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Investigation on chemical composition and optimization of essential oil obtainment from waste *Pinus taeda* L. using hydrodistillation

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ABSTRACT

The extraction of essential oils obtained by the hydrodistillation of needles/twigs waste of Pinus taeda L. was optimized by applying response surface methodology (RSM), with 2^4 full factorial design, in order to improve oil essential production, and aggregate value to the production chain of pine wood. Through the model it was possible to ascertain the influence of the variables in the average amount of essential oil (0.1032 mL), being the variables analyzed: biomass – Bm (x_1), extraction time – ET (x_2), Bm:ET (x_1x_2) and sample size – SS: drying times – DT (x_3x_4). Only linear terms (biomass and extraction time) and your interaction demonstrated significant positive values (0.0344, 0.0206 and 0.0131). The major components of the essential oil identified by GC-MS were: β-phellandrene: (30.39 and 22.44%), tricyclene (26.14 and 20.46%), β-myrcene (14.32 and 11.50%), β-pinene (22.49 and 1.43%) and α-pinene (0.25 and 11.26%) in the years 2011 and 2012, respectively. Our results show that the essential oil obtained from P. taeda represents a way of using some of the waste generated by the timber industry. The process of obtaining doesn't require treatments such as controlled drying or size reduction of the sample, indicating that it can be used in an industrial scale.

Keywords: Pinus, byproduct, essential oil, response surface methodology (RSM), mathematical modeling.

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INTRODUCTION

Essential oils are natural volatile organic complexes that may contain 100 or more compounds. They are characterized by a strong odor, and are responsible for the protection and fragrance of many aromatic plants (Morais et al. 2006; Castro et al. 2004). Essential oils can be synthesized as secondary metabolites by plant parts such as flowers, leaves, fruits, seeds, roots, rhizomes and stems, being stored in secretory cell cavities, channels, epidermal cells or glandular trichomes (Bakkali et al. 2009; Jin et al. 2007).

The components of essential oils may belong to several class of compounds, but terpenes and some other aromatic and aliphatic constituents are the most common, all of them being characterized by a low molecular weight (Castro et al. 2004; Zeng et al. 2012). Chemical compositions and contents of essential oils in plants vary depending on the plant, season, geographical location, harvesting technique, and genotype (Bendaoud et al. 2009; Botrel et al. 2010).

Essential oils are widely used in the industry. Currently, about 3000 essential oils are known, of which 300 are commercially important, being added to food, cosmetics, perfumes, pharmaceuticals, sanitizers and agricultural products. In those products, they act as flavoring, fragrances, fragrance fixatives, antioxidant, antimicrobial, antiparasitic, virucidal, insecticide, and adjuvant in pharmaceutical and oral compositions marketed in their raw or processed form (Bakkali et al. 2009; Jalali-Heravia et al. 2010; Bizzo et al. 2009).

The genus *Pinus*, family *Pinaceae*, is the largest known genus of conifers. With 250 species, it occurs naturally in the Northern Hemisphere and has been cultivated in the temperate regions of the Southern Hemisphere. Pines are evergreen, resinous plants that are often woody, arboreal, varying from 3 to 50 m (Sonibare and Olakunle 2008). Their trunk is straight and cylindrical, their leaves and branches have a cone shaped arrangement, and their leaves are needle-shaped, grouped in fascicles (Morais et al. 2005).

The species introduced in Brazil come mainly from the United States. Among those, *Pinus taeda* L. stands out for its productivity and wood quality (Morais et al. 2005; Souza et al. 2008). *P. taeda* L. has high commercial value and is cultivated in the southern region of Brazil (Carvalho et al. 1998). The wood of this tree is used to make paper and timber, but after harvesting the wood, most of the tree, including twigs and needles, is discarded or burned to generate heat energy for its own industry. Obtaining the essential oil (bioproduct) from this pine would represent a way to utilize some of its byproducts (Oliveira et al. 2006; Ustun et al. 2012; Almeida et al. 2010).

Considering the exiguous literature reports about obtention of essential oils from the byproducts of needles/twigs of *P. taeda* and few reports of their characterization and chemical composition, the aim of this work is to optimize the process using steam hydrodistillation of twigs/needles and carrying out the characterization of the volatile compound of the essential oil. In this regard, this work aims to improve the utilization of the industrial byproducts of the chain production of wood from this pine.

