

A Fundamental Research on Dry Steam Extractives of Japanese Cedar

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In this study, dry steam was applied as a kind of reaction medium to extract organic substance from Japanese cedar leaves. The extractives have been investigated through a continuous flowing dry steam extraction system. The extractives were analyzed by GC-MS. Monoterpene, sesquiterpene (β -eudesmol) and diterpene (kaurene) were found to be the main compounds in cedar leaves extractives. Compared to the extraction in nitrogen gas atmosphere a higher yield of extractives has been obtained in the flowing dry steam. It suggests that dry steam extraction is a promising method for selective extraction and separation of non-polar or low-polar organic materials from Japanese cedar leaves.

1. Introduction

Many studies have been conducted to produce the natural products from plant. The most used method is isolated by steam or hydro-distillation and solvent extraction. The disadvantages of these techniques are low yield, long extraction time, toxic solvent residues, and degradation of unsaturated compounds [1-3]. Recently more research has been conducted to develop some new analytical techniques for effective extraction, such as CO₂-supercritical fluid extraction and vacuum pyrolysis, which were proved to be successful on production of lipophilic substances such as the carboxylic acids, resin acids, aldehydes, ketones, steroids [1, 4-6] and other plant extractives from biomass.

Also water is an environment friendly extraction medium. Besides hot water extraction and steam extraction, researches on superheated water and superheated steam become more conspicuous. Recently superheated water has been applied to analyze the contamination compounds in sediment or soil instead of organic solvent [7-10], and superheated steam in a high temperature range has been used to serve as drying medium of wood or reaction medium for improving adsorption capacities of solid biomass residues [11-15]. Little work, however, has been done up to now on studying the characteristics of dry steam, and also little attention has been paid to use dry steam (heated and unsaturated vapor) as a reaction medium to extract valuable substance from biomass.

In general, dry steam extraction is a technique based on the use of water as extractant, at a temperature range from 100 to 200 °C and a pressure below the saturated vapor pressure. Dry steam lies in low-density water molecular area of steam field and is characterized by a lower dielectric constant (ϵ) than ordinary water. This brings the possibility to accelerate the extraction reaction process of organic substances [8].

Since the 1960's, Japanese cedar has been planted a lots and form an over stock, it is desired to develop some new usages of cedar besides as timber or fuel. Cedar essential oils have been reported to have strong anti-bacterial activity [16]. This implies that Japanese cedar is a potential resource for production of medicament or pesticide. In this study the aims are to develop a fast, cheap and environmentally friendly method for extraction of natural valuable organic substance from Japanese cedar leaves, and to investigate the possibility of selective extraction by control of temperature, pressure of dry steam and reaction time.

2. Experimental

2.1. Apparatus The employed dry steam extraction apparatus system is shown in Fig.1. T₁, T₂, T₄, T₅, T₆ and T₇ are supplied from band heater, and T₃ is supplied from ribbon heater. Gas generator, reactor, sample cups and linked pipes are made of SUS 316. Sample cups are sintered with a meshed bottom (1 mm in diameter of mesh).

