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The Effect of Harvest Time of Sapu-Sapu Leaves (*Baeckea frutescens* L.) the Yield, Characteristics and Composition of Essential Oils Extached Using Steam-Hydro Distillation Method

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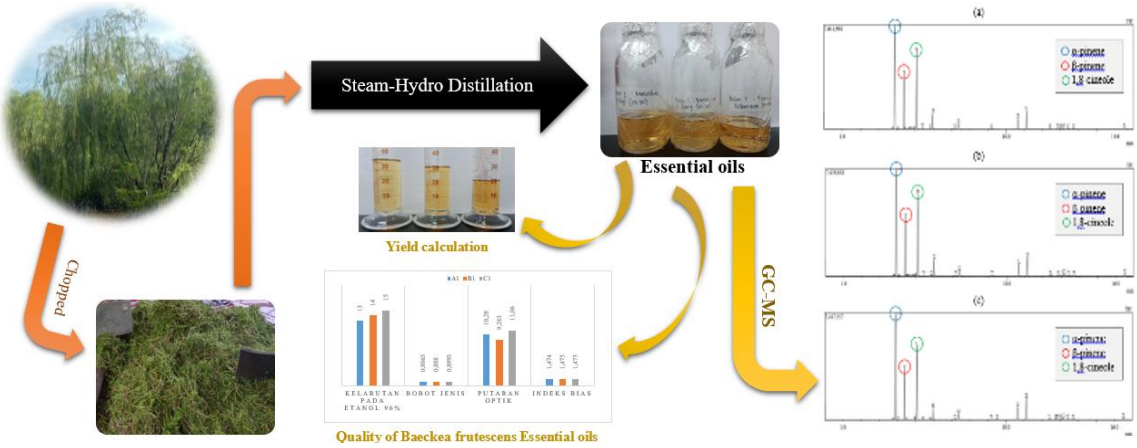
GRAPHICAL ABSTRACT



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ABSTRACT

The sapu-sapu plant (*Baeckea frutescens L.*) is widespread in the Bangka Belitung Islands Province, thriving in sandy areas such as beaches and highlands with less fertile soil conditions. This study aimed to determine the optimal harvest time for sapu-sapu leaves to obtain the highest yield and most desirable characteristics of essential oils. The research focused on the duck-type sapu-sapu leaves, using five variations of harvest time (coded as A1, B1, C1, A2, and A3, representing leaves harvested at 1, 2 and 3 months of growth, respectively). Essential oil extraction was performed using the steam distillation method. The resulting oils were then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to determine their chemical composition. The results showed that sample A1 (1-month growth) produced the highest essential oil yield at 0.74% (w/w). This sample also exhibited the most optimal essential oil characteristics: clear yellow color, characteristic sapu-sapu odor, warm bitter taste, solubility in 96% ethanol at 1:13, specific gravity of 0.8863 g/mL, optical rotation of (+) 10.28°, and refractive index of 1.474. GC-MS analysis revealed that the main compounds in the A1 sample were α-pinene (43.84%), β-pinene (13.56%), and 1,8-cineol (24.26%). The study

concluded that while the variation in harvest time of sapu-sapu leaves did not significantly affect the yield, characteristics, or composition of the essential oils, there were slight differences in yield and color. Sample A1, representing the youngest leaves, produced the most optimal results.

1. INTRODUCTION

Essential oils comprise terpenoids (monoterpenes and sesquiterpenes) with lipophilic properties [1]. Over 300 compound components have been identified in essential oils, with phenolic compounds constituting the most significant percentage [2]. Various plants produce essential oils, including lemongrass (*Cymbopogon nardus*), eucalyptus (*Melaleuca leucadendra*), patchouli (*Pogostemon cablin*), vetiver (*Chrysopogon zizanioides*), ylang-ylang (*Cananga odorata*), and jasmine (*Jasminum sambac*) [3]. The sapu-sapu plant (*Baeckea frutescens L.*) from Bangka Belitung is another essential oil-containing plant of interest.