MATERIAL AND METHODS

Plant Materials

Fresh needles/twigs of *P. taeda* L. were collected, randomly, in April 2011 and April 2012 in same place under the same conditions, in the city of Palmas, southwestern region of the state of Paraná - Brazil, 29°29’ S and 51°59’ W, at an altitude of 1,115 m. The voucher specimen of the plant (nº HPB 371) was prepared and deposited at the herbarium of the Federal Technological University of Paraná (UTFPR). After collection, the samples were transported in hermetic plastic bags to the laboratory and were dried at room temperature.

Experimental Design

It was employed an experimental design, consisting of 16 experiments with three replications at the central point, with four independent variables: amount of biomass (Bm) (X_1), extraction time (ET) (X_2), sample size (SS) (X_3) and drying time (DT) (X_4), these were transformed into coded variables (x_1, x_2, x_3 and x_4), respectively. Dependent variable (Y_1) was the amount of essential oils obtained in mL. The study of the effect of the different factors on the response was carried out using Statistics for Experimenters – Box, Hunter & Hunter complete factorial design (2^4) (Box et al. 1978). The experiments were performed in a random order to minimize the effect of the uncontrolled variables (Sereshti et al. 2011; Burkert et al. 2004).

Mathematical Model

The model used is represented below (1):

$$Y_1 = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ijk} x_i x_j x_k + \varepsilon \quad (1)$$

where Y_1 is dependent variable – amount of oil (mL), β_0 represents the intercept and β_i, β_j and β_k correspond to linear and interaction term effect coefficients, respectively, and ε is an error term (Teófilo and Ferreira 2006; Safaralie et al. 2010; Alcântara et al. 2010).

Statistical Analysis

Optimization of conditions, regression coefficients and analysis of variance (ANOVA) were determined by Statistica v.9.0 Software.

Obtaining the Essential Oil

Essential oil was obtained following experimental design (RSM), from leaves and twigs/needles fresh and/or dried at room temperature and protected from light. The sample sizes (SS) were 2, 3 and 4 cm, (same ratio of leaves and twigs/needles), and first trials of hydrodistillation occurred within 24 hours after collection. New extractions were done with drying times (DT) of the plant material of 48 and 72 hours. The use of this variable is justified based on the sample humidity analysis carried out in three replications (57.26±0.28, 48.49±1.38 and 45.02±0.63% for 24, 48 and 72 hours of drying, respectively). The others variables were extraction time (ET) varying 1.0, 1.5 and 2.0 h and the amount of biomass (Bm) 20, 30 and 40 g. Samples were air dried and mixed with 500 mL of distilled water and the essential oil was obtained by hydrodistillation in a Clevenger type apparatus. Essential oils were extracted from the aqueous phase using ethyl ether (P.A.) and dried over anhydrous sodium sulphate. Essential oil content was determined on a volume to dry weight basis. After extraction, the samples were placed in glass vials with Teflon-sealed caps at 4 ºC in the absence of light and were kept refrigerated prior to the quantitative and qualitative analysis by gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). Yield was calculated using the relationship between the essential oil volume obtained and the dry plant material mass used in the extraction (Eq. 2) (Lago et al. 2008).

$$Y_2 = (v_{eo}/m_{dpm}) * 100 \quad (2)$$

where Y_2 is the yield in %, v_{eo} the essential oil volume obtained (mL) and m_{dpm} the dry plant material mass (g).

Composition of the Essential Oil

Identification of volatile constituents of essential oil from the needles/twigs of *P. taeda* by hydrodistillation was performed using a gas chromatograph–mass spectrometer model Shimadzu© GC–MS QP 2010 Plus, fitted with a Shimadzu AOC-20i auto sampler, and GC–MS solution version 2.0 software (Shimadzu, USA). The GC–MS measurements were performed using a capillary column Rtx®-5MS (5% diphenyl + 95% dimethyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 µm film thickness) and carried out using split/splitless injection, with the injector set at 220 ºC, the column set at 60 ºC, with a heating ramp of 3 ºC/min, a final temperature of 240 ºC and the detector set at 250 ºC, with injection of 1 µL of sample. Helium (He) was used as the carrier gas at 1 mL/min. GC–MS electron ionization system was set at 70 eV. Quantitative analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (GC–FID) under the same conditions previously described. GC–FID quantification was obtained using chromatograms and was expressed as the mean from three samples of each extracted essential oil. Before the injection, all samples were diluted to ethyl acetate (20 mg/mL) for the analyses. Retention indices (RI) were determined by injection of hydrocarbon standards and essential oil samples under the same conditions (Budel et al. 2012). The oil compounds were identified by comparison with data from literature (Adams 2007) and the profiles from the mass spectral libraries (Wiley and NIST).

