Effectiveness of Distillation Models on Bioactivity from Essential Oil Fraction of *Cinnamommum camphora* (L.) J. Presl.

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ABSTRACT
The change of paradigm in forest product management brought out the Forestry Minister's Regulation Number P.35/Menhut-II/2007, concerned Non-Timber Forest Products (NTFPs). Some genus Cinnamomum included in the list of NTFPs, and *Cinnamomum camphora* (camphor) is one species. The recorded data mentioned that this plant was found abundant in Kalimantan and used as a complement in traditional ceremonies, especially for the Dayak Tunjung community, in Pepas Asa village. This research aimed to explore further the potential of *C. camphora* essential oil grown in East Kalimantan as antibacterial. Essential oil fractions obtained from the leave of *C. camphora* (CP), which grew in Pepas Asa village, were fractionated by water and steam distillation based on their distillation models (CP5, CP7, and CP9) and separation times (0-1 h, 1-2 h, and 2-3 h). A hand refractometer physically analyzed the oils to determine their refractive index value. The essential oils were also examined for their antibacterial activity at a concentration of 1%, 10%, and 100% (pure oil fraction) by agar diffusion assay against *Streptococcus mutans*. The results showed the highest yield of oil fractions for each model were 1.62% (CP51), 2.35% (CP71), and 1.23% (CP91. The result of antibacterial activity showed that all fractions from three models potential as antibacterial agents in the concentration of 100% (pure oil), especially the fraction of second-time distillation, could inhibit the growth of *S. mutans* higher than tested positive control.

Keywords: *Essential oil, Cinnamomum champora, distillation model, fraction, bioactivity*

1. INTRODUCTION

The paradigm in forest management is slowly changing because it tends to lead to the management of forest areas (ecosystems) as a whole and to demand diversification of forest products other than wood, this is indicated by the stipulation of Minister of Forestry Regulation Number P.35 / Menhut-II / 2007, Non-Timber Forest Products (NTFPs) are non-wood forest products, both biological and animal along with their derivative products and their cultivation except wood originating from the forest [1]. At present, five types of NTFPs are given priority. However, apart from the five types, the region has the right to develop superior commodity NTFP based on the potential of NTFPs and capabilities in their area [2].

Several types of the Genus Cinnamomum are a group of aromatic plants recorded in the NTFP list. *Cinamommum camphora* is one of the species included in the list as NTFP plants produce essential oils and matter for the forestry department. *C. camphora* has relatively widespread, especially in China, India, Mongolia, and Vietnam. Its natural habitat and naturalized in Florida and New South Wales, and Queensland, Australia [13]. *C. camphora* growth is very suitable for subtropical climate. But in the village of Pepas Asa, West Kutai, Kalimantan, *C. camphora* was scattered around community cultivation. Its use was still limited to a complement in traditional ceremonies, especially by the Dayak Tunjung community, Pepas Asa village.

This research aims to explore further the potential of *C. camphora* which grows in the village of Pepas Asa by obtaining the essential oil fraction that it produces as an antibacterial and is expected to be able to provide an effective alternative in the distillation process, especially to get the results of *C. camphora* oil as an antibacterial activity.

2. MATERIAL AND METHODS

2.1. Plant Materials and Chemicals

C. *camphora* leaves were collected from Pepas Asa village, Kutai Barat, East Kalimantan, Indonesia. The plants were identified by a taxonomist and confirmed by references. The chemicals used in this study were Sodium Sulphate (Na_2SO_4), Nutrient broth, Agar, Glucose, Alcohol 95%, Chlorhexidine, Chloramphenicol, and Aquadest available in the Lab. Forest Product Chemistry and Renewable Energy, Faculty of Forestry Unmul, Samarinda.

2.2. Water and Steam Distillation Method

189 g of powdered samples were soaked with *n*-hexane, ethyl acetate, and 96% ethanol solvents using successive maceration method in a mechanical shaker at room temperature for two days [7]. The solid filtered and evaporated using a rotary vacuum evaporator at 40 ^oC to obtain each solvent's crude extract. The result of extraction reported as percents yield (%) = (weight of extract in grams/weight of sample in grams) x 100 [8].

The essential oils of *C. camphora* were collected by the water and steam distillation method [4]. With modification. *C. camphora* distillation was carried out with three repetition models of CP5 (repetition 1), CP7 (repetition 2), and CP9 (repetition 3), to get the oil fraction of the distillation process carried out for ± 3 hours with separation in each hour. The separation time can be seen in the following table 1.

After the distillation process is complete, water and essential oil will mix. The oil was collected and separated using a separating funnel to separate between oil and water. After waiting for some minutes, the oil was collected until water and oil separated and entirely formed two layers. Na_2SO_4 was used to adsorb the residual of water that was fused with the oil.

Table 1. Time of Separation in the *C. camphora* Refining Process.