Baeckea frutescens L., a member of the Myrtaceae family, is widespread in the Bangka Belitung Islands Province. It thrives in sandy areas such as beaches and sometimes highlands with less fertile soil [4]. This plant has been reported to be effective in treating various ailments, including influenza, epistaxis, coryza, fever, headache, measles, impaired digestion, abdominal pain, irregular menstrual cycles, jaundice, and hemorrhagic dysentery [5]. The primary sapu-sapu leaf essential oil (SSEO) constituents are monoterpenes and sesquiterpenes [6]. The essential oil of *B. frutescens* contains 32 chemical compounds, including five significant compounds and 27 minor compounds such as eucalyptol, α-terpineol, α-humulene, β-ocimene, and β-mycene [7]. In the Bangka Belitung Islands, sapu-sapu plants exhibit two leaf types, which in the local language are referred to as "ducking" and "not ducking" (Note to proofreader: please provide appropriate English translations for these terms). This study focuses on the "ducking" type to identify its characteristics and compounds at various harvest times.

The steam-hydro distillation method is used to extract SSEO at different harvest times. This method offers several advantages over other techniques, including lower cost, shorter distillation time, higher yield, and better quality of the resulting essential oil [8]. However, further research is needed to compare the efficiency and effectiveness of steam-hydro distillation with other extraction methods for SSEO. Previous studies have investigated the effect of harvest time on essential oil yield and quality in various plants. For instance, research on kaffir lime leaves (*Citrus hystrix DC*) harvested at different times of the day (morning at 08:00, midday at 11:00-13:00, and afternoon at 16:30 local time) showed yield percentages of 0.74%, 0.55%, and 0.63%, respectively. The morning harvest produced the highest yield of 0.74% [15,6,5]. Another study on citronella essential oil found that a 3-month harvest age yielded the highest yield of 0.33% with a specific gravity of 8.53g/10mL [9].

Despite these findings in other plants, the effect of harvest time on the yield, characteristics, and composition of essential oils from sapu-sapu leaves (*Baeckea frutescens L.*) in the Bangka Belitung Islands has not been previously studied. Therefore, this research aims to investigate the influence of harvest time on the yield, characteristics, and composition of SSEO using the steam-hydro distillation method in the Bangka Belitung Islands Province.

2. EXPERIMENTAL METHODS

2.1. Material and Apparatus

The tools used in this study were sacks, scales, a set of Zebra Thailand stainless steel SUS 304 36 cm distillation tools, upper hoses, lower hoses, water faucets, heaters, separation funnels, clamps, statics, Erlenmeyer Pyrex 50 mL, aluminium foil, plastic wrapping, sample bottles, Pyrex 50 mL Beaker glasses, Pyrex 25 mL measuring cylinder, glass funnels, test tubes, test tube racks, stirring rod, analytical balance (Pyrex, 10 mL, sensitivity ±0.1 mg), Kruss P3000 polarimeter, Kruss DR6000 refractometer and GC-MS (*Gas Chromatography and Mass Spectroscopy*) Shimadzu QP 2010 SE.

The materials used in this study were fresh sapu-sapu leaves (*Baeckea frutescens L.*), tap water, 96% ethanol (EtOH), and distilled water.

2.2. Sample Preparation

Sapu-sapu leaf samples (*Baeckea frutescens L.)* were harvested from Jalan Ir. H. Eko Maulana Ali, Air Anyir Village, Bangka Regency. The species was authenticated by a botanist from the University of Bangka Belitung in Bangka Belitung. The harvested sapu-sapu leaves were cut and prepared in 5 kg batches for each sample. The variations of sample harvest time were tabulated as follows:

TABLE I. Tabulation of Sample Harvest Time Variation Data.

Sample	Month	Harvest Time
A1	1	Morning (07:30)
B1	1	Noon (10:30)
C1	1	24 hours after morning harvest
A2	2	Morning (07:30)
A3	3	Morning (07:30)

2.3. Sapu-sapu Leaf Essential Oil Extraction

A set of Zebra Thailand stainless steel SUS 304 36 cm distillation equipment was installed. Water was put into the kettle to the limit of the sieve, then a 5 kg sample was placed on the sieve, ensuring it was not in direct contact with the boiling water. The heating rate was set with a heating temperature of 100-110°C and a steam temperature of 150-300°C. The process was continued until the first drop of distillate came out from the condenser. Essential oils and hydrosols were collected during the distillation process using a separation funnel. Distillation was stopped after the heating process had lasted for 4 hours. The essential oil of the sapu-sapu leaves was collected and stored for yield calculation and further testing [10]. The yield was calculated using the Equation 1.

$$Yield\ \left(\%\frac{v}{w}\right) = \frac{\text{Volume of essential oil obtained}}{\text{Weight of plant material}}\ x\ 100\% \qquad (1)$$

$\frac{25}{25}$

2.4. Essential Oil Characteristic Data

2.5. Organoleptic Test

The organoleptic test was conducted through visual observation. The observations included the color, smell, and taste of the essential oil from sapu-sapu leaves at each sample harvest time [11].