RESULTS AND DISCUSSION

Studies of the Effects, Factors and their Interactions

Factor levels were chosen considering the operating limits of the experimental apparatus based on pre-defined ranges in the literature, considering the conditions of steam hydrodistillation (Ammar et al. 2010). Each independent parameter tested is presented in Table 1, with their respective symbols and actual levels, and coded as low level (-1), high level (+1) and center point (0).

Table 1. Level of the un-coded and encoded independent variables used in the experimental design

Independent variables	Symbol	Levels coded		
		-1	0	+1
Bm^A (g)	X_1	20	30	40
ET^B (h)^C	X_2	1.0	1.5	2.0
SS^D (cm)	X_3	2.0	3.0	4.0
DT^E (h)	X_4	24	48	72

	Experiments	x_1	x_2	x_3	x_4	Y_1 (mL) ^{F}
	1	-1	-1	-1	-1	0.05
	2	+1	-1	-1	-1	0.10
	3	-1	+1	-1	-1	0.05
	4	+1	+1	-1	-1	0.18
	5	-1	-1	+1	-1	0.10
	6	+1	-1	+1	-1	0.12
	7	-1	+1	+1	-1	0.07
	8	+1	+1	+1	-1	0.20
	9	-1	-1	-1	+1	0.05
	10	+1	-1	-1	+1	0.10
	11	-1	+1	-1	+1	0.10
	12	+1	+1	-1	+1	0.21
	13	-1	-1	+1	+1	0.05
	14	+1	-1	+1	+1	0.10
	15	-1	+1	+1	+1	0.09
	16	+1	+1	+1	+1	0.10
Central Points	17	0	0	0	0	0.10
	18	0	0	0	0	0.09
	19	0	0	0	0	0.10

^{A} Bm: Biomass; ^{B} ET: Extraction Time; ^{C} Beginning of the extraction time, corresponding to the fall of the first drop of hidrolate; ^{D} SS: Sample Size; ^{E} DT: Drying Time; ^{F} Amount of oil.

The mathematical statistical model, which represents the response function, is equivalent to Equation (3). In the canonical equation fitted to the experimental data, the dependent variable Y_1 is the amount of oil (mL), in which significant terms at the 5% level are shown with an asterisk.

$$\begin{aligned}
Y_1 = {} & \underset{(\pm 0.0041)}{0.1032^*} + \underset{(\pm 0.0045)}{0.0344^* x_1} + \underset{(\pm 0.0045)}{0.0206^* x_2} \\
& - \underset{(\pm 0.0045)}{0.0006 x_3} - \underset{(\pm 0.0045)}{0.0044 x_4} + \underset{(\pm 0.0045)}{0.0131^* x_1 x_2} \\
& - \underset{(\pm 0.0045)}{0.0081 x_1 x_3} - \underset{(\pm 0.0045)}{0.0069 x_1 x_4} - \underset{(\pm 0.0045)}{0.0094 x_2 x_3} \\
& + \underset{(\pm 0.0045)}{0.0044 x_2 x_4} - \underset{(\pm 0.0045)}{0.0144^* x_3 x_4} - \underset{(\pm 0.0045)}{0.0044 x_1 x_2 x_3} \\
& - \underset{(\pm 0.0045)}{0.0106 x_1 x_2 x_4} - \underset{(\pm 0.0045)}{0.0044 x_1 x_3 x_4} - \underset{(\pm 0.0045)}{0.0056 x_2 x_3 x_4}
\end{aligned} \tag{3}$$

It was found that only Bm (x_1), ET (x_2), Bm:ET (x_1x_2) and SS:DT (x_3x_4) were significant at the 5% level, with p values lower at 4%. All terms significance, except for the SS:DT (x_3x_4) had positive coefficients, indicating that they positively influence the amount of essential oil.

