Journal of Applied Sciences

ISSN 1812-5654

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 12 (8): 753-760, 2012
ISSN 1812-5654 / DOI: 10.3923/jas.2012.753.760

Seasonal Variation in the Yield and the Chemical Composition of Essential Oils from Two Brazilian Native Arbustive Species

[1]A.V.M. Castelo, [1]C.H.S. Del Menezzi and [2]I.S. Resck
[1]Department of Forest Engineering, Faculty of Technology, Campus Darcy Ribeiro,
University of Brasília, P.O. Box 04357-70904-970, Brasília, DF, Brazil
[2]Institute of Chemistry, University of Brasília, Campus Darcy Ribeiro, 70910-900, Brasilia, DF, Brazil

Abstract: This study analysed the influence of seasonality on the production and composition of essential oils from *Blepharocalyx salicifolius* (Kunth.) O. Berg and *Psidium myrsinites* DC, which are very common and useful species of the Brazilian Savannah (Cerrado). Steam distillation technique was used to obtain these oils and to investigate their chemical composition, ^{1}H and ^{13}C nuclear magnetic resonance, gas chromatography, mass spectrometry and infrared techniques were employed. The average yields of essential oil were 0.18 and 0.26% from *B. salicifolius* and *P. myrsinites*, respectively; both species tended towards higher production in the dry season. Both oil mixtures were composed primarily of monoterpene and sesquiterpene compounds. The monoterpene linalool was found on the essential oil from *Psidium* species.

Key words: Seasonal variation, linalool, ^{1}H/^{13}C NMR

INTRODUCTION

Secondary metabolites, including essential oils, comprise a class of products related to the physiological development of plants. They represent a chemical interface between the plants and the environment and therefore, their synthesis is often affected by environmental conditions (Kutchan, 2001), such as water and nutrient availability (Alsafar and Al-Hassan, 2009; Azizi and Kahrizi, 2008; Pirzad *et al*., 2006; Supanjani *et al*., 2005) and growing habitat (Verma *et al*., 2011; Azizi and Kahrizi, 2008; Mosayebi *et al*., 2008). Additionally, phenology and harvesting age have been identified as important effects on the essential oil yield (Okoh *et al*., 2007; Ozguven *et al*., 2006; Ayanoglu *et al*., 2005; Telci and Sahbaz, 2005).

According to Brant *et al*. (2008), plant species belonging to the same botanical family do not present a similar behavior as function of environmental conditions. Therefore, it is not possible to establish a pattern and then various environmental factors can differentially alter the quantitative and qualitative aspects of essential oils. As one of the many factors that may influence the characteristics of essential oils, the climatic variations that occur over the course of a year have been the focus of many researchers attempting to identify the most appropriate time of the year for optimal extractions in terms of yield and/or compound concentration. When the set of climatic factors in seasonal climates with two well-defined seasons is modified, these variations act on the plants and generally alter their metabolism (Scherer, 2007).

In research on essential oils, plants of the Myrtaceae family have proven to be of great importance, largely because they naturally exhibit storage structures for essential oils called translucent dots (Joly, 2002). However, in Brazil, the effect of seasonal fluctuations on the chemical composition and yield of essential oils is still unknown for the vast majority of the species in this family in their native environments (Souza, 2009). In general, knowledge about the forest species that produce these oils is still lacking and is especially sparse for the Cerrado (Brazilian savannah), a biome exclusive to Brazil that includes more than 11,000 species of phanerogamic plants.

This study analysed the essential oils of two species of plants from the Myrtaceae family native to the cerrado, the maria preta (*Blepharocalyx salicifolius* (Kunth.) O. Berg) and the araçá do cerrado (*Psidium myrsinites* DC). The study focused on describing the quantitative and qualitative behaviour of essential oil production as a function of the climatic variations that mark the typical season changes observed in Midwestern Brazil.

MATERIALS AND METHODS

Material collection: The samples were collected from a region of the *Stricto sensu* Cerrado, an experimental plot

---

Corresponding Author: C.H.S. Del Menezzi, Department of Forest Engineering, Faculty of Technology, Campus Darcy Ribeiro, University of Brasília, P.O. Box 04357-70904-970, Brasília, DF, Brazil
Tel: +55.61.3107.5634 Fax: +55.61.3107.5642

of the University of Brasilia (Brasília, Brazil) located on the Agua Limpa Farm. Leaves and thin branches from 12 previously marked and identified individuals (six species each of *B. salicifolius* and *P. myrsinites*) were collected. The species were preliminarily identified and later compared with the material deposited in the UNB Herbarium (UB) under the following catalogue numbers: *Blepharocalyx salicifolius*-88293, *Psidium myrsinites*-23116.