2.6. Solubility Test using Ethanol Solvent

One mL of the sample was added to a 10 mL graduated cylinder. 96% ethanol was gradually added, and after each addition, the sample was shaken to obtain a clear solution. The solubility test results were expressed as the ratio of 96% ethanol to sample volume required to obtain a clear solution [12, 13].

2.7. Determination of Specific Gravity

The specific gravity was determined using a pycnometer. The clean, dry pycnometer was weighed empty (m), then filled with distilled water and weighed (m1). After cleaning and drying, the pycnometer was filled with the sample and weighed (m2) [13]. The specific gravity was calculated using the following Equation 2.

$$\text{Specific gravity (g/mL)} = d\frac{25}{25} = \frac{m2-m}{m1-m} \qquad (2)$$

2.8. Optical Rotation Determination

Optical rotation was measured using a polarimeter. The instrument was calibrated using distilled water. The sample was then introduced into the polarimeter tube, ensuring no bubbles were present.

The rotation angle was measured by adjusting the dial until a dark-light-dark position was observed. This process was repeated three times for each sample, and the average rotation angle was recorded.

2.9. Determination of Refractive Index

The refractive index of the essential oils was measured using a refractometer. A sample drop was placed on the prism surface, and the cover was closed. The refractometer was positioned towards a light source, and the refractive index was read from the scale where the bright-dark boundary line intersected [14]. Measurements were performed in triplicate for each sample, and the average value was reported.

2.10. GC-MS Analysis

The phytochemical constituents of the essential oils from sapu-sapu leaves (*Baeckea frutescens L.*) were identified using a Shimadzu QP 2010 SE GC-MS system. A 0.2 µL sample was injected into an Elite Rtx-5 MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The operating conditions were as follows: ionization energy 70 eV, mass scanning range 40-400 amu, detector temperature 280°C, injector temperature 220°C, ion source temperature 230°C, and quadrupole temperature 150°C. The oven temperature was programmed from 35°C (9 min hold) to 150°C at 3°C/min (10 min hold), then to 250°C at 10°C/min, and finally to 270°C at 3°C/min (10 min hold). Helium was used as the carrier gas with a flow rate of 0.5 mL/min [15, 16]. The compounds were identified by comparing their mass spectra with those in the NIST library.

3. RESULTS AND DISCUSSIONS

3.1. Extraction of Essential Oils from Sapu-Sapu Leaves

The extraction of essential oil from sapu-sapu leaves (*Baeckea frutescens L.)* yielded a two-phase mixture: the essential oil and the hydrosol phase. The hydrosol, a by-product of the distillation process, contains water-soluble components. The essential oils were analyzed for yield, characteristics, and compound composition.

3.2. Effect of Harvest Time on Yield

The yield determination aimed to assess the efficiency of the distillation process for extracting sapu-sapu leaf (*Baeckea frutescens L.*) essential oil. The results of the yield calculations for different harvest times are presented in Table 2.

TABLE II. Yield Data of Essential Oil from Sapu-Sapu Leaves (*Baeckea frutescens L.*).

Sample	Initial Weight (kg)	Volume (mL)	Yield (% v/w)
A1	5.000	37	0.74
B1	5.000	33	0.66
C1	5.000	22.5	0.45
A2	0.009	-	-
A3	0.650	-	-

Sample A1 produced the highest yield of 0.74% with a volume of 37 mL from 5 kg of plant material. The yields from the three harvest times in the first month (A1, B1, C1) did not show significant differences. This similarity might be attributed to consistent environmental conditions during the harvest period, such as light intensity, temperature, and weather. The distillation process parameters, including duration and temperature, likely played a crucial role in the extraction efficiency. However, the influence of these parameters on the optimum evaporation of essential oils requires further investigation to draw definitive conclusions. Samples A2 and A3 did not yield sufficient essential oil for accurate measurement due to the limited sample mass available for the steam distillation process. The minimum required sample mass for the distillation system used was 5 kg. To address this limitation in future studies, it is recommended to adjust the distillation apparatus or methodology to accommodate smaller sample sizes, or to express the yield as a ratio to the standard 5 kg sample size. This approach would allow for a more comprehensive comparison across all harvest

times. These results highlight the importance of considering both harvest time and sample quantity in the distillation process. Further research is needed to optimize the extraction process for smaller sample sizes and to investigate the effects of environmental and procedural factors on essential oil yield.

