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Harvest time and plant age on the content and chemical composition of the essential oil of *Alpinia zerumbet*

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ABSTRACT

Alpinia zerumbet is an aromatic and medicinal plant rich in essential oil, known as colônia. Essential oils are derived from secondary metabolism and may be a source of raw materials for cosmetic, pharmaceutical, food and perfumery industry. The plant secondary metabolism and biosynthetic activity can vary according to endogenous and exogenous factors to which it is exposed. In this context, in this study we evaluated the influence of harvest time and plant age of *Alpinia zerumbet* on biomass and essential oil production. For the harvest time experiment the plants of *A. zerumbet* were harvested at different times (8, 10, 12, 14, 16 and 18 h), using a completely randomized design with four replications. In the plant age experiment the seedlings were propagated by division of rhizomes and grown in a completely randomized design with treatments consisting of four ages (3, 6, 9 and 12 months after transplanting), with seven replications. The extractions of the essential oil were performed by oil hydrodistillation in Clevenger apparatus and chemical analysis by gas chromatography coupled with mass spectrometry (CG-MS). There was an effect of harvest time on the essential oil content with the highest value (0.48%) found at 14:33 h with no change in the chemical composition. In relation to plant age, there was a significant increase in aboveground biomass of plants, accompanied by increases in height, number of shoots, and essential oil content and yield. The major compound terpinen-4-ol was present in higher concentrations in plants harvested between six and nine months old.

Keywords: Zingiberaceae, 'colônia', medicinal plant, GC analysis, terpinen-4-ol.

RESUMO

Horário de colheita e idade da planta sobre o teor e a composição química do óleo essencial de *Alpinia zerumbet*

Alpinia zerumbet é uma planta medicinal e aromática rica em óleo essencial, conhecida popularmente como 'colônia'. Os óleos essenciais derivam do metabolismo secundário e podem ser fonte de matérias-primas para as indústrias de cosméticos, farmacêutica, alimentícia e de perfumaria. O metabolismo secundário e a atividade de biossintética de uma planta podem variar de acordo com fatores endógenos e exógenos aos quais ela está exposta. Neste contexto, o presente estudo objetivou avaliar a influência do horário de colheita e da idade de plantas de *Alpinia zerumbet* sobre a produção de biomassa e de óleo essencial. Para avaliar os horários de colheita, as plantas de *A. zerumbet* foram colhidas em diferentes horários (8, 10, 12, 14, 16 e 18 h), em delineamento inteiramente casualizado com quatro repetições. No experimento sobre idade da planta as mudas foram propagadas por divisão de rizomas e cultivadas em delineamento inteiramente casualizado com os tratamentos constituídos pela colheita em quatro idades (3, 6, 9 e 12 meses após o transplante das mudas), com sete repetições. As extrações de óleo essencial foram realizadas por hidrodestilação em aparelho de Clevenger e a análise química por meio da técnica de cromatografia gasosa acoplada ao espectrômetro de massa. Houve efeito do horário de colheita sobre o teor de óleo essencial com maior valor (0,48%) encontrado às 14:33 h, mas sem alteração da composição química. Em relação à idade da planta, verificou-se um aumento significativo da biomassa da parte aérea das plantas, acompanhada pelo aumento em altura, quantidade de brotos emitidos, teor e rendimento do óleo essencial. O composto majoritário terpinen-4-ol esteve presente em maior concentração nas plantas colhidas entre os seis e nove meses de idade.

Palavras-chave: Zingiberaceae, 'colônia', planta medicinal, análise CG, terpinen-4-ol.

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Alpinia zerumbet is a species of Asian origin, from Zingiberaceae family, which is grown in all the Brazilian states, mainly as an ornamental plant, and also for medicinal use. This species is popularly known in the northeastern Brazil as 'colônia' or 'leopoldina' and is used for home treatment of hypertension and also as calming and diuretic (Lorenzi & Matos, 2008). Among confirmed pharmacological properties which show the medicinal importance of this species are the hypotensive effects (Barcelos *et al*., 2010), antimicrobial activity (Stefanini *et al*., 2009) and antinociceptive activity (Pinho *et al*., 2005).