Quality of the equation obtained was verified through ANOVA (Table 2), in which the model is significant at the 5% level (p = 2.5%), with experimental and adjusted coefficients of determination equal to 96.80 and 85.61%, respectively, and a non-significant regression deviation (p = 5.2%), confirming the validity of the model for predictive purposes.

Table 2. Analysis of variance for the response variable

	Degrees of freedom (*df*)	Sum of Squares (SS)	Mean Squares (MS)	*F-value*	$F_{tab.}$	*p-value*
Model	14	0.03854	0.00275	8.65^A	5.87	0.025
Linear	4	0.02603	0.00651	20.46^A	6.39	0.006
Interaction	10	0.01251	0.00125	3.93^B	5.96	0.100
Residual error	4	0.00128	0.00032	-	-	-
Lack-of-fit	2	0.00121	0.00060	18.10^B	19.00	0.052
Pure error	2	0.00007	0.00003	-	-	-
Total	18	0.03981	-	-	-	-

$R^2 = 0.9680$ / $R^2_{adj} = 0.8561$

^{A} Significant at the 5% level; ^{B} Not significant at the 5% level.

Considering that the model was validated, it was used to generate the response surfaces, with the objective to optmize the process. Figure 1 shows the effects of the main variables Bm (X_1) and ET (X_2), whereas the other variables, SS (X_3) and DT (X_4), were fixed at their optimum. The boundary region of the response surface demonstrates that there is more essential oil when variables Bm (X_1) and ET (X_2) are increased.

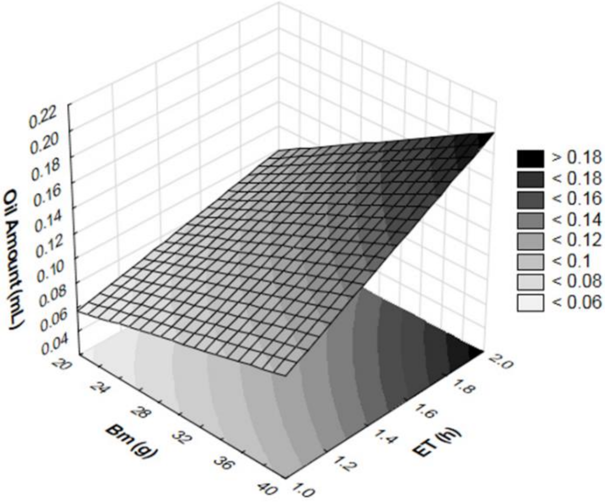

Figure 1. Effects of Bm (X_1) and ET (X_2) in the amount of essential oil obtained, with SS (X_3) and DT (X_4) fixed at their optimum values.

In Figure 2, in which the surface was generated by the variables SS (X_3) and DT (X_4) setting Bm (X_1) and ET (X_2) at their values optimum, it can be observed that it is not necessary to reduce the size of the sample when drying the raw material for 24 h, because these procedures do not significantly influence in the amount of essential oil.

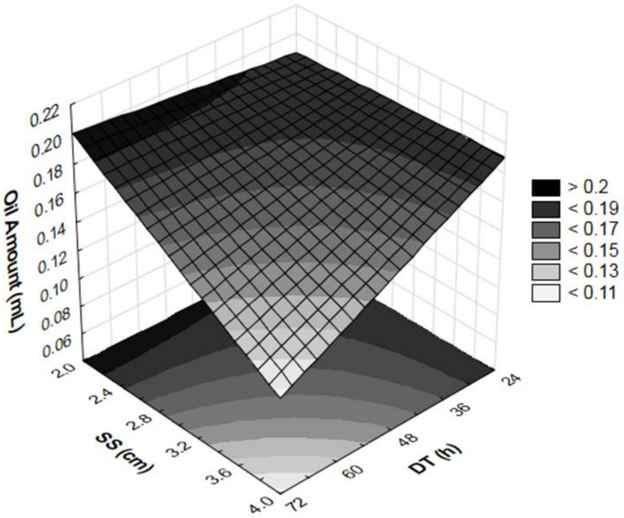

Figure 2. Effects of SS (X_3) and DT (X_4) in the amount of essential oil obtained, with Bm (X_1) and ET (X_2) fixed at their optimum values.