3.3. Effect of Harvest Time on Essential Oil Characteristics

The essential oil samples were analyzed for organoleptic properties, solubility in 96% ethanol, specific gravity, optical rotation, and refractive index. The results of these analyses are presented in Table 3.

TABLE III. Characteristic Data of Sapu-Sapu Leaf Essential Oil (*Baeckea frutescens L.).*

Parameter	Sample A1	Sample B1	Sample C1
Color	Clear yellow	Clear, dark yellow	Clear, dark brownish yellow
Odor	Characteristic sapu-sapu	Characteristic sapu-sapu	Characteristic sapu-sapu
Taste	Pungent and slightly bitter	Pungent and slightly bitter	Pungent and slightly bitter
Solubility in 96% ethanol (v/v)	1:13	1:14	1:15
Specific gravity (g/mL)	0.8863	0.8880	0.8893
Optical rotation (degrees)	(+) 10.28	(+) 9.28	(+) 11.06
Refractive index	1.474	1.475	1.475

The data in Table 3 indicate that the three sample variations from the first month did not show substantial differences in most characteristics of the sapu-sapu leaf essential oils. However, some subtle variations were observed:

a) Color: The samples progressed from clear yellow (A1) to dark brownish yellow (C1), suggesting potential changes in oil composition over time.
b) Solubility: Solubility decreased slightly from A1 to C1, as indicated by the increasing ethanol-to-oil ratio required for complete dissolution.
c) Specific gravity: A minor increase was observed from A1 to C1, possibly due to changes in oil composition.
d) Optical rotation: Sample B1 showed a slightly lower value than A1 and C1, but the difference may not be statistically significant without further replication and analysis.
e) Refractive index: Samples B1 and C1 showed marginally higher values than A1, but the difference is minimal.

While the differences observed are relatively small, they suggest that harvest time may subtly influence the essential oil characteristics. However, further statistical analysis and replicate studies would be necessary to definitively state that one harvest time produces more optimal results. The current data provide a foundation for future, more comprehensive investigations into the effects of harvest time on sapu-sapu leaf essential oil properties.

3.3.1. Organoleptic

Organoleptic observation of essential oils is one of the physical characteristics that is a reference for the quality of the essential oils produced. Observations include the color, smell, and taste of the essential oil of the drooping sapu-sapu leaf presented in Table 3. The results of data observation show that sample A1 is clear yellow, sample B1 is dark clear yellow, and sample C1 is dark clear yellow-brownish, as seen in Figure 1 below.

Figure 1. Produced Sapu-Sapu Leaf Essential Oil.

Usually, essential oils are colorless or yellowish, but some are reddish, green, brown, and blue [13]. The color change of the oil becomes darker due to the storage of samples that are left for too long and exposed to sunlight so that oxidation of terpene compounds occurs and forms resin compounds during the heating process [17, 18].

3.3.2. Solubility in 96% Ethanol

The solubility test was conducted to determine the amount of alcohol required to dissolve a specific quantity of essential oil. As shown in Table 3, sample A1 exhibited the highest solubility with a ratio of 1:13 (1 mL of essential oil to 13 mL of 96% ethanol), followed by samples B1 (1:14) and C1 (1:15). A previous study reported that sapu-sapu leaf essential oil was soluble in 96% ethanol at a ratio of 1:8, resulting in a clear solution [13]. The lower solubility observed in our samples suggests a higher content of unoxygenated terpenes and sesquiterpenes, which typically have lower solubility in ethanol [19].

3.3.3. Specific Gravity

Specific gravity is determined by comparing the weight of the oil to the weight of an equal volume of water at the same temperature. The specific gravity measurements were conducted at 25°C ± 0.5°C. As presented in Table 3, the specific gravity values for samples A1, B1, and C1 were 0.8863 g/mL, 0.8880 g/mL, and 0.8893 g/mL, respectively. These values did not show significant differences among the various harvest times. Our results are comparable to a previous study that reported a specific gravity of 0.878 g/mL for sapu-sapu leaf essential oil [13].

The specific gravity of an essential oil is an indicator of its purity and composition. Generally, a higher fraction of heavy molecular weight components in the oil results in a higher specific gravity. Conversely, an increase in specific gravity can also occur due to the presence of impurities or undesirable materials [20]. The consistency in specific gravity values across our samples suggests a relatively stable composition of the essential oil regardless of harvest time, though minor variations may exist.