The plant is herbaceous, perennial, reaches up to 2.5 m high, presents rhizomes with short aboveground stem, lanceolated leaves on disposal couplet, acute base and apex cuspidate, pubescent on the edges, short-petiolated, with long open sheaths in which a pseudostem is originated (Albuquerque & Neves, 2004). The leaves present scattered oil cells in the mesophyll and abaxial epidermis (Albuquerque & Neves, 2004) in which the essential oils, rich in oxygenated monoterpenes, are produced and stored, the main compounds being terpinen-4-ol, 1,8 cineol and γ-terpineol (Stefanini *et al*., 2009).

Factors related to medicinal plant growing like plant spacing, cutting height, seson, age and harvest time may influence directly in the essential oil production and, therefore, in its pharmacological properties. Some studies state the influence of these factors in aromatic species like *Cymbopogon citratus* (Nascimento *et al*., 2003), *Lippia alba* (Santos *et al*., 2004), *Cymbopogon winterianus* (Blank *et al*., 2007) and *Aloysia triphylla* (Brant *et al*., 2009). However, studies on plants of Zingiberaceae family are still rare. Phytochemical studies have advanced considerably, but the same is not true for agronomic studies. That is why the researches related to the agronomic aspects became essential (Innecco *et al*., 2003) to guarantee a regular supply of a great amount and quality of raw material for industries.

In this context, this study evaluated the influence of harvest time and plant age of *Alpinia zerumbet* on biomass and essential oil production.

MATERIAL AND METHODS

The experiment was carried out in the Universidade Estadual de Santa Cruz, Ilhéus, Southern Bahia state (14°26'S, 39°02'39°30'W), Brazil. The plant material was properly identified and the voucher specimen is registered in HUESC herbarium under number 14088.

Two experiments were carried out separately:

Harvest time – *A. zerumbet* leaves were harvested at different times (8, 10, 12, 14, 16 and 18 h) to evaluate the essential oil content and chemical composition. The experiment was set in a completely randomized design, with six treatments and four replications.

Daily, between 6 and 17 h, the photosynthetically active radiation (PAR) was monitored with a light radiation sensor S-LIA-M003, coupled to a meteorological station Hobo Micro Station Data Logger (Onset, USA), while the temperature (Ta) and air relative humidity (RH) were recorded using a microprocessed sensor Hobo H8 Pro Series (Onset, USA).