Chemical Composition of the Essential Oil

In order to enable a comparison with the chemical composition of the essential oil obtained in 2011 and 2012, samples were collected in place under the same conditions and their data are shown in Table 3, together with experimental retention indices (RI), including their percentage peak areas. The characterization of the essential oil from 2011 to 2012 was carried out with the samples that showed higher amount of essential oil determined by RSM (40 g – Bm, 2 h – ET, 2 cm – SS and 72 h – DT). We chose to investigate only the optimal condition for obtaining the essential oil of the same species at different times, since the other experiments may have shown different qualitative and quantitative chemical composition.

Table 3. Chemical composition of the essential oil of *P. taeda* L. collected in 2011 and 2012.

Nº	Components	(%) Composition		RI^A		RI^B
		2011	2012	2011	2012	
1	Tricyclene	26.14	20.46	926	928	926
2	*α*-Pinene	0.25	11.26	939	943	939
3	Camphene	0.91	-	953	-	954
4	Sabinene	0.04	-	976	-	975
5	*β*-Pinene	22.49	1.43	980	979	979
6	*β*-Myrcene	14.32	11.50	991	992	990
7	*δ*-2-Carene	0.07	-	1001	-	1002
8	*β*-Phellandrene	30.39	22.44	1031	1030	1029
9	*γ*-Terpinene	0.05	-	1062	-	1059
10	*p*-Mentha-2,4(8)-diene	0.55	-	1086	-	1088
11	Linalool	0.04	-	1098	-	1096
12	Exo-Fenchol	0.04	-	1117	-	1121
13	*γ*-Terpineol	1.04	-	1189	-	1199
14	Linalool acetate	-	2.14	-	1248	1257
15	Bornyl acetate	0.11	0.37	1285	-	1288
16	*α*-Terpinyl acetate	-	0.32	-	1346	1349
17	*γ*-Himachalene	-	0.50	-	1484	1482
18	Germacrene A	0.06	-	1503	-	1509
19	*γ*-Cadinene	-	0.74	-	1507	1513
20	Zonarene	-	1.50	-	1539	1529
21	Spathulenol	0.96	3.24	1580	1589	1578
22	*epi*-*α*-Cadinol	-	2.13	-	1647	1640
23	*epi*-*α*-Muurolol	-	4.22	-	1649	1642
24	*α*-Muurolol	-	2.57	-	1651	1646
25	*α*-Cadinol	1.02	5.08	1658	1660	1654
26	Manool	0.18	-	2056	-	2057
27	Docos-1-ene	-	0.10	-	2176	2189
28	*n*-Docosane	-	0.21	-	2198	2200
	Monoterpenes					
	Hydrocarbons	95.21	67.09			
	Oxygenated	1.23	2.83			
	Sesquiterpenes					
	Hydrocarbons	0.06	2.74			
	Oxygenated	1.98	17.24			
	Diterpenes					
	Oxygenated	0.18	-			
	Other	-	0.31			
	Identified	98.66	90.21			
	Not identified	1.34	9.79			
	TOTAL	100	100			

Notes: ^{A} Calculated Retention Index; ^{B} Tabulated Retention Index available in literature (Adams 2007).

Essential oil yield (Y_2) obtained from the needles and twigs of *P. taeda* varied between 0.25-0.53% (results of 2011 and 2012). Content of essential oil, expressed in absolute value, taking into account the experimental design performed in 2011, consisting of 19 trials, with four independent variables (amount of biomass, extraction time, sample size and drying time), varying between 0.05-0.21 mL. Essential oils from 2011 and 2012 showed 28 components. In 2011 were identified 18 components which represent 98.66% of the total, and in 2012, 18 components were identified, representing 90.21%. However, 1.34% and 9.79% of the total oil remained unidentified in 2011 and 2012, respectively (Table 3). The non-identified compounds have also revealed typical mass spectra of hydrocarbons monoterpenes with molecular ions in *m/z* 136; hydrocarbons sesquiterpenes with molecular ions in *m/z* 204; oxygenated sesquiterpenes with molecular ions in *m/z* 218, 220 and 236.

