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Effect of tree age on the yield, productivity, and chemical composition of essential oil from *Cinnamomum burmannii*

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ABSTRACT

One of the factors that influence the yield of cassia oil is the age of tree. Cassia bark is normally harvested at a tree age of 5 years old and continue to be harvested until 15 years of age. This study investigated the effect of tree age of *Cinnamomum burmannii* (5, 12 and 20 years old) on the yield, productivity and chemical composition of essential oil from leaf, branch and trunk bark. The essential oil was extracted using steam distillation and liquid-liquid extraction methods. The results showed that the optimum yield of *Cinnamomum burmannii* oil was obtained when the water content was in the range of 36-47%. The optimum yield of essential oils from the leaf was obtained at 1.36±0.31 wt% (5 years old) and for the branch and the trunk bark were obtained at 3.2±0.07 wt% and 2.95±0.30 wt% (both were 12 years old). Chemical composition of the essential oil was also analysed. The major components of *Cinnamomum burmannii* oil were determined as cinnamaldehyde (68.3%-82%), cinnamyl acetate (2.5%-16%), cinnamyl alcohol (2.25%-4.6%), and cinnamic acid (3%-8%). The productivity of essential oil was estimated at 336 kg/ha.year (5 years old), 577 kg/ha.year (12 years old) and 387 kg/ha.year (20 years old).

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1. Introduction

Cinnamon is a plant with aromatic properties and great demand in the food and additive market. Cinnamon product derivatives can benefit as a source of antimicrobial component for drugs, biopesticides, antifungal, as well as food preservatives. Sri Lanka, China, and Indonesia are known to be the main producers of cinnamon and cassia, with *Cinnamomum zeylanicum* being the most popular species. Indonesia has great potential for cinnamon production, with cinnamon exports reaching 55,027 tons out of 149,909 tons of total export, with a trade value of IDR 1.38 trillion in 2015 (TRADE MAP, 2017). The type of cinnamon that grows in Indonesia is *Cinnamomum burmanii*, or also known as cassia.

The value of cassia products from Indonesia is relatively lower than other countries such as China (Daswir, 2005). One method of increasing the product value is by extracting essential oils. Generally, essential oil from cassia (cassia oil) is synthesized and accumulated in the bark, leaves, calyx, and seeds (Ravindran et al., 2003). Cassia oil of bark is located in the cortex and phloem inside the inner bark (1-3 years), and inside the secondary phloem (4-11 years) (Geng et al., 2012). In leaves, essential oil is mainly synthesized inside the chloroplast. Essential oil is composed of volatile compounds with aromatic and antimicrobial properties. Cassia oil can be valued commercially for an amount of USD 30-35/kg (Coppen, 1995).

The yield and chemical composition of cassia oil varies in accordance to the part of the plants which are extracted. Cassia oil extracted from stem bark, branch bark, and leaves has the yield up to 4.5%, 2.38%, and 1.17% of sample dry weight, respectively (Krishnamoorthy et al., 1999; Rusli and Ma'mun, 1990). The main chemical constituents of essential oil from Indonesian cassia (cassia oil) is cinnamaldehyde, which can be extracted from bark (70-90%) and leaves (50-60%). Other terpene components such as eugenol, 1,8-cineole, α-terpineol, 4-ol, borneol, α-pinene, and β-caryophyllene are also present in cassia oil (Hasanah et al., 2003). Cassia oil is standardized according to its cinnamaldehyde content. The minimal content of cinnamaldehyde is 50% for National Standard of Indonesia (BSN/SNI. 06-3734-2006, 2005), 55-78% for USA Essential Oil Association (EOA) (Coppen, 1995), and 70% for international standard (IS/ISO 3216, 1997).

There are several factors which influence the yield of cassia oil, such as distillation method, moisture content, environmental, and biological aspects of the sample, such as microclimate condition and the age of tree. The growth phase of stem bark, branch bark, and leaves influence the amount of essential oil. The phase which gives the optimal yield of essential oil is within the accumulation and saturation of oil phase (Li et al., 2013). The optimal yield will vary with the difference of physiological age of each tree in different plant organs. Knowing the optimal age to extract cassia oil will give advantage as a reference in optimizing harvest strategy and increasing productivity in a larger production scale.

The aim of this research was to study the influence of tree age of *C. burmannii* on the yield and chemical composition of cassia oil extracted from stem bark, branch bark, and leaves. The analysis of microclimate and the physicochemical properties of soil were also conducted to support the experiment.