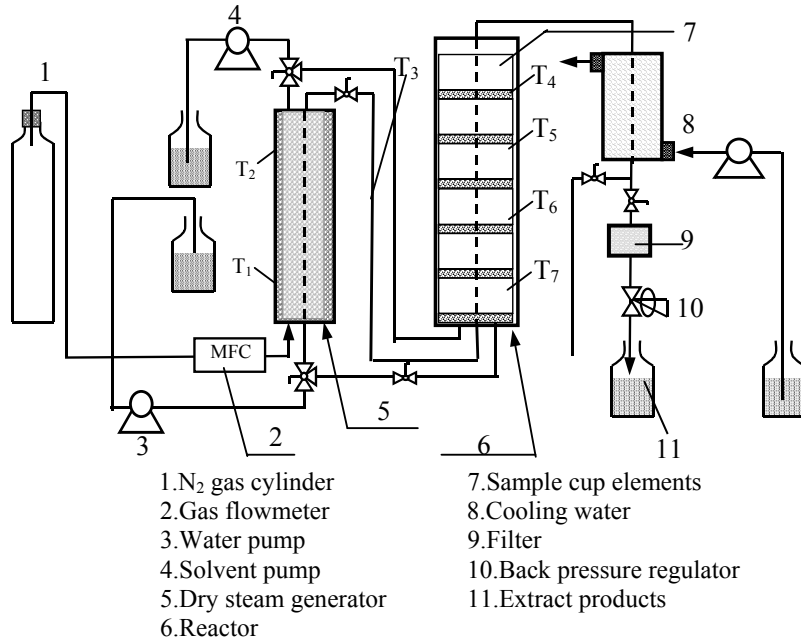


Fig. 1. Schematic flow diagram of the extraction apparatus

Nitrogen gas is served as a carrier and regulator of dry steam. While nitrogen gas is supplied from a gas cylinder, water is pumped into the gas generator from water pump, where water was instantly heated by countless round brass (3.18 mm in diameter) balls accreted inside of the wall and dry steam was generated. Density of dry steam is adjusted by controlling the back pressure regulating valve and the mixing ratio of vapor and nitrogen gas. Dry steam is carried by N₂ gas into the extraction reactor vessel, where sample cups filled with the Japanese cedar leaves are installed. And then the dry steam is allowed to flow upwards through the sample cups and react with leaves. Therefore, the volatile compounds and other lipophilic substances can be extracted with the flowing of the dry steam and flow into the cooler, where the exhausted steam is cooled below 20 °C. Afterward the cooled samples are directly fed into a gas-liquid phase separator, and separated into gas phase and liquid phase for further analysis.

A control panel system is installed for measuring and controlling the gas flow rate, extraction temperature and reaction pressure.

2.2. Materials Fresh leaves of Japanese cedar (the water content of the cedar was about 60 %) were

broken into about 5-10 cm pieces for the extraction reaction.

2.3. Procedure 100 g of fresh cedar leaves was packed into sample cups. The cups were installed into extraction reactor vessel, and then the vessel was closed tightly. After the leakproofness of the system was confirmed by nitrogen gas with flow rate 1 l/min, the gas generator and extraction vessel were heated to the desired extraction temperature, and then the appropriate water (0.48-11 ml/min) was fed for preparing dry steam. Reaction temperature of reactor was set at a relatively low temperature range from 100 to 200 °C. The desired pressure is controlled by the back pressure valve below the saturated pressure at the current reaction temperature. Finally the cooled samples after separation were collected immediately and demixed by mixing *n*-hexane, to form hexane-soluble phase and water-soluble phase.

2.4. Analysis Extractives were analyzed by GC-MS (Hewlett-Packard Co., 5890 series II -- 5980B mass spectrometer). Helium is the carrier gas. The column (HP-1) temperature, which was employed to separate the extracted oils in hexane phase, was increased from 50 to 150 °C at 6 °C/min, from 150 to 280 °C at 4 °C/min, and then kept for 10 minutes

at 280 °C. The detector temperature was maintained at 280 °C. The temperature of column (HP-innowax) served to separate the organic components in water phase, was increased from 40 to 230 °C at 5 °C/min and the detector temperature was 250 °C. 1 μ l portion of extractives was injected into the GC for determination.

3. Results and discussion

3.1. Analysis of Extractives The extraction of Japanese cedar leaves chips by dry steam was done at 170 °C, 0.5 MPa for 120 min. GC chromatograms of extractives in hexane-soluble and water-soluble extractives are shown in Fig. 2. The results show that most of extractives obtained from dry steam extraction are found to exist in hexane-soluble phase. The structural formulas of these main components were verified by mass spectrum analysis. As shown in Table 1, the main products are the derivatives of terpine, which include monoterpene (Sabinene, α -terpinene, 3-cyclohexen-1-ol), sesquiterpene (β -eudesmol, elemene, cadinene), and diterpene (15-kaurene, 16-kaurene). In addition, two selinene, aromadendrene, aromatic esters and high fatty acids were identified. And the extractives components in water phase were mainly composed of low carboxylic acids and some furan-aldehydes, which are considered to be

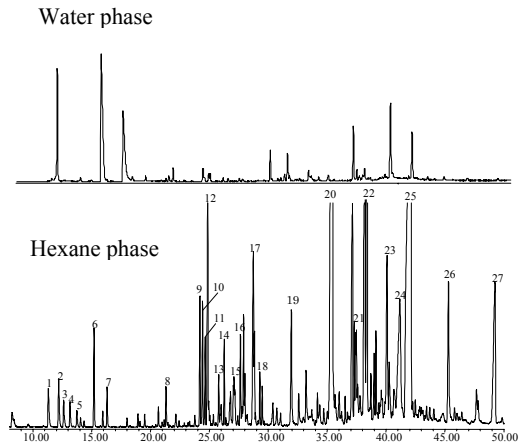


Fig.2. GC chromatograms of dry steam extractives from Japanese cedar leaves

concerned with the hydrolyzation of cellulose [17]. It suggests that the hydrolysis unavoidably occur with the extraction of organic components if reaction time is long enough.

