CELLULOSE CHEMISTRY AND TECHNOLOGY

VALORIZATION OF MARITIME PINE WOOD (*PINUS PINASTER*) WASTE BY VACUUM EXTRACTION OF VOLATILE COMPOUNDS. COMPARISON WITH CONVENTIONAL METHODS

ALICE MEULLEMIESTRE,[*] ZOULIKHA MAACHE-REZZOUG,[*]
RICHARD CAPART[**] and SID-AHMED REZZOUG[*]

[*]*LaSIE, FRE-CNRS 3474, University of La Rochelle, Avenue Michel Crépeau,*
17042 La Rochelle, France
[**]*Chemical Engineering Laboratory, University of Technology of Compiègne,*
Research Center of Royallieu, BP 20529. 60205 Compiègne Cedex, France

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This study is a part of a work on the double valorization of industrial waste from the wood sector. The proposed process is composed of two-step extraction of volatile compounds and production of activated carbon from the residue. The innovative aspect of this project is that the first step of the process acts as a pretreatment for the second step. The presented study concerns only the first step.

Essential oil of *Pinus pinaster* was extracted by conventional steam distillation and hydrodistillation techniques at normal temperature and pressure. For the two techniques, vacuum extraction was also performed. Yields of 0.25 and 0.15% on dry basis were obtained, respectively, by steam distillation and hydrodistillation for processing time of 10 hours. For the extraction by vacuum steam distillation, the essential oil yield remained sensibly the same by increasing processing pressure from 60 mbars (0.24% dry basis) to 200 mbars (0.23% dry basis) and these maximum values were reached after 8 hours. The chromatographic analysis confirmed that the contents of the main components, such as caryophyllene and L-fenchone, were more important for the vacuum extraction, indicating a better quality of the obtained oil. Due to these improvements, the extraction of *Pinus pinaster* essential oil under vacuum can attract considerable interest for its application in high-class perfumes, flavours and other formulations.

Keywords: *Pinus pinaster*, volatile compounds, vacuum extraction, steam distillation, hydrodistillation

INTRODUCTION

Scientific advances in plant transformation technology and biotechnology can be viewed as products of “green chemistry”. Biotechnology products are interchangeable and can compete with fine chemistry as well as energy materials derived from fossil fuel. A primary objective of a chemistry research today is “clean” chemistry.

The first step in this project involves isolating volatile and extractable compounds, which are present in a very small amount of lignocellulosic materials, with high added value. The second step consists in transforming the solid residue into activated carbon, which is another material with high added value. The proposed method, with an integrated approach to waste recycling, relates to “green chemistry” field.

At present, in France, 11% of total wood waste (equivalent to 1 million tons) are going to landfill or are destroyed. Maritime pine is the coniferous type of wood, which is most prevalent in the Poitou Charente area. According to a recent report (ADEME, 2004) over 30000 tons of wood waste is not valued. This vegetable material is cheap or free and, above all, it is inexhaustible, because it is renewable. This lignocellulosic biomass is mainly composed of three types of polymers: cellulose (40-45%), hemicelluloses (26-64%) and lignin (3-5%), as well as extractible compounds in low quantity.[1] These kinds of volatile compounds are called essential oils.

In contrast to other compounds, the extractibles are the only fraction that can be isolated without affecting the other compounds. The AFNOR NF T 75-006 (AFNOR, 1980) standard defines the essential oil (EO) as "a product obtained from plant material, either by steam distillation or by "dry" distillation". Essential oils are separated from the aqueous phase by physical means and are widely used in pharmaceuticals, perfume, soap and for unconventional medicinal purposes, as well as in aromatherapy.[2,3] Their virtues are largely cited by many authors.[4,5] The essential oil of maritime pine is known for its many properties and health benefits. It is notable as an oxygenating agent for the respiratory system and muscles. It also has analgesic properties that relieve muscle and articular pain. This oil is mainly composed of α-pinene, β-pinene, caryophyllene and germacrene D.[6,7] These compounds, present in a low quantity, have a high added value[8] and they are highly sought by industrials. It should be noted that there are many studies on the extraction of essential oil from different aromatic plants,[9,10,11] but few authors[12,13] are interested in the extraction of essential oil from wood and especially from wood waste.