2. Results and Discussion

2.1. Yield of essential oil at different level of moisture content

The yield of essential oil based on the dry weight of leaves, stem bark, and branch bark was influenced by the change in moisture content (Fig. 1).

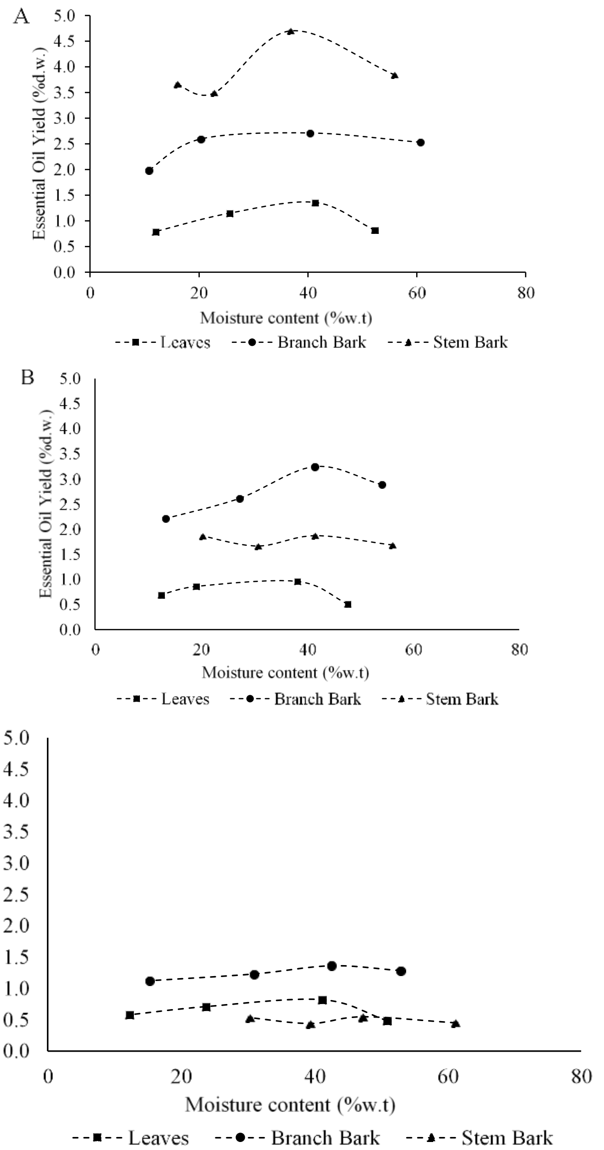

Fig. 1. Essential oil yield at different moisture content for *C. burmannii* tree at age of 5 years (A), 12 years (B), 20 years (C).

In general, the evaporation rate of essential oil correlates positively with the drying period, causing the decrease of essential oil content. However, Fig.1 shows that the essential oil content does not follow this trend. The optimum essential oil content from leaves, stem bark, and branch bark was achieved at a moisture content range of 36-47%, at a drying period of 24-48 hours. This shows a correlation that a certain range of moisture content from samples dried at room temperature (25^{o}C) and a short duration (24-48 hours) gave the optimum essential oil yield (Rahimmalek and Goli, 2013). However, the precise optimum moisture content depends on the difference of species, oil cell localization, and variation of compounds that constitutes the essential oil (Khangholi and Rezaeinodehi, 2008). According to another study, shade drying of *Warionia saharae* shows an optimal yield of essential oil (1.1%) at a moisture content range of 33-37% (Essaqui et al., 2016).

One of the factors inhibiting the evaporation of essential oil at the initial phase of shade drying was due to the mucilage and tissue structure of the plant organs. The mucilage, a water colloid and exopolysaccharide, which is present inside the transmembrane space of cells, caused inhibition of water and essential oil (Nobel et al., 1992). The presence of water, which bound certain volatile compounds, also contributed in inhibiting evaporation of essential oil in the drying and distillation process (Clifford et al., 2002). The above factors that caused inhibition tend to decrease due to an increase in the deterioration of mucilage which releases binding water (Ahmed et al., 2017; Singh and Bothara, 2014), change in tissue structure (Clifford et al., 2002), and the increase of enzymatic activity inside cells due to defensive response (Petrov et al., 2015). The yield of essential oil would increase until it reached an optimum point, which was then followed by the increase of evaporation, causing the amount of essential oil extracted by steam distillation to decrease.