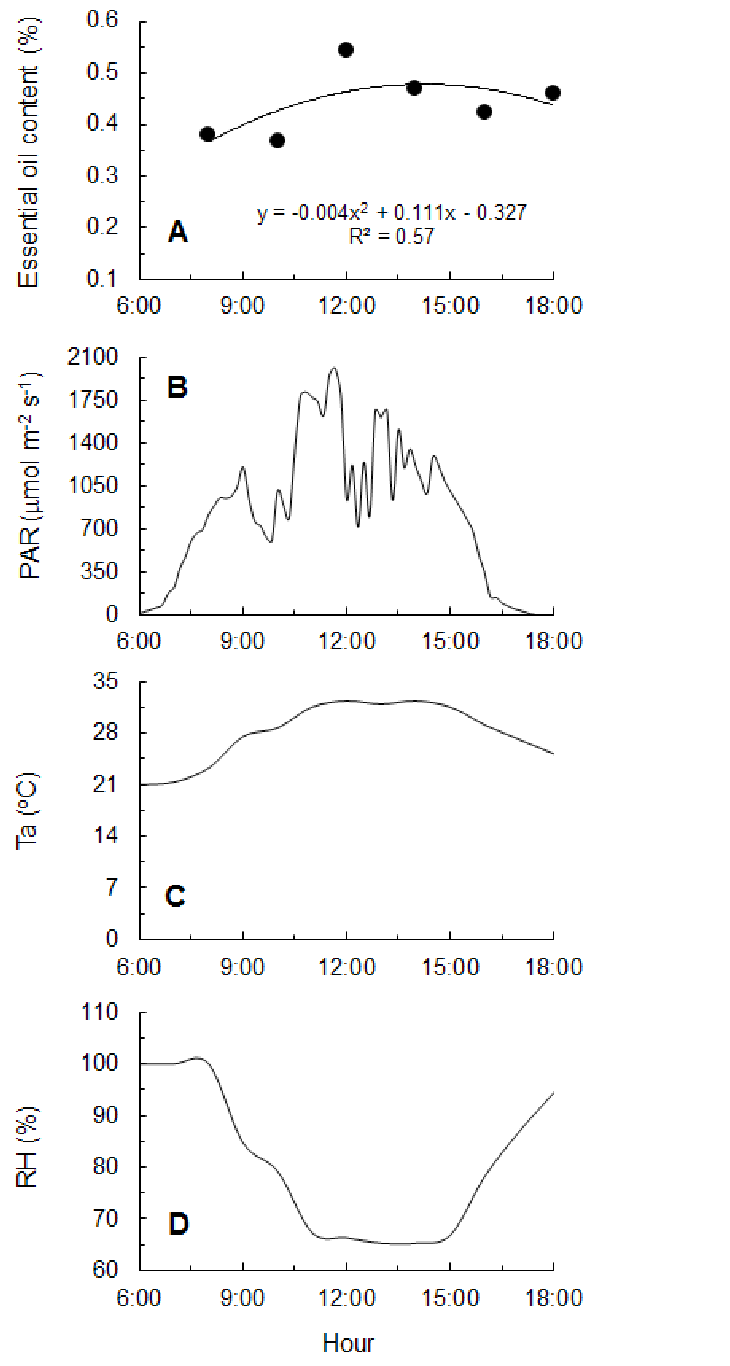

Figure 1. Essential oil content of *A. zerumbet* in different harvesting times (A), photosynthetic active radiation (PAR) (B), air temperature (Ta) (C) and relative humidity (RH) (D) throughout the day (teor de óleo essencial *de A. zerumbet* nos diferentes horários de colheita (A), radiação fotossinteticamente ativa (PAR) (B), temperatura do ar (Ta) (C) e umidade relativa do ar (RH) (D) ao longo do dia). Ilhéus, UESC, 2010.

Plant age - The 'colônia' seedlings were obtained through the division of rhizomes, previously grown in sand beds and subsequently transplanted to field. The experiment was carried out in a completely randomized design with four treatments corresponding to plant ages (3, 6, 9 and 12 months) and four replications with seven plants. The plant height, the number of shoots higher than 10 cm, aboveground dry biomass divided into leaves and pseudostem, the content and chemical composition of the essential oil of the leaves were evaluated.