In this context, this work is a comparative study of the extraction of maritime pine wood essential oils, using traditional methods, such as steam distillation (SD) and hydrodistillation (HD), and the same techniques under vacuum. We are especially interested in the major components of maritime pine essential oil issued from samples obtained from a sawmill company near La Rochelle (France): caryophyllene and L-fenchone.

EXPERIMENTAL

Raw material

Maritime pine (*Pinus pinaster*) wood was collected in the South West of France in the Landes area, after the Xynthia storm. It came from Archimbaud sawmill, as sawdust, in September 2010. Particle size distribution was assessed by an electric sieve shaker (Fritsh-Analysette 3 Pro), the samples were calibrated with an average particle size of 0.8 to 1 mm in diameter. Sawdust was used to its residual water content, W_i = 25.2% on dry basis (db). Volatile compounds were extracted by traditional and vacuum steam distillation and hydrodistillation.

Extraction of volatile compounds

Steam distillation

The steam extraction used in the present study was a modified Clevenger method. Some modification was allowed to the equipment to be adequate for carrying out various treatments and quantifications of the amount of essential oil thus extracted. 1 Liter of distilled water was boiled to be injected to a glass reactor containing 100 grams of raw material and the condensate of maritime pine wood flowed within the raw material. For the extraction under vacuum, a vacuum pump was connected between the condenser and separation flask. Vacuum was fixed at 60 mbars and 200 mbars.

The extraction proceeded for 16 hours, from the moment the first drop of distillate fell into the separation flask until the raw material had been completely consumed. The total duration of the extraction was determined previously by studying the extraction kinetics, using the measurement of the essential oil yield versus time (Figure 1).

Hydrodistillation

100 grams of raw material were immersed in 1 Liter of distilled water in a 2 Liter distillation reactor flask. The extraction was performed for 16 hours, from the moment the first drop of distillate fell into the separation flask until the raw material had been completely consumed. For the vacuum hydrodistillation, a vacuum pump was connected between the condenser and separation flask. Vacuum was fixed at 60 mbars and 200 mbars. As for steam distillation, the duration of the extraction was determined previously by studying the extraction kinetics.

Isolation of essential oil

An amount of hexane was added in order to capture, separate and quantify the extracted essential oil present in the aqueous phase. The organic phase was separated from the aqueous phase by settling, the condensation water reached the bottom of the separation flask, so the mixture of essential oils/hexane could be recovered. The mixtures were dried with anhydrous sodium sulfate and purified by using microfiber filters. The combined filtrates were evaporated to quantify the oil yield by using a Rota vapor machine. The extraction yield was calculated in % weight dry basis by the following formula:

$$\text{Yield of extraction (\% db.)} = \left(\frac{\text{weight of extract}}{\text{weight of raw material} \times (1 - W_i)}\right) \times 100 \qquad (1)$$

To avoid any degradation due to the light and heat, the essential oil recovered, to which a certain amount of hexane was added, was stored in a tightly closed amber vial at 4 °C in a refrigerator, for analysis.

GC/MS analysis

A Varian 3900 chromatograph coupled to a Saturn 2100 T mass spectrometer equipped with a fused silica capillary column was used to analyse the samples. A non-polar stationary phase of CP-Sil 8 CB (30 m x 0.25 mm, 0.25 microns) was used. The measurements were carried out under the following conditions: the carrier gas (helium) at 1 mL.min^{-1}, ratio 1:100; injection volume 1 µL; injection temperature 250 °C; oven temperature ranging from 50 to 250 °C using a gradient temperature of 2 °C.min^{-1}, kept for 5 min at 250 °C.

Ionization and fragmentation were performed by impact ionization at 70 eV, with an injection temperature of 250 °C. The scanning range of m/z was from 30 to 400 uma at 2.2 scan.s^{-1}. The identification of compounds was achieved by comparing their mass spectra with the database and spectral libraries (Varian 2002 NIST MS Data Library). A quantitative analysis was carried out by integrating each peak of the chromatograms. A quantitative analysis was carried out by integrating each peak of the chromatograms.

SEM characterization

Micro-structures were observed using an environmental type JEOL 5410LV FEI Quanta 200F Scanning Electronic Microscope (SEM) at the Joint Analysis Center (*Centre Commun d'Analyses* – CCA) of the University of La Rochelle. The samples were placed on a covered stud using carbon adhesive. The samples were scanned in a partial vacuum (7 Pa) with an acceleration tension of 20 kV.