To analyse the influence of seasonality on the essential oils of these species, collections were scheduled to be conducted in four months, November, March, July and September, between 2009 and 2010. However, it was not possible to complete the collection during the last month (September 2010) because of sufficient plant material to obtain samples from both species. *P. myrsinites* is deciduous and its foliation period occurs only between August and September (Silva-Junior, 2005). All materials collected in the field were placed in plastic bags and the samples were marked. Then, the material was stored in a cold chamber (10°C, 50% relative humidity) until essential oil extraction, which occurred 1 day after collection for *P. myrsinites* and 2 days after collection for *B. salicifolius.*

Climatological data, including temperature, rainfall, sunlight and relative humidity for each sampling month, were obtained from the National Institute of Meteorology website (www.inmet.gov.br).

Essential oil extraction, yield and composition analysis: Extractions were performed by steam distillation using laboratory equipment to obtain small extractions of the essential oils (Linax, D1). Approximately 250 g of fresh leaves was used in each extraction. Each extraction was performed for 90 min to allow for maximum yield. By the end of each extraction, approximately 900 mL of the hydrosol was obtained and stored in a properly identified amber glass bottle. All bottles were conditioned in a cold chamber until the next step of separating the aqueous and organic portions of the hydrosol.

The separation of each sample was performed using a decanting funnel and four portions of ethyl acetate (100 mL each), with an electromagnetic stirrer used in 10 min cycles at each addition of solvent. During each cycle, the organic fraction of the hydrosol was reused and the aqueous fraction was discarded. Next, 50 mL of saturated sodium chloride solution (NaCl) was added to the organic fraction to dry the material and decrease any emulsification formed during the initial process, allowing for the removal of any aqueous component still present. To remove possible water residue, sodium sulphate (Na_2SO_4) was also added. Finally, the organic fraction was filtered through a funnel and the solvent was recovered using a rotary evaporator.

To calculate the essential oil yield, a relationship between the wet leaf mass used in distillation and the essential oil mass obtained was determined using a scale accurate to 1×10^{-4}. To evaluate the effect of the collecting season on the essential oil yield, an analysis of variance (ANOVA) and a multiple comparison test, the Least Significant Difference (LSD) test, both at a significance level of 5%, were used.

After obtaining the pure oil samples, the samples were divided according to the time of year of collection. Initially, to identify the chemical profiles of the oils, spectroscopic Nuclear Magnetic Resonance (NMR) analyses, ^1H (300 MHz) and ^{13}C (75 MHz) were used to identify the functional groups. In all experiments, a 5 mm internal diameter ATB probe at room temperature with a 45° pulse was used. The ^1H and ^{13}C spectra, referenced to TMS and $CDCl_3$ (77.0 ppm), respectively, with chemical shifts (δ) expressed in ppm, scalar couplings (J) in Hz and multiplicities defined for singlet (s), doublet (d), double doublet (dd), multiplet (m) and heptet-triplet (th), were obtained using a Varian Mercury Plus spectrometer. Infrared analyses were performed using a Bomen MB-100 spectrometer and vibrational frequencies were expressed in cm^{-1}.

To identify the major components of the oils, the same previously used samples were injected into a gas chromatograph coupled to a mass selective detector (GC/MS) operated in the electron ionisation mode (70 eV). A Shimadzu 7890 A chromatograph with a 5%-phenyl/95%-methyl-silicone (HP5, 30 mm×0.32 mm×0.25 μm) capillary column and helium as the carrier gas (1.0 mL min^{-1}) was employed to analyse the oils. The oven temperature was programmed from 60 to 240°C at a heating rate of 3°C min^{-1}. The oil was diluted to 1% in ethyl acetate and 1 μL of the solution was injected into the injector at 250°C in split flow mode (1:20). The mass spectra obtained were compared to data from the Wiley library, sixth Edn.

RESULTS AND DISCUSSION

Quantitative evaluation: The *B. salicifolius* species did not demonstrate a variation in yield between the first two analysed periods, November and March; the average yield for both months was 0.16% but the yield increased to 0.21% in July. However, this difference was not statically significant according to LSD test. For *P. myrsinites*, there was a variation statistically significant between the first two periods and the average yields for November and March were 0.18 and 0.22%, respectively. During July, the average yield further increased to 0.38%, the highest value observed.