For both experiments, the extraction

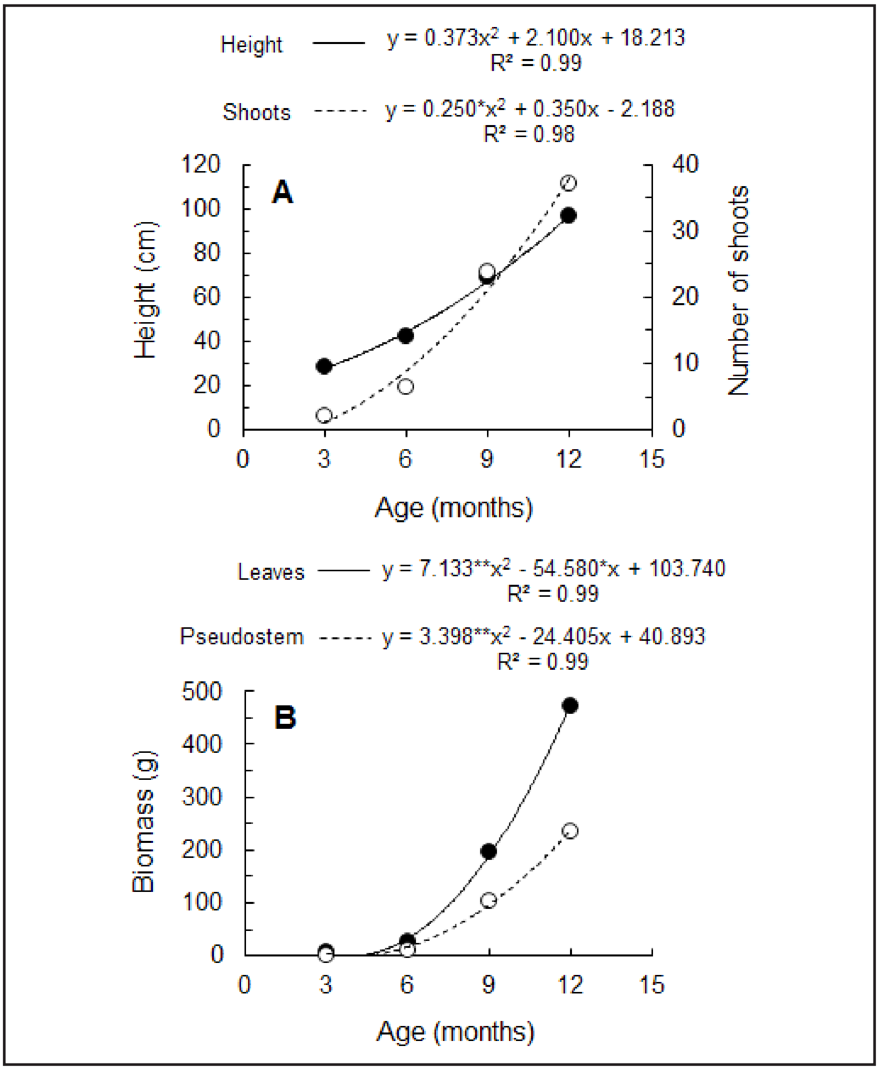

Figure 2. Height and number of shoots (A) and aboveground biomass (B) of *A. zerumbet* harvested at different plant ages (altura e número de lançamentos (A) e biomassa seca da parte aérea (B) de *A. zerumbet* em diferentes idades de colheita). Ilhéus, UESC, 2010.

of essential oil was performed through hydrodistillation, using a Clevenger apparatus, with 100 g of leaf dry biomass, in quadruplicate, for one hour. The hydrolate obtained was extracted through liquid-liquid partition with dichloromethane and, dried with anhydrous sodium sulfate.

The essential oil was analyzed by gas chromatography, using Varian Saturn 3800 apparatus equipped with a flame ionization detector (GC-FID), using VF-5ms capillary column (30 mm x 0.25 mm x 0.25 µm film thickness) (helium as gas drag, flow of 1.4 mL/min. The injector and detector temperatures were 250 and 280°C, respectively. The column temperature programming began with 60°C, followed by an increase of 8°C/min up to 240°C, keeping the temperature during five minutes. 1 µL of solution at 5% of oil in chloroform was injected in the 1:10 *split* mode. The concentration of the volatile constituents was calculated based on the full area of their respective peaks, related to the total area of all the constituents of the samples. The qualitative analysis of the essential oil was carried out using mass spectrometer Chromopack 2000/MS/MS. The same VF-5ms column and the same column programming were used. The transferline temperature was 250°C and the trap temperature was 220°C. The mode of operation was electron impact at 70eV. Various chemical constituents of the essential oil were identified through computer comparison to the apparatus library, literature and Kovats retention index (Adams, 1995). Kovats retention indices were calculated injecting a series of patterns of n-alkanes (C_8-C_{26}), injected in the same chromatographic conditions of the samples.