No.	Model	Separation Times (hour)	Code
1	CP5	0 – 1	(CP51)
		1 – 2	(CP52)
		2 – 3	(CP53)
2	CP7	0 – 1	(CP71)
		1 – 2	(CP72)
		2 – 3	(CP73)
3	CP9	0 – 1	(CP91)
		1 – 2	(CP92)
		2 – 3	(CP93)

2.3. Refraction Index of C. camphora oil

Essential oils were subjected to tests index of refraction using a hand refractometer. A few drops of oil are dropped on the prism, the measurement results are observed with readings that look at eyepieces directed towards the light. The value on the border between blue and white is the refractive index value of oil.

2.4. Antibacterial Assay

The Agar diffusion method used the nutrient agar method for bacteria and fungi [4]. The method was used for the *in vitro* antibacterial activity of the essential oil against oral pathogen. The microorganism used in this study was *Streptococcus mutans.* The final concentration used in this assay was 100% (pure oil), 10%, and 1%, diluting 40% ethanol. As a positive control, two kinds of antibiotic standards were used in this study, Chlorhexidine (CHX) and Chloramphenicol (CHP). The 40% ethanol was served as a negative control. After incubation at 32^oC for 18-24 hours, the antibacterial activity values were determined. The diameter of inhibition zones (IZ) was measured [5] and calculated [6] in this study. All experiments were performed in triplicate.

Inhibition Zone (IZ) = IZ_{sample} / $IZ_{standard}$ (1)

3. RESULT AND DISCUSSIONS

In this study, the fractionated essential oil was obtained from water and steam distillation of leaves *C. camphora*. The result of the yields is presented in Table 2. Table 2 shows that the oil yield will decrease with the duration of refining. The fractions of *C. camphora* atsiri oil showed a high output at 0-1 time separation with a value of each fraction of 1.62% (CP5), 2.35% (CP7), and 1.23% (CP9). The refractive index states the ratio (ratio) of the speed of light in a vacuum to light in a material. The refractive index is one of several parameters to determine the quality of an oil.

Table 2. The yield of *C. Camphora* essential oil fraction

Test	Separation Time (hour)	Fraction Code	% Yield	Refractive Index
CP5	0 -1	CP51	1.62	1.409
	1-2	CP52	0.71	1.415
	2-3	CP53	0.14	1.434
CP7	0 -1	CP71	2.35	1.462
	1-2	CP72	1.2	1.465
	2-3	CP73	0.38	1.468
CP9	0 -1	CP91	1.23	1.425
	1-2	CP92	0.85	1.422
	2-3	CP93	0.3	1.428

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The antibacterial activity test was divided into three parts of the test. There were three fractions of *C. camphora* oil with each test with concentrations of 1%, 10%, and 100% (pure oil). The positive controls used are Chlorhexidine (CHX) and Chloramphenicol (CHP). The results of antibacterial testing of *C. camphora* oil fraction are shown in Table 3.

Table 3 shows that most of the inhibition is demonstrated by the oil fraction at 100% oil concentration. The CP52 fraction showed the most significant inhibition in the CP5 model with a value of 17.9 ± 2.01 mm, then in the CP7 model, the most considerable inhibition was demonstrated by the CP72 fraction of 22.9 ± 1.68 mm, and the CP9 model showed the most significant inhibition of CP92 with a value of 18.7 ± 6.17 mm.

The inhibition zone shown by *C. camphora* essential oil against *S. mutants* above is in line with earlier tests report essential oil of *C. camphora* shows inhibition of 22 mm in the testing of *S. mutans* [8].

Table 3. Antibacterial activity of *C. camphora* essential oil fraction

Bacterial Strains	Code	Zone of Inhibition (mm)		
		1%	10%	100%
S. mutans	CHX		13.1 ± 1.39	
	CHP		16.0 ± 0.33	
	CP51	na	na	12.6 ± 1.93
	CP52	na	1.8 ± 3.08	17.9 ± 2.01
	CP53	na	na	14.0 ± 0.58
	CHX		14.8 ± 1.17	
	CHP		16.3 ± 1.86	
	CP71	na	na	17.7 ± 2.08
	CP72	na	na	22.9 ± 1.68
	CP73	na		17.2 ± 6.35
	CHX		11.8 ± 2.04	
	CHP		16.0 ± 0.33	
	CP91	na	na	15.0 ± 5.78
	CP92	na	na	18.7 ± 6.17
	CP93	na	na	12.1 ± 1.26

Remarks: na (not active), CHX (Chlorhexidine), CHP (Chloramphenicol) with concentration of 5µg/mL.

Besides the most significant inhibition, this test is always shown in the fraction of oil obtained from refining at 1-2 hours. Various factors can affect plant bioactivity as an antibacterial, a compound produced during the oil refining process. *C. camphora* is one of the camphor compounds producers [9] and has been tested in several tests as potentially antibacterial [10].

4. CONCLUSIONS

In conclusion, the essential oil fraction Camphora produces the highest yield found in the separation time 0-1 hour. All fractions from three models potentially as antibacterial agents in the concentration of 100% (pure oil), especially the fraction of second-time distillation, could inhibit the growth of *S. mutans* higher than tested positive control.

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