3.3.4. Optical Rotation

The determination of optical rotation is derived from the value obtained by the essential oil in the polarization of light that is rotated to the right (*dextrorotary*) or the left (*laevorotary*). The chemical components in oil that are optically active can rotate the polarization field. This direction is a combination of the direction of rotation of the polarization field of each component [21]. Based on the data in Table 3, the optical rotation value of each sample includes the A1 sample of (+) 10.28, the B1 sample of (+) 9.28 and the C1 sample of (+) 11.06. The three samples produced a positive optical rotation (+), indicating that the components of the compound contained in it could rotate the polarization field to the right (*dextrorotary*). The direction and degree of rotation are essential to determine the purity characteristics of the oil. Other studies have stated that the magnitude of the optical rotation value in the oil depends on the type, concentration, measurement temperature, and the length of the path that the light travels through the compound [22]. The smaller the optical rotation value, the better the quality of the essential oil [23]. Essential oils of B1 samples, namely samples harvested during the day, have a smaller optical rotation value than A1 and C1 samples.

3.3.5. Refractive Index

The determination of the refractive index is carried out through a comparison between the speed of light in the air (sine of the angle of arrival) and the speed of light in a substance (sine of the angle of refraction) at a certain temperature using a refractometer [19]. Based on the data in Table 3, the refractive index values for samples A1, B1, and C1 were 1.474, 1.475, and 1.475, respectively. These results fall within the range of 1.471 to 1.475 specified by the Essential Oil Association (EOA) for essential oils, indicating that our samples meet the EOA requirements. Our findings are consistent with a previous study that reported a refractive index value of 1.474 for sapu-sapu leaf essential oil [13]. The consistency in refractive index values across our samples suggests that the harvest time did not significantly affect this property of the essential oil. This consistency may indicate that the fundamental composition of the oil, particularly in terms of double bonds and carbon chain lengths, remained relatively stable across different harvest times. The slight variation observed (0.001) between sample A1 and samples B1 and C1 is minimal. It may be attributed to minor differences in water content or slight variations in the proportion of compounds with different refractive properties. These results, combined with our findings on other physical properties and chemical composition, provide a comprehensive characterization of the sapu-sapu leaf essential oil and demonstrate its consistency across different harvest times in the first month of growth.

3.4. Effect of Harvest Time on Compound Composition

The composition of chemical compounds in the sapu-sapu leaf essential oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS). This technique separates components in a mixture through gas chromatography and provides precise mass spectra for each component. The GC-MS chromatograms and the percentage areas of the main compound compositions are presented in Figure 2 and Table 4, respectively.