The data were subjected to the regression analysis of the different variables depending on the time of the day and the plant age, according to the experiment.

RESULTS AND DISCUSSION

The essential oil content of *A. zerumbet* showed a quadratic tendency in relation to the harvest time (Figure 1A). The lowest values were observed in the morning, followed by an increase in the afternoon, reaching the maximum concentration at 14:33 h (0.48%). This behavior is possibly related to a diurnal variation of climatic conditions such as PAR, Ta and RH (Figures 1B, 1C and 1D). The higher values of the oil contents occurred at the same time when PAR and Ta were higher and RH was lower. This result may be associated with terpene production that occurs in the chloroplasts through methyl-erythritol-phosphate via and it is dependent on photosynthesis (Reis & Mariot, 2000). Temperature, relative humidity and total duration of sun exposure can act as a direct influence on the production of the essential oil (Simões & Spitzer, 2000). Similar result was observed in *Cymbopogon winterianus* in which, specifically in the rainy season, the increase of the essential oil content at noon followed by a decrease at 17:00 h was associated to the diurnal variation of temperature and light (Blank *et al*., 2007), as well as in *Ocimum basilicum* in which the joint influence of the season and harvest time was verified (Silva *et al*., 2003).

According to a general consensus, essential oils generally reach their highest contents in the morning (Marchese & Figueira, 2005), however, with the advancement of the researches, different responses have been verified, depending on each species. For *Cymbopogon citratus*

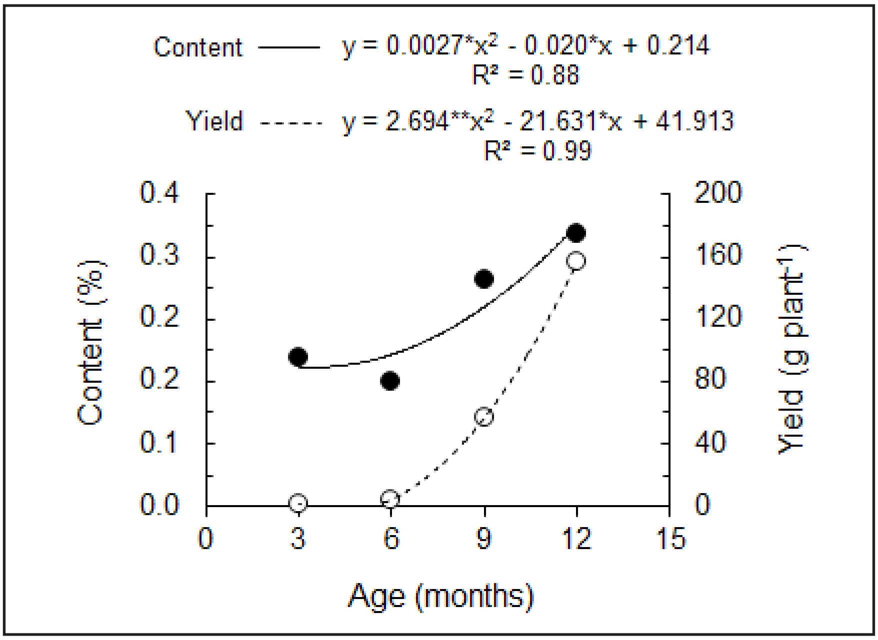

Figure 3. Content (%) and yield of the essential oil of *A. zerumbet* (g plant^{-1}) at different plant ages of harvest (teor (%) e rendimento de óleo essencial de *A. zerumbet* (g planta^{-1}) nas diferentes idades de colheita). Ilhéus, UESC, 2010.

(Nascimento *et al.*, 2003), *Ocimum selloi* (Gonçalves *et al.*, 2009) and *Cordia verbenacea* (Souza *et al.*, 2009), the highest content of essential oil was found in the morning. Nevertheless, for *Lippia alba* and *Rosmarinus officinalis* (Santos *et al.*, 2004; Gonçalves *et al.*, 2009), as well for the studied species, the highest essential oil content occurred in the afternoon.