3.2. Characterization of extraction rates In Fig.3 effect of treatment conditions on yield of extractives are discussed. At the given experiment condition (170 °C, 0.5 MPa and water flow rate of 4.4 ml/min), change of extracted amount with time

Table 1. Structural formulas of extractives in oil phase.

1	Sabinene		10	Endobornyl acetate		19	Neophytadiene	
2	α -3-carene		11	Elemene		20	Elemol	
3	β -Myrcene		12	3-cyclohexen-1-ol		21	Guaiol	
4	α -Terpinene		13	Germacrene B		22	β -Eudesmol	
5	dl-limonene		14	β -Farnesene		23	15-kaurene	
6	γ -terpinene		15	Linalyl propionate		24	Hexadecanoic acid	
7	α -terpinolene		16	β -Cubebene		25	16-kaurene	
8	2-Furancarboxaldehyde		17	δ -Cadinene		26	2-Hexadecen-1-ol	
9	2-furancarboxaldehyde, 5-methyl		18	(+)Aromadendrene		27	2(1H)-naphthalenone	

is shown in Fig.3 (a). In the initial extraction stage (<20 min), most of smaller-molecule organic substance can be extracted out, such as monoterpene (sabinene and 3-cyclohexen-1-ol). However, extraction of larger-molecule compounds as sesquiterpene (β -eudesmol, elemene, cadinene), and diterpene (15-kaurene, 16-kaurene), need longer reaction time. Also it shows that extraction of oxide of terpene (3-cyclohexen-1-ol and elemol) is more slowly than that of terpene (sabinene and elemene). It suggests that extraction rate is concern with molecule structure of extractives, and non-polar substance is more easily extracted in medium of dry steam.

While reaction pressure and time remain invariant at 0.4 MPa for 120 min, the yield of extractives increases with the increase of reaction temperature from 110 to 190 °C, as shown in Fig.3 (b). The yield at 170 °C gets the maximum value for all extracted organic compounds, and then decreases at 190 °C. Especially this trend is more obvious for larger molecule extractives (elemol, β -eudesmol and 16-kaurene). It suggests that relative high temperature can accelerate extraction of

organic substance from cedar leaves, but excessive high temperature above 170 °C could result in a decrease of extractives, which was thought to be a result of pyrolyzing of extractive.

Figure 3 (c) shows the results at a selected extraction condition at 170 °C, a total pressure of 0.85 MPa for 120 min with change of water flow rate from 0-11 ml/min. As the nitrogen gas flow rate is fixed at 1 l/min, the partial pressure of vapor can be calculated to be from 0-0.8 MPa. Effect of different density of water vapor on extraction is compared with those obtained in pure nitrogen gas atmosphere. It was found that vapor with different density has strongly influenced the distribution and kinds of extractives. As shown in Fig.3 (c), the yields of large molecular compounds as β -eudesmol and 16-kaurene have dramatically increased with the increase of partial pressure of water vapor. For sesquiterpene and diterpene, the yields in dry steam with a partial pressure of vapor of 0.8 MPa are 2-3 times higher than that in nitrogen gas atmosphere. The higher yield of extractives obtained in denser water vapor, should be related to the ability of water vapor to penetrate