For both species, the dry season, represented by July, produced a slight increase in essential oil production. This result possibly occurred because of the more severe weather conditions that generally influence plant physiology by causing the plants to defend against adverse external conditions (Evans, 1996; Salisbury and Ross, 1991). The last day of rain before the dry season occurred on 27 May 2009, followed by 46 days without rain until the collection date in July. These conditions resulted in an adverse situation for basic plant physiological functions in terms of the relative humidity present.

It is possible that essential oils in these species play an important role in the defence of the plants against adverse weather conditions because for both species, the period with the greatest yield had the worst relative humidity and rainfall conditions. Water supply can often be critical for essential oil production when associated with other climatic factors such as high temperatures. However, in this study, although July had the lowest relative humidity during the collection months (55%), the average variation in the maximum temperature for each period was low (25-27°C) (Table 1). This condition may have resulted in only an alteration in leaf stomatal activity, thereby reducing oil volatilisation.

As stated by Taiz and Zeiger (2004) under water-deficient circumstances, in a slow and long-term process, plants generally develop resistance strategies by diminishing leaf area and deepening root depth. When the onset of stress is rapid or the plant has already established these adaptations, stomatal activity can be drastically reduced. The stomata, which are cellular structures responsible for controlling air intake and air/water output as mentioned by Appezzato-da-Gloria and Carmello-Guerreiro (2003), are formed by two guard cells that control the stomatal opening, which is the channel between this organ and the environment. According to Appezzato-da-Gloria and Carmello-Guerreiro (2003), the opening and closing of the stomata depend on turgor variation and the stomata open in the presence of higher amounts of potassium ions in the guard cells. Located on the leaf epidermis, the guard cells lose turgor by releasing water to the atmosphere. This release is likely triggered by low relative humidity, causing hydropassive closure (Taiz and Zeiger, 2004). The absence of water or reduced quantities of water leads to stomatal closure, which reduces the amount of water and air leaving the cells. Thus, the loss of volatile material may be less than what would be found during periods of high relative humidity, which favour stomata opening and high-intensity rainfall, which can leach volatile material from the plants.

Table 1: Average weather conditions for each collection month

	Collection period			
	2009	2010		
Weather condition	November	March	July	September
Max. temperature (°C)	26.0	27.0	25.0	28.0
Min. temperature (°C)	17.0	17.0	12.0	16.0
Relative humidity (%)	78	78	55	53
Rainfall (mm month^{-1})	200	250	0.0	0.0
Sunlight (h day^{-1})	4.7	5.9	8.5	8.3

The dry season in central Brazil can last for up to 7 months and each plant adapts by developing survival strategies to avoid suffering from the lack of rain. As stated by Eiten (1972) the diverse vegetative types of the Cerrado are marked by differences in floristic composition as a function of the soil characteristics, such as nutrient stock and concentration, depth and drainage. For the development of arboreal species in the more closed-vegetation types, deeper soils are required to provide a greater nutrient supply (Henriques, 2005) and a higher water content in the top soil is needed during the dry season relative to that needed by the more open-vegetation types (Franco and Luttge, 2002). Along with these soil characteristics, analyses of the arboreal vegetation in the Cerrado have revealed that most woody plants produce deep root systems that can access the soil layers that store water, providing these plants access to water for vegetation throughout the year in the restricted formations of the Cerrado (Ferri, 1944). Even in the dry season, these arboreal plants do not suffer from a lack of water and possibly exhibit diminished leaf water content only due to the reduction in relative humidity. Accordingly, the plants that have established the necessary adaptations can continue to produce essential oils and only exhibit reduced stomatal activity, reducing oil volatilisation but not production.

In addition to water stress, other aspects may be related to the physiology in *B. salicifolius* and *P. myrsinites* species that make the dry period the most favourable for essential oil production. Full development of the glandular trichomes that store essential oils in some species may be light dependent, as observed for basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.) (Gobbo-Neto and Lopes, 2007). In July, there was a higher incidence of sunlight, reaching an average of 8.5 h day^{-1}, versus 4.7 and 5.9 h in November and March, respectively Future studies of the leaf anatomy of these plants as they relate to the production of essential oils can help better identify which climatic factors actually interfere with the production of these oils.