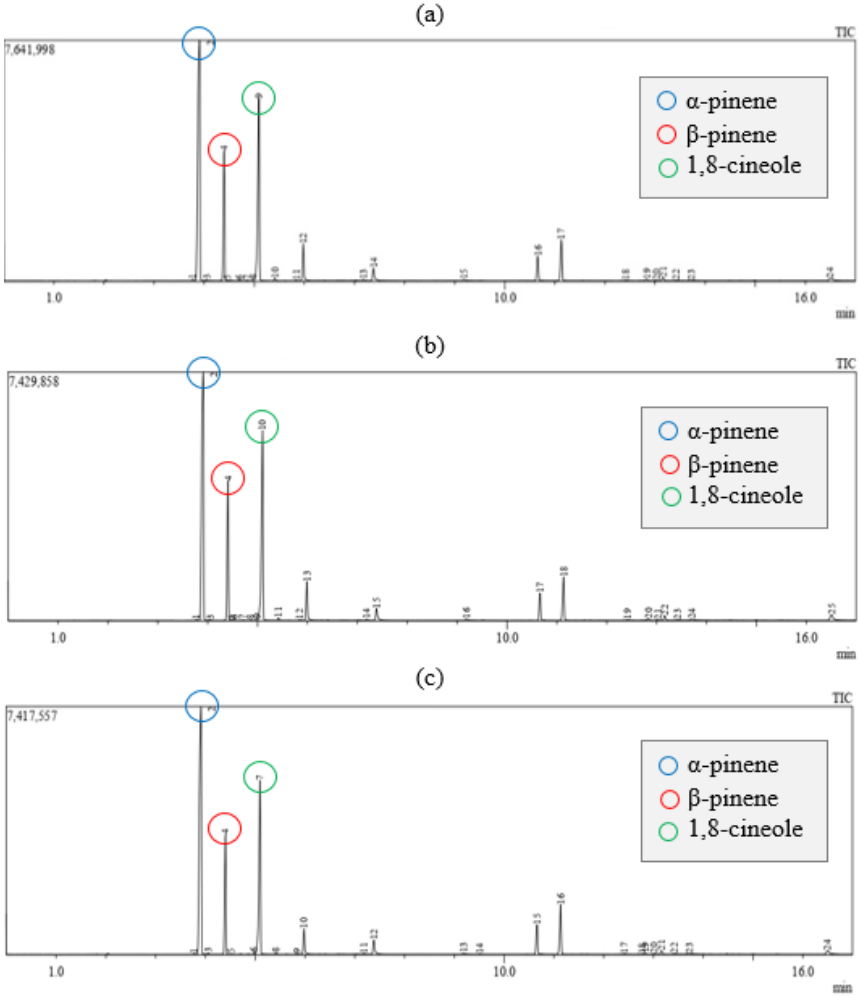

Figure 2. GC-MS chromatogram of (a) Sample A1, (b) Sample B1, and (c) Sample C1.

TABLE IV. Comparative Data on the Composition of Major Compounds at Different Harvest Times (First Month Samples).

Compound Name	A1	B1	C1
α-pinene	43.84	41.29	44.84
β-pinene	13.56	14.49	12.37
1,8-cineole	24.26	24.41	23.27
Total	**81.66**	**80.19**	**80.48**

GC-MS analysis identified 24 compound peaks in sample A1 (morning harvest, 07:30), 25 peaks in sample B1 (noon harvest, 10:30), and 24 peaks in sample C1 (24-hour harvest). While we focused on the three major components, it's worth noting that α-pinene, 1,8-cineole, and β-pinene were present only in the sample (A1/B1/C1), potentially serving as a marker for sapu-sapu essential oil harvested at this specific time. The three major compounds α-pinene, 1,8-cineole, and β-pinene, showed slight variations across harvest times:

- α-pinene : 43.84% (A1), 41.29% (B1), 44.84% (C1)
- 1,8-cineole : 24.26% (A1), 24.41% (B1), 23.27% (C1)
- β-pinene : 13.56% (A1), 14.49% (B1), 12.37% (C1)

The percentage differences among harvest times were relatively small: α-pinene (1-3.55%), β-pinene (0.93-2.12%), and 1,8-cineole (0.15-1.14%). This stability in composition might be attributed to consistent light intensity and temperature during the harvest period, which could maintain stable molecular formation of the essential oil components. However, further research is needed to confirm this hypothesis and explore the specific environmental factors influencing essential oil composition.

The morning harvest (A1) yielded the highest total composition of major compounds at 81.66%. Compared to a previous study reporting α-pinene (26.95%), β-pinene (21.55%), and 1,8-cineole (18.04%) as the main compounds [13], our samples showed higher percentages of α-pinene and 1,8-cineole, but lower β-pinene content. These differences might be due to variations in growing conditions, extraction methods, or genetic factors of the plant material used. While the harvest time of sapu-sapu leaf essential oil did not show statistically significant effects on yield, characteristics, and compound composition, subtle differences were observed. The morning harvest (A1) demonstrated slightly higher yield and a more pronounced yellow color, suggesting potential benefits of early harvesting. However, more comprehensive studies with replicated trials and statistical analysis are needed to confirm these observations and establish optimal harvesting practices for sapu-sapu leaf essential oil production.

4. CONCLUSIONS

The highest yield of essential oil of sapu-sapu leaves was obtained from the A1 sample, which was 0.74% at the time of harvest in the morning. The most optimal characteristics of the essential oil of sapu-sapu leaves were obtained from the A1 sample, namely the sample at harvest time in the morning which had a clear yellow color, a distinctive smell of sapu-sapu, a warm bitter taste, solubility in ethanol 96% 1:13, a specific weight of 0.8863 g/mL, an optical rotation (+) of 10.28 and a refractive index of 1.474. The total composition of the main compounds of the essential oil of the drooping sapu-sapu leaves was obtained from the A1 sample, namely the sample at harvest time in the morning of 81.66%, including α-pinene (43.84%), β-pinene (13.56%), and 1.8-cineole (24%).

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