2.2. Yield of essential oil from leaves, stem bark, and branch bark at various ages

Plants experience growth and development at a physiological, morphological, and molecular level as they grow older. Analysis of essential oil content from cinnamon trees at the age of 5, 12 and 20 years resulted in the difference of essential oil yield distribution from leaves, stem bark, and branch bark (Fig. 2).

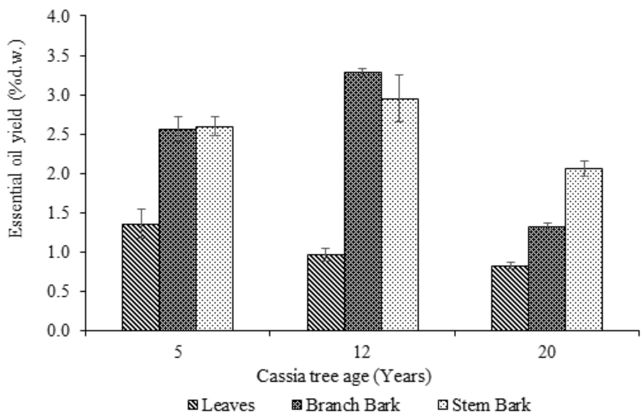

Fig. 2. Essential oil yield of *C. burmannii* from 5, 12, and 20 years old cassia tree.

The essential oil composition from *C. burmannii* and *C. cassia* are very diverse. *C. cassia* is used as a comparison due to its proximity to *C. burmannii* in physiology and bark morphology (Parry, 1969). Essential oil yield of leaves ranges from 0.5-1.2% (Wang et al., 2009), while the essential oil yield of cinnamon bark varies greatly within the range 0.5-5% (Geng et al., 2011; Hasanah et al., 2003; Krishnamoorthy et al., 1999; Xu et al., 2004). The samples used as a comparison were shade dried for ±3 weeks until they reached a moisture content range of 8-12% (Geng et al., 2011). These values were based on the ISO 939 standard on the maximum moisture content of cinnamon bark (National Institute of Industrial Research (India). Board of Consultants & Engineers., 2006). By comparing the standardized value and the results of observation, it was found that the yield range was still in accordance with data from various sources.

From three age variations (5, 12, and 20 years) of *C. burmannii* trees, it was shown that the essential oil yield from leaves decreased through age, whereas the essential oil yield of stem bark and branch reached its maximum yield at 12 years old (Fig. 2). As the main source of cinnamaldehyde production is in leaves, photosynthetic capacity is one of the indicators of metabolic activity. As the tree grows older, the width and number of branches of the tree will also increase. This causes the leaves, which occupy the area closer to the stem or distal region of the tree to draw further away from the sun. Photosynthetic capacity will increase in accordance to the surface area of the leaves that is exposed by sunshine, hence decreasing the carbon flux needed for producing primary and secondary metabolites (Kitajima et al., 2002). This phenomenon is similar to the decreasing trend of essential oil content through the aging of tree branches. As the tree branches grow older, the number of oil cells entering the degradation phase will increase relative to the number of oil cells in leaves from young tree branches (Li et al., 2013).

The experimental data indicates a small difference of essential oil yield from stem bark and branch bark at 5 and 12 years old, whereas the essential oil yield has a minimum value at 20 years old (Fig. 2). Contributing factors to the accumulation of essential oil inside stem bark and branch bark are not solely influenced by the age of the tree but is also the product of synergistic effects between several factors such as the secondary growth of trees and production of secondary metabolite due to the intense activation of tree defence mechanism at a young age. Secondary growth of the trees promotes differentiation of secondary phloem, a cellular space inside bark where oil cells are localized. The utilization of secondary metabolite of trees at young ages is a vital part of plant adaptation and mechanical defence through various environmental conditions (Chapin et al., 1993). The defence activity will decrease when the tree has a stronger defence mechanism, indicated by stable environment and secondary growth, which increases through the age of the tree (Paine et al., 2010).