These values are consistent with those reported in previous studies conducted with other species of *Pinus*, with yields ranging from 0.02 to 2.33% (w/w or v/w) (Sonibare and Olakunle 2008; Dob et al. 2005; Macchioni et al. 2003; Amri et al. 2012; Rezzi et al. 2001; Kurose et al. 2007; Abi-Ayad et al. 2011), the amount of compounds detected in the order of 22 to 100 (Sonibare and Olakunle 2008; Dob et al. 2005; Macchioni et al. 2003; Amri et al. 2012; Rezzi et al. 2001; Kurose et al. 2007; Abi-Ayad et al. 2011; Stevanovic et al. 2005; Petrakis et al. 2001) and the proportion of compounds identified from 67.0 to 99.1% (Sonibare and Olakunle 2008; Dob et al. 2005; Macchioni et al. 2003; Amri et al. 2012; Rezzi et al. 2001; Abi-Ayad et al. 2011; Stevanovic et al. 2005; Petrakis et al. 2001).

The chemical composition of the essential oil had a high proportion of monoterpenes (96.44% in 2011 and 69.92% in 2012), due to the high concentration of hydrocarbon monoterpenes (95.21% in 2011 and 67.09% in 2012). The *β*-phellandrene was the major component, representing 30.39% (in 2011) and 22.44% (in 2012), followed by tricyclene (26.14%), *β*-pinene (22.49%) and *β*-myrcene (14.32%) in 2011; and tricyclene (20.46%), *β*-myrcene (11.50%) and *α*-pinene (11.26%) in 2012. According to Pagula and Baeckström (2006) the main fraction of the essential oils from pine species is monoterpene hydrocarbons (*α*-pinene, camphene, *β*-pinene, *δ*-3-carene, *β*-myrcene, limonene and *β*-phellandrene), which is in agreement with published results. Researches with *Pinus* species show that the main components of the oils are monoterpenes, mainly the hydrocarbon type, composing 60-70% of the oil, followed by oxygenated monoterpenes, sesquiterpenes oxygenated and hydrocarbons (Amri et al. 2012; Stevanovic et al. 2005; Petrakis et al. 2001).

Oxygenated monoterpenes were also detected (1.23% in 2011 and 2.83% in 2012) in *P. taeda*. Other compounds, such as sesquiterpenes (hydrocarbons and oxygenated) and diterpenes are represented in minor quantities (2.22%), in the 2011. On the other hand, in analyzes made with the essential oil obtained in 2012, other compounds, such as sesquiterpenes hydrocarbons and oxygenated were represented in considerable amounts (2.74% and 17.24%, respectively). Some identified compounds have very similar chemical structures ((21) spathulenol; (22) *epi*-*α*-cadinol; (23) *epi*-*α*-muurolol; (24) *α*-muurolol and (25) *α*-cadinol), therefore the chromatogram had to be expanded in the region of retention times of those peaks according Figure 3. Sesquiterpenes (hydrocarbons and oxygenated) as *β*-caryophyllene, *α*-cadinene, *γ*-cadinene, *γ*-muurolene, *α*-humulene, *α*-muurolol, *α*-cadinol and caryophyllene oxide, are usually detected in essential oils of pines in low amounts (Pagula and Baeckström 2006). This did not occur with the sample of 2012, *α*-muurolol (2.57%), *α*-cadinol (5.08%), *epi*-*α*-muurolol (4.22%) and *epi*-*α*-cadinol (2.13%) were detected.

Differences in the concentration of essential oil constituents can vary according to genetic, physiological and climatic factors, type of soil and extraction technique (Bakkali et al. 2009). Méndez-Tovar et al. (2016) emphasizes that environmental factors influence the composition of essential oils, and must be taken into account. Another factor to consider is that the essential oil was obtained from residual plant material (twigs and needles) which, after cutting the tree trunk, were deposited on the ground exposed to inclement weather. As reported by Bier et al. (2016) the low concentration of some constituents in the waste may be due to the results for the essential oil of *Pinus* being obtained from fresh samples and not from agricultural residues from forest soil.