Micro-structural changes in the maritime pine wood were studied on the raw material and on the samples extracted by classic and vacuum hydrodistillation and steam distillation.

RESULTS AND DISSCUSION

Extraction kinetics by steam distillation and hydrodistillation

The essential oil obtained by steam distillation and hydrodistillation of maritime pine samples had a very light yellow color and a pleasant pine odor.

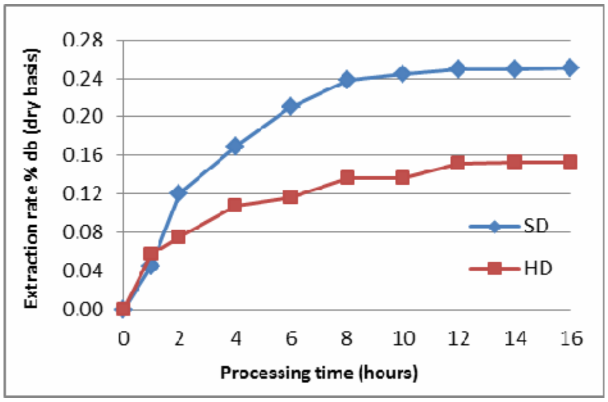

Figure 1: Extraction kinetics of maritime pine wood waste by steam distillation (SD) and hydrodistillation (HD)

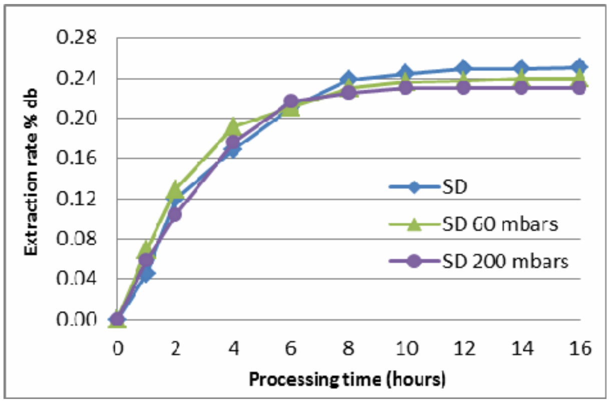

Figure 2: Extraction kinetics of maritime pine wood waste by steam distillation (SD) at atmospheric pressure, 60 mbars and 200 mbars

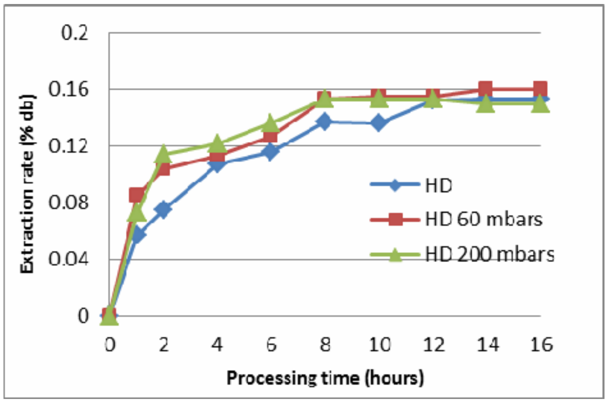

Figure 3: Extraction kinetics of maritime pine wood waste by hydrodistillation (HD) at atmospheric pressure, 60 mbars and 200 mbars

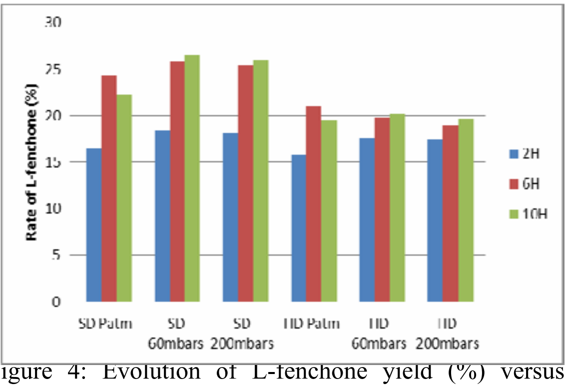

Figure 4: Evolution of L-fenchone yield (%) versus extraction time by different methods: steam distillation (SD) and hydrodistillation (HD) at atmospheric pressure, 60 mbars and 200 mbars