2.3. Chemical composition of essential oil from leaves, stem bark, and branch bark

Essential oil extracted from samples of each age variety at the optimal moisture content range of 36-47% was analysed and the chemical composition is shown in Table 1. It was found that not all components from previous experiments were present in the essential oil, such as coumarin (1-11%), D-borneol (30-70%) and 1,8-Cineole (7-55%) (Ji et al., 1991; Woehrlin et al., 2010). However, the four main components commonly present in the volatile oil of cinnamon bark in Indonesia, cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, and cinnamic acid was identified. Cinnamaldehyde content lies in the range of 30-85% in essential oil extracted from cassia barks from various areas in Indonesia, such as Jambi, North Sumatra, Central Java and East Java (Batu, 2015; Pebrimadewi, 2011; Wijayanti et al., 2009; Yuliarto et al., 2012). On the other hand, essential oil from leaves contain cinnamaldehyde which lies in the range of 30-60% (Wang et al., 2009). Cinnamaldehyde content in this study is greater than the content of cinnamaldehyde (63.61%) in the essential oil attained from *C. burmannii* leaves of Indonesian varieties as reported by Tampubolon (2011).

Table 1. Chemical composition of essential oil from leaves, branch bark, and stem bark at 5, 12, and 20 years old tree.

Compound	Leaves fraction (%)		
	5 Years	12 Years	20 Years
trans-cinnamaldehyde	73,80	84,12	68,30
Cinnamyl acetate	16,10	2,97	5,59
Cinnamyl alcohol	3,98	2,97	3,80
trans-cinnamic acid	0.00	0.00	0.00
α-Linoleic acid	0.00	0.00	0.00
α-Copaene	0,60	0,56	0,46
Benzopyrene	0.00	0.00	10,00
Other components	5,52	9,38%	11,85
	100,00	100,00	100,00

Compound	Branch bark fraction (%)		
	5 Years	12 Years	20 Years
trans-cinnamaldehyde	77,06	84,71	77,10
Cinnamyl acetate	15,59	12,65	14,16
Cinnamyl alcohol	2,25	0.00	4,59
trans-cinnamic acid	0,18	0.00	0,26
α-Copaene	0,16	0.00	0,19
Other components	4,76	2,64	3,70
	100,00	100,00	100,00

Compound	Stem bark fraction (%)		
	5 Years	12 Years	20 Years
trans-cinnamaldehyde	79,25	81,61	75,97
Cinnamyl acetate	11,95	10,22	9,07
trans-cinnamic acid	4,21	3,81	8,60
α-Linoleic acid	2,44	0,04	0,03
α-Copaene	0,50	1,03	0,60
Other components	1,65	3,29	5,73
	100,00	100,00	100,00

The variation of chemical composition present in aromatic plants such as cassia trees are influenced by organ development (Akula and Ravishankar, 2011; Chapin et al., 1993; Geng et al., 2012). Differences in chemical composition between the results obtained in this study and previous reports were caused by the difference in age, species, and environmental conditions, such as geographical and microclimate conditions. However, in general, the main chemical components present in the essential oil were in accordance with the previous study by Batu (2015). In addition, the difference in soil type also played a role in effecting the components of essential oil. The type of soil grown by the tree at the age of 5 and 12 years is silt loam, whereas the 20 years old tree grows on clay (Table 2).

Table 2. Soil analysis of 5, 12, and 20 years old cassia tree.

Parameter	Unit	Age of Tree		
		5 Years	12 Years	20 Years
Moisture content	%	35,71	33,80	16,38
Texture				
- Sand	%	30,14	42,66	29,47
- Silt	%	64,58	51,58	14,82
- Clay	%	5,28	5,76	55,70
pH(H_2O)	-	7,12	7,28	7,18
C-Organic	%	1,84	2,01	3,42
Nitrogen (N)	%	0,26	0,22	0,38
Phosphorous (P)	mg/kg	362,45	282,99	221,32
Potassium (K)	mg/kg	80,17	68,66	74,47

The results of soil analysis results can be an indicator of the presence of cinnamic acid in the branch bark. A high fraction of cinnamic acid indicates a high lignin demand by plants. By comparing tree conditions at ages 5 and 12 with age 20, it was found that a much larger stem diameter at age 20 correlates to the volume of lignin needed to support xylem tissue. Thus, it can be inferred that at the age of 20, the need for lignin was much greater than in the previous ages, especially in supporting xylem rod tissue, considering that the xylem network of tree stem cannot produce the lignin precursor compound due to lignification process on xylem tissue forming cells.