The results obtained here are different from those found by Pirzad *et al.* (2006). They studied the effect of different irrigation regimes on the yield of essential oil from *Matricaria chamomilla* and observed that the highest oil yield was obtained for irrigation at 85% of field capacity, while the lowest yield was at 55%. Surely, these studies cannot be directly compared since they were conducted in very different environment. Nevertheless, they highlight that water supply is an important factor affecting the essential oil production.

Analysing only the quantitative aspect of essential oil production, the role of these essential oils in protection against predators and attraction of pollinators/dispersers may not be as relevant. It can be inferred since that the period of increased intake did not coincide with the flowering (November to December for *P. myrsinites* and August to January for *B. salicifolius*) and fruiting (November to February for *P. myrsinites* and January to March for *B. salicifolius*) seasons (Fig. 1). Ozguven *et al.* (2006) observed that the yield of essential oil form *Origanum syriacum* var. *bevanii* was higher during the full blooming period. However, the effect of phenological period can vary according to the site as observed by Ayanoglu *et al.* (2005) for yield essential oil from *Melissa officinalis*. They observed an opposite behaviour: depending on the site, the yield can be higher before or after flowering.

Comparing data from the literature, it is noted that the average yield of 0.18% for *B. salicifolius* was very close to the value of 0.17% described by Marques (2007) but was higher than those found by Castelo *et al.* (2010) (0.10%) and Limberger *et al.* (2001) (0.09%). For the *P. myrsinites* species, the average yield of 0.26% was twice that found by Castelo *et al.* (2010) (0.13%) in a study in the cerrado but was lower than that found by Freitas *et al.* (2002) (0.4%) in a study in the Caatinga.

Although, a trend toward a greater yield in the dry period was observed with the two species during the periods analysed, the LSD test revealed that this variation was statistically significant only for *P. myrsinites*. By comparing the three periods, a statistically significant difference was observed between the yield from the rainy season, November (0.18%) to March (0.22%) and the dry season in July (0.38%).

For *B. salicifolius*, the variation was not statistically significant among the periods analysed: November (0.16%), March (0.16%) and July (0.21%). One hypothesis for the non-significant yield variation in this species is that unlike most plants used in essential oil studies, which have annual or biennial life cycles and which are found in herbaceous or shrubby habitats, *B. salicifolius* is an arboreal plant with a perennial life cycle. The difference in the physiological development between plants with a perennial life cycle and those that are annual or biennial (Arthur and Wilson, 1967) may be a relevant factor that can cause plants to have shorter life cycles during the peak years of essential oil production. The development of these plants must be faster so that all stages of growth, development and reproduction can be completed for the successful propagation of the species. Annual plants, usually weeds and vegetables, have a cycle from seed to adult plant in which they form new seeds within a single growing season that can last for several weeks. Biennials have cycles of two growing seasons, the first near the ground with root formation, a small stem and leaves, followed by the second phase with flowering, fruiting and death; these plants rarely become woody (Raven *et al.*, 1996). Perennials can be woody or herbaceous and they have a vegetative structure that survives year after year, with a longer life cycle and the evolution of all of the developmental stages occurring over several seasons (Raven *et al.*, 1996). This attribute can therefore be a factor that leads to more continuous production throughout the year because there is no urgent need to complete all of the developmental stages in only a few seasons.

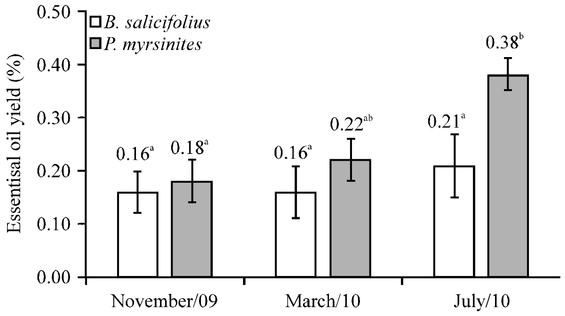

Fig. 1: Yield of essential oil according to the month of collection, means followed by same letter within each species are not statistically significant, bars are standard deviation

Qualitative evaluation

GC/MS: In the GC/MS analysis, the oils of *B. salicifolius* contained the following main components: p-cymene (aromatic hydrocarbon), monoterpenes (α-pinene and α-terpineol), sesquiterpenes (aromadendrene, globulol and caryophyllene oxide) and others, such as cis-3-hexane-1-ol and 2(1H)-naphthalene, with variation occurring between periods.