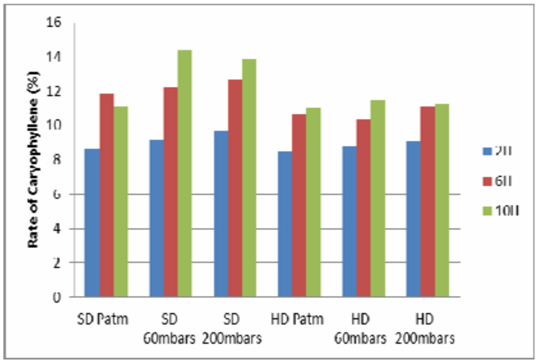

Figure 5: Evolution of caryophyllene yield (%) versus extraction time by different methods: steam distillation (SD) and hydrodistillation (HD) at atmospheric pressure, 60 mbars and 200 mbars

The average yield of maritime pine wood essential oil extracted by steam distillation and hydrodistillation were, respectively, 0.25 g EO/100 g dry basis and 0.15 g EO/100 g dry basis.

Figure 3 shows the variation of the maritime pine oil yield versus time, extracted by steam distillation and hydrodistillation. More than 97% of the total yield of essential oil was recovered after 6 and 8 hours, respectively, for the SD and HD (Figure 1). Therefore, these respective duration times were considered as the reference/ equilibrium time, for each extraction method.

For the SD extraction, the extraction yield increased faster at the beginning of the extraction, then from 6 hours, it gradually bended to reach the equilibrium point, which was 0.25% dry basis. The yields obtained by hydrodistillation increased gradually with the duration time until the equilibrium point was reached. The maximum yield was 0.15% dry basis. The trend curves for each extraction method with the best representation were third-order polynomial curves with a correlation coefficient above 0.99. We observed that whatever the extraction method used, the essential oil yield increased with extraction time. However, the maximum yield obtained by steam distillation was almost 1.6 times higher, compared to the hydrodistillation method. As a consequence, this difference was due to a better accessibility of water vapors within the sample.

Comparative extraction yields between classic extraction and vacuum extraction

Whether by conventional extraction or under vacuum at 60 mbars and 200 mbars, extraction yields remained sensibly stable, 0.24 ± 0.01% db for an extraction by steam distillation and 0.15 ± 0.01% db for an extraction by hydrodistillation. However, for both SD and HD extraction techniques, the equilibrium time to reach the maximum yield decreased for vacuum extraction. For vacuum techniques, regardless of the vacuum level, the equilibrium time was 8 hours against 10 hours for traditional techniques. Figures 2 and 3 show that the extraction was faster under vacuum, indicating that the air acted as a barrier for extraction.

Composition of maritime pine essential oil

The global chemical analysis of the essential oil of maritime pine wood waste is presented in Table 1. A total of 27 compounds were identified, similar for all extraction methods but at different concentrations. A majority of L-fenchone and caryophyllene was detected in steam distillation and hydrodistillation at normal temperature and atmospheric pressure (NTP) and for extractions at 60 mbars and 200 mbars. Most often, the higher rates were reached by vacuum steam distillation at 60 mbars and 200 mbars, 26.47 and 25.94% for the L-fenchone compound and 14.36 and 13.83% for caryophyllene. While, for the extraction by hydrodistillation, for the same pressure and temperature conditions, the concentration for both these compounds were lower. So the concentrations were always higher when the vacuum was used.

For both major compounds present in essential oil obtained under vacuum, the concentration increased with extraction time, except with steam distillation and hydrodistillation at normal temperature and pressure, where the rates decreased after 10 hours of extraction (Figures 4 and 5). The steam distillation at NTP reached its maximum after 6 hours (24.3% for L-fenchone and 11.83% for caryophyllene) and decreased to attain 22.25% (L-fenchone) and 11.12% (caryophyllene). The compounds underwent some degradation with extraction time. This degradation was not visible for the vacuum extraction whatsoever the pressure used, it was avoided due to the lower temperature, compared to the traditional methods; the boiling temperatures were, respectively, 40 °C and 60 °C, for 60 mbars and 200 mbars.