The results show that the total fraction of cinnamaldehyde and trans- cinnamaldehyde was the highest in essential oil from 12 years old trees, followed by 5 years old trees and 20 years old trees. This large difference in component fraction supports the previous argument that the function of essential oil is closely related to plant defence against pests or weeds (Vijayan and Thampuran, 2003). As the plant reached maturity, the urgency to accumulate essential oil decreased, and diversification of compounds occurred which can be seen from the number of peaks resulted from the GC-MS analysis of essential oil from leaves, stem bark, and branch bark, which shows that the number of peaks increase as the trees grow older.

The content of cinnamyl acetate showed a decreasing trend with an increasing age of cassia tree. This showed the stability of citrate acetate regulation in cassia. The stability of this regulation related to the function of cinnamyl acetate as the attractant of insects in assisting the cinnamon flower fertilization (Jayaprakasha et al., 2003; Nishida, 2014). After the initial phase of flowering, which occurred when the tree reached around 5 years old, cinnamyl acetate content reached stability and decreased through age. On the other side, coumarin derivative (α-benzopyrone) was only identified in essential oil of the leaves attained from 20-year-old tree with an amount of 10%. Coumarin in essential oil from 5 years old and 12 years old trees are almost non-existent. Coumarin may raises health concern, as high consumption can cause hepatotoxicity (Loprinzi et al., 1997). The content of coumarin in essential oil of cassia leaves can reach up to 13.39% (Wang et al., 2009).

The essential oil of *C. burmannii* from leaves, stem bark, and branch bark at various ages met the standard requirement of cassia oil based on SNI 06-3734-2006 standard, with the content of cinnamaldehyde ≥ 55% and ISO 3216: 1997 standard with the content of cinnamaldehyde ≥70% (BSN/SNI. 06-3734-2006, 2005; Nguyen, 2003). In addition, the absence of coumarins in the sample was also an important point in meeting the requirements of existing essential oil standards.

2.4. Productivity estimation of essential oil

Essential oil productivity was estimated based on a mathematical model that uses a diameter breast height (DBH) data (Poudel et al., 2013). Based on the cultivation of cassia plants in PPTK Gambung, it was found that the average tree circumference of DBH for each age category was 35-40 cm (5 years), 60-70 cm (12 years) and 85-100 cm (20 years). All measurements were made at an altitude of 1.35 meters above ground level. The assumption of plant spacing was 2.2 m x 3.5 m, with a density of 1000 trees (5 years), 800 trees (12 years) and 625 trees (20 years) per hectare (Hasanah et al., 2003), and the result are shown in Table 3.

Table 3. Productivity estimation of essential oil from cassia tree at 5, 12, and 20 years old.

Parameter	Organ	5 Years	12 Years	20 Years
Mass d.w (kg/ha)	Leaves	9848.7	19183.2	21083.5
	Branch bark	7456.2	18288.6	22901.1
	Stem bark	41118.1	7191	7434.5
Yield of essential oil (%)	Leaves	1.36	0.96	0.82
	Branch bark	1.28	0.99	0.26
	Stem bark	2.60	2.95	2.06
Productivity of Essential oil (kg/ha-year)	Leaves	133.7	184. 6	173
	Branch bark	95.2	180.3	60.6
	Stem bark	107.0	212.1	153.1

The productivity of leaves, stem and branch bark dry weight increases with tree age. The productivity of essential oil from 8 years old trees can reach up to 7 kg/tree with the potential of essential oil production of 200-300 kg/ha from 2000 trees/ha (Nguyen, 2003). The essential oil productivity of *C. zeylanicum* and *C. verum* leaves, each grown in cinnamon fields in Thailand and India were 35 kg/ha-year and 100 kg/ha-year, respectively (CSIR, 2017). The productivity of essential oil estimated from each tree organ was in accordance to this value. Differences in productivity values were influenced by essential oil yield, estimation method, and harvest intensity.

3. Conclusion

The optimum yield of essential oil from the leaves was obtained at 1.36±0.31 wt% (5 years old) and for branch and trunk bark were obtained at 3.2±0.07 wt% and 2.95±0.30 wt% (both were 12 years old). The chemical composition of the essential oil was also analysed. The major components of *Cinnamomum burmannii* oil was determined as cinnamaldehyde (68.3%-82%), cinnamyl acetate (2.5%-16%), cinnamyl alcohol (2.25%-4.6%), and cinnamic acid (3%-8%). The productivity of essential oil was estimated at

336 kg/ha.year (5 years old), 577 kg/ha.year (12 years old) and 387 kg/ha.year (20 years old). Cinnamaldehyde content of the essential oil met the SNI and ISO commercial standard for cassia oil. Age of trees, as well as environmental and physiological factors produces a synergistic influence on the production of essential oil from leaves, branch bark, and stem bark of *C. burmannii* tree.