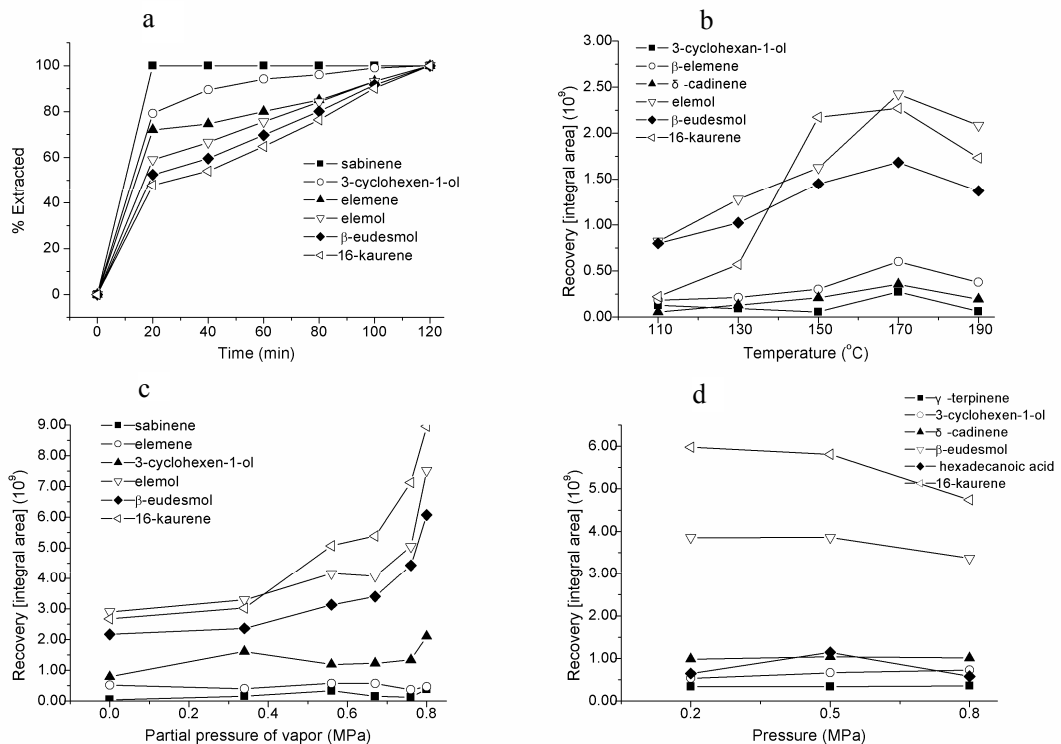


Fig.3. Effect of treatment conditions (temperature, density of vapor, total pressure, time) on the yield of extractives.

Table 2. The yield of extractives from 100g of fresh cedar leaf at different partial pressure of vapor

Partial pressure (MPa)	0	0.20	0.30	0.40	0.45
Hexane-soluble (g)	0.5684	0.6603	0.8853	0.6693	1.4285
Water-soluble (g)	0.0543	-	0.1425	0.3006	0.332
Total extractives (g)	0.6227	-	1.0278	0.9699	1.7605
β -Eudesmol (g)	0.0602	0.0628	0.0717	0.0959	0.1388
Acetic acid (g)	0.0031	0.0076	0.0163	0.0171	0.0349

Total extractives (g) = Hexane-soluble + water-soluble

the solid cedar leaves and to help desorption and distillation.

As showed in Fig.3 (d), at the constant temperature, extraction time (170 °C, 120 min) and fixed water flow rate of 3.36 ml/min and N₂ flow rate of 1.0 l/min, it was found that even total pressure of reaction were changed, the yields of main extractive components had little of changes. It was verified again that the total pressure in a lower pressure range (<1 MPa) isn't so important factor as temperature or density of vapor. The extraction reaction is directly related with partial pressure of vapor, not the total pressure of dry steam.

3.3. Quantitative analysis The extractives obtained at 170 °C, 0.5 MPa for 120 min, was demixed by addition of hexane. Hexane-soluble fraction and water-soluble fraction were then desolventized by a vacuum absorber separately, and by weighting the yield amount of extractives was obtained. GC was used for quantitative analysis of β -eudesmol and acetic acid. The yields with the change of vapor density of dry steam are shown in Table 2. Considering the content of water of fresh leaves of cedar (about 58.8%), the yields based on dry weight base are 4.5% for the total oils, 0.34% for β -eudesmol and 0.1% for acetic acid. The largest amount of extractives was found in the hexane-soluble fractions. It also be seen that in the samples extracted in nitrogen gas atmosphere the yield of hexane-soluble fraction are 10 times higher than those of water-soluble fraction. With the increase of density of vapor, the yield of hexane-soluble fraction increase, but the ratio of hexane-soluble amount to that of water-soluble decreases, it suggests that high yield of total extractives can be achieved in a flow of dry steam with higher density of vapor, but it should be attend that hydrolization become intense with the increase of water.

4. Conclusion

The present paper reports a new extraction process for the production and recovery of useful plant components from Japanese cedar leaves by dry steam. In view of the results so far achieved, dry steam extraction is suggested as an effective process because it is able to separate and concentrate some specific organic components at a low temperature range just by adjusting reaction time and density of vapor, especially for those non-polar products, such as terpene and some high fatty acid. More than thirty kinds of components in hexane-soluble fraction can be identified with significant abundance by GC-MS methods. Low carboxylic acids and some furan-aldehydes were determined to be the main compounds in water-soluble fraction. High yield of total extractives can be achieved in a flow of dry steam with higher density of vapor and at a high temperature below 170 °C. Extraction for large molecular substance needs a long time more than 3 hours.

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