Table 1
Chemical composition of maritime pine essential oil extracted by steam distillation (SD) and hydrodistillation (HD) at atmospheric pressure, at 60 mbars and 200 mbars after 10 hours of extraction

N°	Retention time (min)	Peak name	CAS Number	SD Patm (%)	SD 60 mbars (%)	SD 200 mbars (%)	HD Patm (%)	HD 60 mbars (%)	HD 200 mbars (%)
1	5.77	1R-alpha-Pinene	7785-70-8	1.39	3.17	2.64	1.12	1.65	1.29
2	7.29	beta-Pinene	127-91-3	0.60	1.21	0.68	0.63	1.16	0.80
3	9.106	4,6-Decadiene	55682-65-0	0.28	0.33	0.30	0.31	0.84	0.48
4	9.24	m-Cymene	535-77-3	3.62	5.56	5.03	3.01	3.54	3.18
5	9.41	Camphene	79-92-5	1.37	2.89	2.36	1.25	1.78	1.42
6	12.46	L-Fenchone	126-21-6	22.25	26.47	25.94	19.56	20.09	19.73
7	15.69	Camphor	464-49-3	4.60	6.12	5.59	4.13	4.66	4.30
8	17.39	Origanol	15358-88-0	0.42	0.63	0.35	1.69	2.22	1.86
9	1.64	Terpinen-4-ol	562-74-3	6.52	9.49	8.96	6.52	7.05	6.69
10	18.665	alpha-terpineol	98-55-5	9.22	12.31	11.78	8.98	9.51	9.15
11	27.53	Tricyclo[5.4.0.0(2,8)]	58989-08 -2	0.75	0.64	0.78	0.57	1.10	0.74
12	29.18	Copaene	3856-25-5	0.95	0.89	0.81	1.01	1.54	1.18
13	31.09	Longifolene	475-20-7	7.32	8.79	8.26	6.96	7.43	7.13
14	31.77	Caryophyllene	87-44-5	11.12	14.36	13.83	11.03	11.50	11.26
15	33.91	alpha-Caryophyllene	6753-98-6	2.86	3.01	2.99	2.24	2.71	2.47
16	37.77	delta-Cadinene	483-76-1	2.35	3.78	3.25	2.16	2.63	2.39
17	41.30	Caryophyllene oxide	1139-30-6	1.11	1.86	1.33	1.02	1.49	1.25
18	42.453	1,4-Methanoazulen	27551-75-3	0.59	0.66	0.61	0.00	0.47	0.23
19	43.967	Cubenol	21284-22-0	0.76	0.98	0.88	0.36	0.83	0.59
20	59.605	Biformene	5957-33-5	1.05	1.68	1.15	1.13	1.60	1.36
21	62.89	Epimanoyl oxide	1227-93-6	6.45	9.12	8.59	6.44	6.91	6.67
22	64.34	Cembrene	1898-13-1	1.00	2.45	1.92	0.85	1.32	1.04
23	66.066	Kaurene	34424-57-2	0.59	0.83	0.68	0.49	0.96	0.68
24	67.14	Sclareol	515-03-7	3.63	4.61	4.08	3.55	4.02	3.74
25	72.11	1R-pimaral	472-39-9	5.27	6.72	6.19	5.04	5.51	5.23
26	73.48	Rimuene	1686-67-5	4.24	5.49	4.96	4.18	4.67	4.35
27	94.19	Squalene	94016-35-0	0.28	0.46	0.35	0.00	0.49	0.17

Microstructure of residual untreated and treated wood waste

The SEM images of maritime pine before and after extraction revealed considerable structural changes. Before extraction, the cells were almost full, the empty cavities were probably damaged when the tree was sawn, as the wood used was a waste (Figure 6).

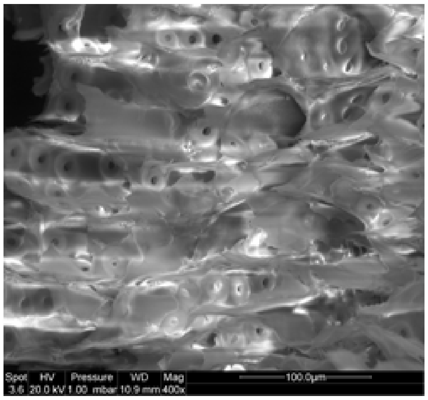

Figure 6: Untreated maritime pine wood (400x)