4. Materials and Methods

4.1. Materials

Stem bark, branch bark, and leaves samples were obtained from 5, 10, and 20 years old trees from The Centre of Tea and Quinone Research (PPTK) Gambung, South Bandung, Indonesia. Soil sample was taken 60 cm below ground surface of each tree for further analysis. Na_2SO_4 anhydrate, dichloromethane, and Whatman filter paper were obtained from Brataco Chemicals.

4.2. Methods

4.2.1. Shade drying of samples

Bark from stem and branches were peeled and dried at room temperature without exposure to direct sunlight with a relative humidity of 70-90%. Moisture content (%wt) of samples were measured using a moisture analyser on a daily basis for 5 days, with the following equation used:

$$MC\ (\%)= \frac{M_{initial}\ (g) - M_{final}(g)}{M_{initial}\ (g)} \times 100\% \quad (1)$$

where MC is moisture content (%), and m is mass of the sample (g).

4.2.2. Essential oil extraction

Stem bark, branch bark, and leaves were crushed and shredded with a blender and filtered until the size was ±36 mesh. 50 grams of stem bark and 60 grams of branch bark and leaves were distilled with steam distillation at a boiling temperature 96.5°C at 0.92 atm pressure and 780 m above sea level, with the condenser temperature at 0-10°C. Distillate collected inside the separator funnel had a milky white colour, indicating the presence of mixed essential oil and water. The distillation process was continued until the distillate collected was 200 mL or showed clear colour, indicating that most of the essential oil was extracted into the distillate.

Essential oil mixed in the distillate was further purified by liquid-liquid extraction with methylene chloride or dichloromethane (DCM) at a ratio of 1:1 as the solvent and shaken inside the separator funnel. After the distillate showed a clear colour, the mixed solution of essential oil and dichloromethane were then stirred with Na_2SO_4 anhydrate as a drying agent to remove the remaining water inside the mixture. DCM was vaporized using a rotary evaporator at a temperature of 50°C until the yellow colour of cassia oil was shown, which was then collected inside a vial and weighed.

4.2.3. Quantification of essential oil yield

Essential oil yield was determined based on the mass of the essential oil relative to the dry weight (DW) of the samples. Essential oil yield (Y) is quantified with this expression:

$$Y\ (\%)= \frac{M_{essential\ oil}\ (g)}{M_{sample}(g).(100\%\text{-}MC(\%))} \times 100\% \quad (2)$$

4.2.4. Chemical composition analysis of essential oil

Chemical composition of the volatile constituents of essential oil was analysed with Gas Chromatography-Mass Spectrometry (GC-MS) Agilent GC MSD which was operated at a temperature of 40-280°C, and used a glass column of 5% phenyl methyl silox with a dimension length of 30 m, diameter of 0.25 mm, and thickness of 0.25 μm. One μL of sample was diluted with 999 μL chloroform and injected inside the GC-MS with splitless mass mode and a split ratio of 100:1. Compound identification is done using the NIST library.

4.2.5. Productivity estimation of essential oil

Biomass of leaves, stem and branch bark from each tree was estimated with an allometry expression obtained from biomass estimation of *Cinnamomum tamala* (Poudel et al., 2013).

Leaf biomass (W_{leaf}),

$$W_{leaf}\ (kg)= -13.671+2.8725 \times DBH(cm) \quad (3)$$

Stem bark biomass ($W_{stem\ bark}$),

$$W_{stem\ bark}\ (kg)= \alpha\times[-5.8194+1.27\times DBH(cm)] \quad (4)$$

Branch bark biomass ($W_{branch\ bark}$),

$$W_{branch\ bark}\ (kg)= -23.0700+[3.5204\times DBH(cm)] \quad (5)$$

Productivity of essential oil (P) was then estimated by correlating mass of each sample with yield of essential oil (Y) and harvest intensity ($N_{harvest}$).

$$P\left(\frac{kg}{ha.yr}\right) = W_{sample}\ (kg)\ x\ N_{harvest}\ (\frac{trees}{ha.yr})\ x\ Y\ (\%) \quad (6)$$

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Conflict of Interest

The authors declare no conflict of interest.

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