In a study by Limberger *et al.* (2001) with the same species, 1,8-cineole, linalool and β-caryophyllene were found to be the major compounds. Moreira *et al.* (1999)

studied species in southern Brazil and reported 1,8-cineole, (-)-β-pinene and (-)-limonene as the main components. In a study in Argentina, Tucker *et al.* (1993) found 1,8-cineole, limonene and linalool. It is important to note that although these studies examined the same species, many factors, such as genetics, soil characteristics, extraction techniques and the climatic characteristics of the region, affect the production quantity and quality of the essential oils. Therefore, it is normal and even expected that there would be variations between the results from different studies with varied characteristics.

Because there was no significant variation in yield between the periods examined, essential oil extraction from *B. salicifolius* in the region should be directed according to the desired chemical compounds, i.e., depending on the type of market that will use the oils or the type of markets the producers hope to reach.

GC/MS analysis of *P. myrsinites* revealed a predominance of principal components that included sesquiterpenes (caryophyllene oxide, β-caryophyllene, β-guaiane, α-humulene and viridiflorol) and monoterpenes (myrcene). There was also variation in the principal components among the periods analysed. Despite the predominance of sesquiterpenes in the species composition, the presence of a monoterpene was detected among the major components. This monoterpene, β-myrcene, is a natural olefin compound found in several plants that is used in the production of fragrances. In the study by Freitas *et al.* (2002) with the same species, major components, such as sesquiterpenes, β-caryophyllene and caryophyllene oxide, were also identified. Additionally, July was the period of highest yield, that is, with the greatest commercial potential and the diversity of organic compounds among the oil's major components was demonstrated using chromatography. Analysing these two aspects it is concluded that this dry period is the most suitable for the extraction of essential oils from *P. myrsinites*.

^{1}H and ^{13}C NMR: Using ^{1}H and ^{13}C NMR spectroscopic techniques, it was observed that essential oils from these two species are complex mixtures of saturated and unsaturated hydrocarbons with oxygenated functional groups. In the ^{13}C NMR spectra of oils from the *B. salicifolius* species, signs of a ketone carbonyl and traces of ester were observed in the three periods analysed. These compounds greatly affect the aromatic aspect of the essential oils. Regions of olefinic and aromatic carbons were also observed, structures that also influence the aroma by being very volatile. Aliphatic and oxygenated carbons were also found. In ^{1}H NMR spectra, the presence of aliphatic, oxygenated and olefinic hydrogen was also confirmed (Table 2, 3).

Table 2: The functional groups identified by ^{13}C NMR for *Blepharocalyx salicifolius*

	^{13}C NMR		
	November/09	March/10	July/10
Groups	(δ_C (ppm))		
Aliphatic CH	15.1-48.5	13.8-48.5	15.1-48.6
Oxygenated CH	50.4-105.9	50.4-109.5	50.6-105.7
Olefinic CH	111.5-142.8	112.7-142.9	112.7-142.9
Aromatic-olefinic CH	120.4-142.8	120.4-142.9	119.8-142.9
Aromatic C	154.5	149.0-152.8	149.0-152.8
C = O ester	174.9	174.9-175.4	175.2
C = O ketone	198.5; 210.8	191.3; 191.4; 191.8; 198.3; 198.5; 210.8	197.5; 213.0

Table 3: The functional groups identified by ^{1}H NMR for *Blepharocalyx salicifolius*

	^{1}H NMR		
	November/09	March/10	July/10
Groups	(δ_C (ppm))		
Aliphatic CH	0.84-2.92	0.84-2.92	0.78-2.91
Oxygenated CH	3.08-4.10	3.10-4.10	3.11-4.13
Olefinic CH	4.61-5.92	4.61-5.80	4.61-5.70
Aromatic-olefinic CH	6.89-7.40	6.80-7.21	7.12-7.21

According to Stewart (2006), ketone and ester carbonyls have different physical and chemical properties than simple hydrocarbons. The ketone functional group is responsible for some of the most powerful aromas and flavours in natural products. This functional group can act therapeutically as a sedative, analgesic and relaxant but can also be dangerous for humans. An example is the oil from *Salvia officinalis* L. which is rich in camphor, a neurotoxic product. In plants, the compounds act as pheromones and are excellent natural insecticides when used in large quantities. According to the same author, the presence of esters greatly influences the fragrance of the essential oils, even in small quantities. A wide variety of fragrances is composed of esters and esters form the most popular class of compounds in the perfume industry. Esters are generally not toxic and produce relaxing effects. Some of the compounds detected in GC/MS were found in the ^{13}C NMR spectra using the compound spectroscopic data, verifying the presence of these compounds in the essential oils of the species (Table 4).