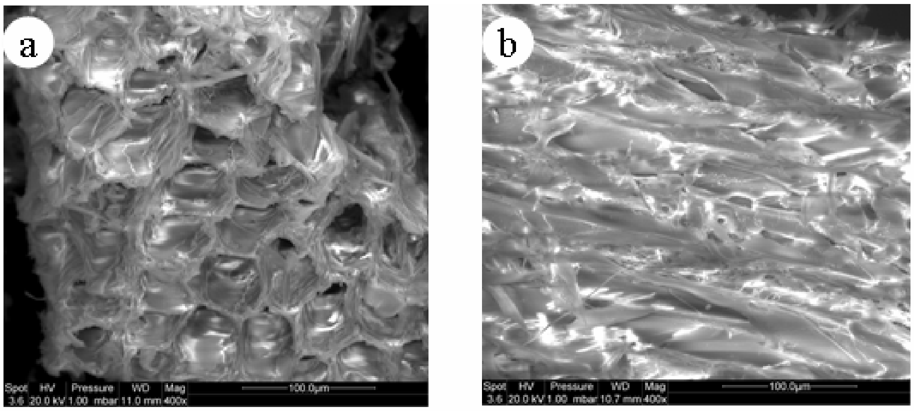

Figure 7: Maritime pine wood after 10 hours of steam distillation (a) and hydrodistillation (b) at normal pressure and temperature (400x)

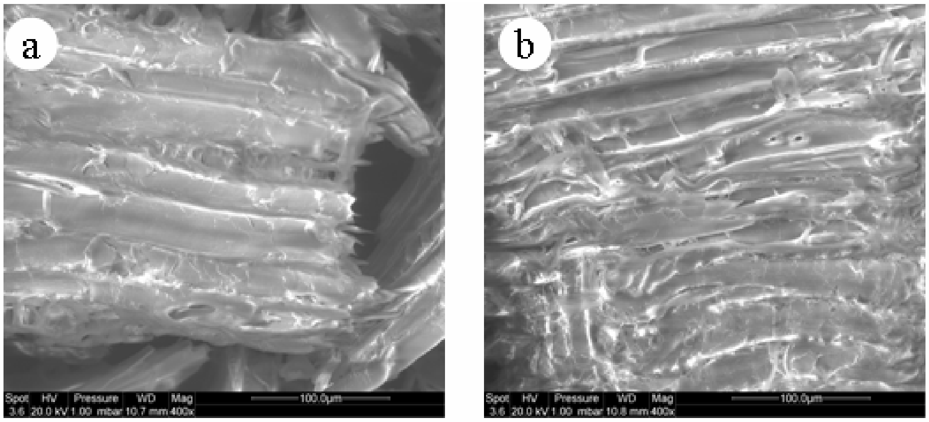

Figure 8: Maritime pine wood after 10 hours of steam distillation under vacuum (a) and hydrodistillation under vacuum (b) at 60 mbars (400x)

But approximately 80% of the cells were full. After 10 hours of extraction by steam distillation and hydrodistillation at NTP (Figure 7), practically all the matter was extracted, the cells were empty and the wood fibers began to tear. For the vacuum extraction (Figure 8), a different phenomenon appeared, the complete structure of wood was folded on itself, and the wood was absorbed, meaning that all the cell contents were extracted.

CONCLUSION

The present work proved the high relevance of vacuum for the conventional extraction of essential oils. From the viewpoint of extraction yield, this technique had no significant effect. It remained sensibly the same, 0.24 ± 0.01% db for an extraction by steam distillation and 0.15 ± 0.01% for an extraction by hydrodistillation after 10 hours of processing. However, vacuum technology can achieve the maximum yield in less time (8 hours) than the conventional methods. This first observation permits to argue that the vacuum steam distillation should be preferred.

This conclusion was confirmed by the second important point in evaluating essential oils: chemical analysis. Indeed, steam distillation, either vacuum or not, showed a higher level of volatile compounds. For both major compounds, L-fenchone and caryophyllene, the higher yields were reached after 10 hours of extraction by vacuum steam distillation at 60 mbars, respectively, 26.47% and 14.36%. The fact is that decreasing temperature by vacuum prevents the degradation of volatile compounds during extraction. Finally, to optimize the quantity and the quality of maritime pine essential oil from wood waste, vacuum distillation at 60 mbars was used with a duration time of 8 hours.

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