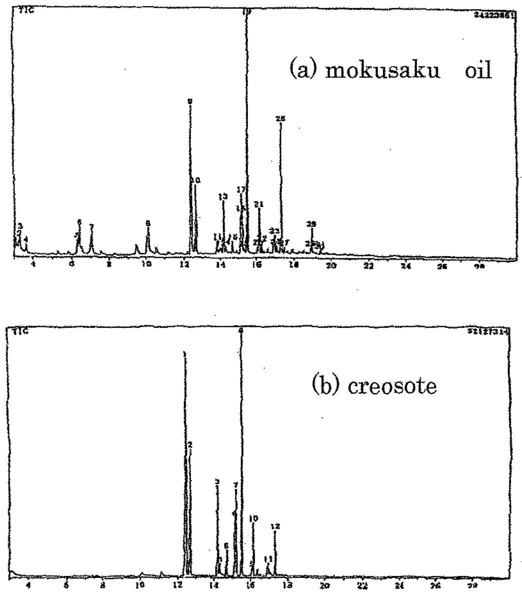

Fig. 9 Gas chromatograms of (a) mokusaku oil and (b) creosote

3.4 Particle Diameter Distribution of Emulsion of Mokusaku Oil (O/W)

To obtain water-soluble emulsion from mokusaku oil, the preparation was produced by the mixture of mokusaku oil (10wt%), emulsifying agent (20wt%),ethanol (10wt%) and water (60wt%). Particle diameter distribution was measured with dynamic light scattering-type particle diameter distribution equipment. An average of particle diameter was found to be about 18.1 nm (data not shown).

E. coli ATCC 25922, MRSA and *Aspergiluus* sp. (one of clinical isolate) were investigated by disk method using above-mentioned water-soluble preparation of mokusaku oil and 10% ethanol solution of mokusaku oil. Antimicrobial potency was determined by dilution method. The results are summarized in Fig. 4. As shown in Fig.4, both preparations showed antimicrobial activities against three kinds of microbe. In particular, both preparations showed clear antimicrobial activities on MRSA and *Aspergillus* sp. even at low dilution concentration of five hundred and twelve times.
Antimicrobial activity of 10% ethanol solution of mokusaku oil on *E. coli* ATCC 25922 was weaker than that of water-soluble preparation of mokusaku oil, and its maximum dilution rate was two hundred and fifty-six times. On the other hand, the maximum dilution rate of antimicrobial activity of water-soluble preparation of mokusaku oil was five hundred and twelve times.

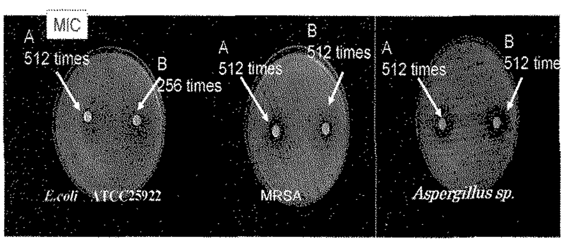

Fig.4 Antimicrobial Activity of Mokusaku Oil

4. CONCLUSION

(1) Detection of formaldehyde, which is discussed as the social problems in the council of specification pesticide, was examined using Hinoki (*Chamaecyparis obtusa*)in Kiso district. From the analytical results by trap method of TG-GCMS, the peak of formaldehyde was detected at the retention time of 2.1 min. on chromatogram. However, formaldehyde and 3,4-benzyprene were not detected in mokusaku oil, which is developed in this study.
(2) Seventy-two percent of phenol components are obtained by distillation of wood tar with mokusaku oil. These phenol components are almost equal to those of wood creosote.
(3) Water-soluble preparation of mokusaku oil was composed from the ratio of weight of which mokusaku oil: emulsifying agent: ethanol: water is 10: 20: 10: 60, and its particle diameter was confirmed to be 15.7nm. On the other hand, particle diameter of 10% ethanol solution of mokusaku oil was found to be 65.9 nm.

5. REFERENCE

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Table 1. Phenolic components in mokusaku oil and creosote by GC-MS

	Creosote			Mokusaku oil		
Phenolic Compounds	Peak No	t_R (min)	GC(%)[1)]	Peak No.	t_R (min)	GC(%)[2)]
guaiacol	1	12.47	30.68	9	12.41	17.21
phenol	2	12.66	14.32	10	12.66	8.06
o-cresol	3	14.17	8.07	13	14.18	4.49
xylenol	4	14.3	0.92	14	14.3	0.4
methylguaiacol	5	14.66	2.26	15	14.66	1.17
p-cresol	6	15.12	4.75	16	15.12	3
m-cresol	7	15.16	8.76	17	15.17	6.44
metylguaiacol	8	15.5	21.19	19	15.49	19.56
2-ethylphenol	9	16.09	0.43	20	16.1	0.54
xylenol	10	16.17	4.19	21	16.17	3.37
ethylguaiacol	11	16.95	0.85	23	16.98	2.64
ethylguaiacol	12	17.34	3.57	26	17.35	9.56

1) GC(%) : relative peak area percentage of phenolic compounds by GC-MS for creosote
2) GC(%) : relative peak area percentage of phenolic compounds by GC-MS for mokusaku oil.