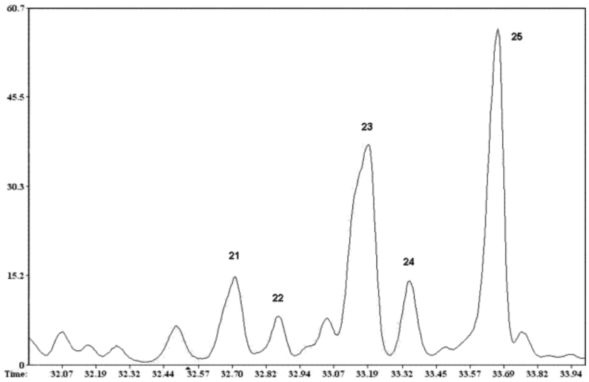

Figure 3. Chromatogram in expanded view of the essential oil from *P. taeda* L. collected in 2012.

The mass spectra corresponding to each compound in the expanded chromatogram were inserted in the Figure 4 (A-E), where mass spectra corresponding to peaks 21 (spathulenol), 22 (*epi*-*α*-cadinol), 23 (*epi*-*α*-muurolol), 24 (*α*-muurolol) and 25 (*α*-cadinol), respectively.

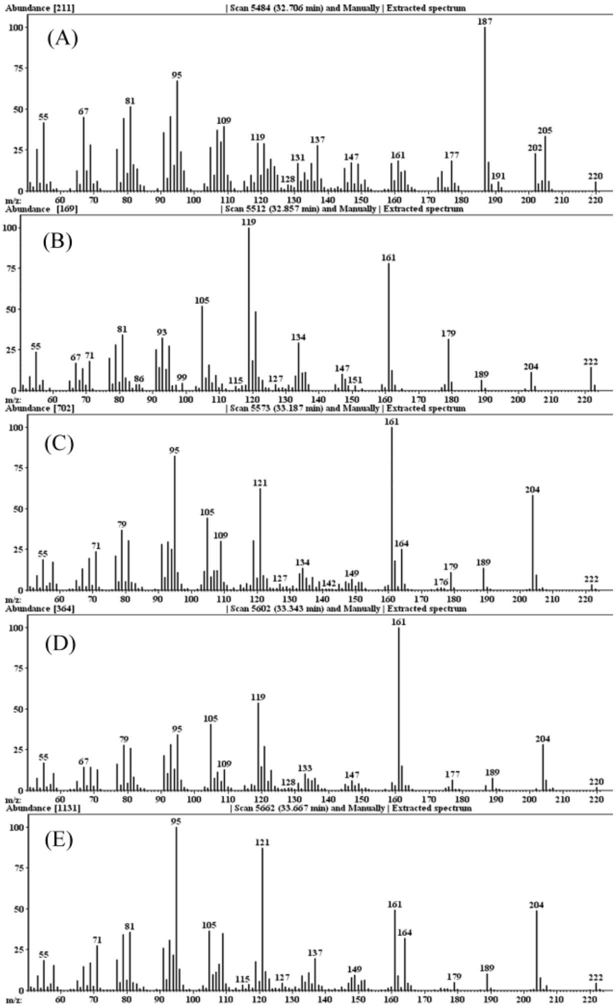

Figure 4. Mass spectrum of the spathulenol (peak 21) (**A**); *epi*-α-cadinol (peak 22) (**B**); *epi*-α-muurolol (peak 23) (**C**); α-muurolol (peak 24) (**D**); α-cadinol (peak 25) (**E**).

Bier et al. (2016) reports studies (forest wastes) of the volatile composition of residues are rare in the literature. The residues of *P. taeda* (needles) and *P. ellioti* (wood shavings) was study for the use as source of terpenoids (limonene, α-pinene and β-pinene) by pressurized liquefied petroleum gas (LPG) extraction, at yields of 2.32% for the pine needle and 0.6% for the pine wood shavings, obtaining 0.48 and 0.38% of limonene; 0.57 and 2.93% of α-pinene; 7.37 and 6.34% of β-pinene, respectively. However, the LPG extraction displayed less decomposition or modification of the compounds. Adams et al. (2014), evaluated the essential oil composition from forestry byproducts of *P. taeda* in mid-April to mid-September 2013. The major components of the oil were α-pinene (36.61 to 42.48%), β-pinene (11.96 to 14.70%), limonene (1.55 to 4.90%), terpineol (2.78 to 7.14%), and caryophyllene (7.24 to 10.23%). The camphene, β-myrcene, β-phellandrene and β-caryophyllene oxide, were not detected in any month. As occurred in the present work with some components. Furthermore, the results demonstrated that the essential oils had antimicrobial activity against four *Staphylococcus aureus* strains (Adams et al. 2014).