The essential oils obtained in different harvest times had similar chemical characteristics (Table 1). Sixteen compounds in the oil were identified, the majority being terpinen-4-ol, followed by 1,8-cineol, γ-terpinene and sabinene. The harvest time did not interfere in the concentration of the essential oil of *A. zerumbet* chemical constituents as well as reported for *Cymbopogon winterianus* (Blank *et al.*, 2007) and *Ocimum basilicum* (Silva *et al.*, 2003).

Depending on the plant age, increase in height and in number of shoots was verified with quadratic fit. These results showed maximum values of 96.43 cm of height and 37 shoots after 12 months of transplant (Figure 2A). The production of leaves and pseudostem biomasses also showed a quadratic fit along the evaluated period but with a greater production of leaves than pseudostem biomass, showing averages of 473.6 g plant^{-1} and 234.8 g plant^{-1}, respectively, at twelve months old (Figure 2B). With the development of the plant, in ideal growing conditions, occurred a greater investment on the production of leaves, which are responsible for the synthesis of photoassimilates. This proportional increase in amount of leaf dry mass along the time has been reported in other aromatic species such as *Cymbopogon winterianus* (Marco *et al.*, 2006), *Cymbopogon citratus* (Leal *et al.*, 2003) and *Lippia alba* (Steffanni *et al.*, 2006).

The content and yield of the essential oil of 'colônia' showed quadratic response according the plant age with a great increase (Figure 3). Twelve months of evaluation were not enough to identify the production peak. For *Cymbopogon winterianus* harvested at 4, 6 and 8 months, a greater essential oil yield was obtained in the last two seasons due to a higher biomass accumulation (Marco *et al.*, 2006). However, these results may vary according the species, as in other aromatic plants like *Ocimum selloi* (Costa *et al.*, 2007) and *Ocimum gratissimum* (Rocha *et al.*, 2005), the maximum oil production was verified at 117 and 83 days, respectively, followed by a decrease, whereas for *Cymbopogon citratus* (Leal *et al.*, 2003), there was a decreased production of oil with aging of the plant.

Most of the species studied are annual with a shorter life cycle which allows to monitor the entire phenological process. This study, however, was not possible for 'colônia' since it is a perennial species and even the evaluation period being a relatively long one (12 months), was not enough to reach the plant flowering stage, which, possibly, could influence the essential oil production.

The plant age was a major interference factor in the chemical composition of essential oil of 'colônia'. At three months old only four compounds were identified, one of them, γ-cadinene which disappeared subsequently. At six and nine months old plants, the composition is very similar concerning the identification of 19 compounds, including the major component terpinen-4-ol with higher contents at twelve months old, when more other four components come out (myrcene, δ-2-carene, α-terpinene and terpinolene) and an increase in contents of 1.8-cineol and γ-terpinene is noticed (Table 2).

In the conditions in which the experiment was carried out, it was possible to verify that the harvest time of *Alpinia zerumbet* interfered in the essential oil content without changing its chemical composition. One year old seedlings showed increase in height, shoot emission and dry biomass of leaf and stem. Parallel increase was also verified for the essential oil content with some noticeable variations in chemical composition with a greater concentration of terpinen-4-ol between six and nine months. No flowering was observed in one year of evaluation which was not also enough to determine the highest value of production of the essential oil.

Table 1. Relative concentration (%) of the chemical constituents of the essential oil obtained from dry biomass of 'colônia' leaves harvested at different times (concentração relativa (%) dos constituintes químicos do óleo essencial obtido da biomassa seca de folhas de 'colônia' colhidas em diferentes horários). Ilhéus, UESC, 2010.