Pagula and Baeckström (2006) analyzed essential oil composition of fresh needles from *P. taeda* grown in Mozambique. In their report, 27 compounds were identified, totalizing 95.0% of identified compounds at yield of 0.83% of oil. The major components of the essential oil identified were: tricyclene (3.8%), α-pinene (62.3%), β-pinene (7.1%), limonene (2.0%), β-myrcene (1.8%) and β-phellandrene (3.7%), respectively. Which is in agreement with published results of Table 3, with exception of limonene (not detected).

In their research, Kurose et al. (2007) analyzed the chemical composition of essential oil from leaves and cones of eleven species of *Pinus*, including *P. taeda*. Essential oil of the *P. taeda* had 35 compounds formed mainly by monoterpenes (98.7%) and sesquiterpenes (0.7%), α-pinene being the major component (51.8%), followed by *p*-mentha-1,5-dien-8-ol, verbenone, (both amounting 4.8%) and β-pinene (3.8%). Tricyclene, β-myrcene and β-phellandrene were also present, however, in lower proportions than those obtained in this work, being 0.8, 0.3 and 0.2%, respectively.

In others pine species the composition of oil from needles/twigs/cones of four species (*Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra*) from Italy (Macchioni et al. 2003), five species (*P. brutia*, *P. halepensis*, *P. nigra*, *P. pinea*, and *P. sylvestris*) from Turkey (Ustun et al. 2012) and five species (*P. attenuata*, *P. heldreichii*, *P. peuce*, *P. pinaster* and *P. radiata*) from Greece (Petrakis et al. 2001) were analyzed. With exception of two species from Greece (*P. peuce* and *P. pinaster*), all the other species presented β-pinene (0.7 to 47.5%), α-pinene (6.2 to 96.5%), β-myrcene (0.2 to 42.1%) and β-phellandrene (0.3 - 7.5% and traces). Tricyclene (0.1-0.5% and traces) was not detected in one specie from Italy (*P. pinea*) and some from Turkey (*P. halepensis*, *P. nigra*, *P. pinea*, and *P. sylvestris*). The chemical composition of the essential oils of *P. nigra* (Rezzi et al. 2001), *P. caribaea* (Sonibare and Olakunle 2008), and *P. mugo* (Stevanovic et al. 2005) have also been previously investigated. β-

pinene was present in all species, amounting 0.5 to 25.1%, *β*-phellandrene from 1.1 to 67.9% and *α*-pinene from 5.4 to 70.0%. Moreover, tricyclene was detected only in *P. mugo* (0.4%) and *β*-myrcene in *P. nigra* and *P. mugo* (0.7 to 30.0%). High concentration of *β*-phellandrene in the oil suggests that it might work as a fragrance because of its pleasing aroma (Sonibare and Olakunle 2008). However, further studies are required to determine the applicability, cost, safety and toxicity of these bioproducts.

The composition of the essential oil of *P. halepensis* (Dob et al. 2005) and *P. pinea* (Amri et al. 2012) showed *β*-pinene (0.2 and 3.4%), *α*-pinene (1.2 and 7.7%) and *β*-myrcene (3.1 and 2.7%), respectively. Only 0.36% of *β*-phellandrene was detected in *P. pinea* and not detected in *P. halepensis*, whereas tricyclene was found in both species in traces. Abi-Ayad et al. (2011) did not detect any of the five main compounds of *P. taeda* in *P. halepensis*.

CONCLUSION

Twigs and needles are wasted because there is no information on what can be done with them and how to do so. The model allowed us to demonstrate the influence of the variables on the amount of essential oil obtained, showing that the linear factors Bm (x_1) and ET (x_2) and the binary combination Bm:ET (x_1x_2) had a positive effect, whereas SS:DT (x_3x_4) had a negative one. We demonstrated, through the RSM methodology, that in order to obtain the essential oil there is no need to decrease the size of the sample or controlled drying. Results indicate a great chemical variability in the essential oil of *P. taeda* L., with high proportion of monoterpenes, while other compounds such as sesquiterpenes and diterpenes are minority. Main constituents of the essential oil obtained in 2011 and 2012 were *β*-phellandrene, tricyclene, *a*-pinene, *β*-myrcene and *β*-pinene.

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