Compounds	Harvest time (hours)						
	*IK	8	10	12	14	16	18
α-thujene	929	2.73	3.09	3.37	3.20	3.05	3.69
α-pinene	939	1.09	1.24	1.34	1.31	1.22	1.45
Sabinene	977	11.88	11.90	15.08	14.08	11.52	13.56
β-pinene	984	3.82	3.98	4.15	4.08	3.91	4.19
α-terpinene	1021	3.56	3.42	4.09	4.17	4.49	4.16
p-cimene	1029	3.58	5.67	4.64	4.52	5.85	5.54
Limonene	1034	1.98	1.89	2.02	2.01	1.93	2.02
1,8-cineol	1039	18.08	17.21	17.74	17.21	18.68	17.67
ɣ-terpinene	1062	13.87	13.30	13.76	14.94	13.24	14.62
Cis-sabinene hydrate	1075	2.56	1.87	2.59	2.59	2.21	1.72
Terpinolene	1087	2.02	1.90	2.12	2.20	1.89	2.11
Trans- sabinene hydrate	1098	1.24	1.18	1.16	1.26	1.24	1.16
Linalool	1104	3.04	2.33	3.02	3.14	2.72	2.02
Cis-β-terpineol	1148	0.75	0.79	0.67	0.69	0.76	0.67
Terpinen-4-ol	1187	23.86	25.83	20.23	20.55	24.08	22.23
(E)-caryophyllene	1427	0.68	-	0.81	1.15	0.69	0.62
Monoterpenes		44.53	46.39	50.57	50.51	47.10	51.34
Oxygenated monoterpenes		49.53	49.21	45.41	45.44	49.69	45.47
Sesquiterpenes		0.68	-	0.81	1.15	0.69	0.62
Total identified (%)		94.71	95.60	96.79	97.10	97.48	97.52

*IK= Kovats experimental index = not detected (indice experimental de Kovats= não detectado)

Table 2. Relative concentration (%) of the chemical constituents of essential oil obtained from dry biomass of 'colônia' leaves harvested at different ages (concentração relativa (%) dos constituintes químicos do óleo essencial obtido da biomassa seca de folhas de 'colônia' colhidas com diferentes idades). Ilhéus, UESC, 2010.

Compounds	Plant age (months)				
	*IK	3	6	9	12
β-pinene	982	-	0.69	0.78	4.43
Mircene	990	-	-	-	0.75
δ-2-carene	995	-	-	-	0.47
α-terpinene	1021	-	-	-	1.31
p-cymene	1030	-	5.23	5.67	5.98
Limonene	1034	-	-	0.46	1.43
1,8-cineol	1040	-	8.66	9.29	13.97
ɣ-terpinene	1063	-	0.54	0.57	8.1
Trans-4-tujanol	1076	-	2.57	2.69	3.36
Terpinolene	1088	-	-	-	1.37
Cis-4-thujanol	1149	-	2.45	2.54	1.68
α-fenchol	1106	-	3.54	3.65	4.22
Terpineol	1131	-	2.56	2.54	1.88
Cis-β-terpineol	1149	-	2.06	2.12	1.41
Terpinen-4-ol	1192	-	52.63	53.01	38.28
α-terpineol	1201	-	4.35	4.26	2.89
Trans-pulegol	1215	-	1.16	1.15	0.8
Sabinene hydrate acetate	1249	-	0.95	0.60	1.08
Bornyl acetate	1288	-	0.77	0.79	0.50
(E)-caryophyllene	1427	-	2.34	2.08	2.07
E-nerolidol	1562	2.27	0.52	0.41	0.43
ɣ-cadinene	1562	5.76	-	-	-
Longiborneol	1593	27.06	4.43	3.81	3.03
α-eudesmol	1655	9.12	0.55	0.36	0.47
Monoterpenes			11.48	12.71	28.88
Oxigenated monoterpenes			67.25	75.91	64.53
Sesquiterpenes		44.21	8.61	7.45	6.50
Total identified (%)		44.21	87.34	96.07	99.91

IK= Kovats experimental index = not detected (indice experimental de Kovats= não detectado).

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