The qualitative analysis of the ^{13}C NMR spectra of *B. salicifolius* demonstrated that the profiles differ amongst themselves and an expansion of the spectra made the difference more evident. Of the periods analysed, the March extract was the richest in volatile compounds, with the greater signal in the ketone carbonyl regions (Table 4). The July extract exhibited lower signal from the differentiated carbons and the extract from November was in the intermediate range. A possible explanation for the March extract being the richest in compounds is that this month marks the fruiting period of the species, which begins in January. Although, the individuals analysed did not bear fruits, the presence of

Table 4: The ^{13}C NMR chemical shifts of compounds obtained from *Blepharocalyx salicifolius** and from *Psidium myrsinites***

Compound	Literature δ_C (ppm)	This study δ_C (ppm)
α-pinene	22.8; 23.0; 26.4; 31.3; 31.5; 38.0; 40.9; 47.2; 116.1; 144.5	22.6; 22.8; 23.2; 23.3; 26.4; 26.1; 29.8; 31.5; 37.7; 37.8; 40.9; 41.0; 47.0
α-terpineol	23.1; 23.6; 25.9; 26.7; 30.9; 44.6; 71.9; 120.7; 132.9	23.3; 23.7; 25.7; 25.9; 26.7; 30.8; 44.4; 71.9; 72.4; 44.7; 120.4; 120.7; 132.2; 133.6;
p-cymene	20.9; 24.1; 33.8; 126.3; 129.0; 135.1; 145.8	20.8; 24.0; 24.1; 33.8; 126.5; 128.8; 135.0; 145.3
Caryophyllene oxide	16.4; 22.6; 26.6; 29.2; 29.5; 33.3; 38.4; 39.1; 48.0; 50.1; 59.1; 63.0; 112; 151.0	16.3; 16.4; 22.5; 26.5; 26.6; 29.0; 29.1; 29.3; 29.5; 33.5; 38.3; 38.8; 39.0; 39.4; 48.0; 48.2; 50.4; 50.5; 58.6; 63.7; 112; 112.6; 151.0; 151.1; 151.3
Myrcene	17.1; 25.1; 26.1; 30.8; 112.9; 115.5; 124.4; 134.1; 139.0; 145.9	17.2; 17.5; 25.0; 25.1; 26.1; 26.2; 30.7; 30.8; 112.6; 112.7; 112.8; 115.5; 115.8; 124.3; 124.4; 134.0; 134.6; 139.1; 139.5; 145.7; 145.9

*The *Blepharocalyx* species presented pinene, α-terpineol, p-cymene and caryophyllene oxide compounds. **The *Psidium* species presented only caryophyllene oxide and myrcene compounds, Source: Silverstein *et al*. (2005), Note: Experimentally obtained chemical shift values were obtained from the ^{13}C spectra of the three periods analysed

Table 5: The functional groups identified by ^{13}C NMR for *Psidium myrsinites*

	^{13}C NMR		
	November/09	March/10	July/10
Groups	(δ_C (ppm))		
Aliphatic CH	14.9-48.95	13.6-48.9	14.5-48.6
Oxygenated CH	50.4-105.9	50.6-109.6	50.4-108.6
Olefinic CH	111.5-151.5; 144.8	111.1-155.0; 144.8	112.6-154.3; 144.8
Aromatic-olefinic CH	174.9	174.9	174.9
Aliphatic CH	-	-	212.0

Table 6: The functional groups identified by ^{1}H NMR for *Psidium myrsinites*

	^{1}H NMR		
	November/09	March/10	July/10
Groups	(δ_C (ppm))		
Aliphatic CH	0.84-2.92	0.84-2.92	0.78-2.91
Oxygenated CH	3.08-4.10	3.10-4.10	3.11-4.13
Olefinic CH	4.61-5.92	4.61-5.80	4.61-5.70

ketones in the oils may be related to the need to attract dispersers or to protect the plant from predators. A possible explanation of the July results is that the essential oil production was enhanced in the sexual organs and reduced in the leaves during the month preceding the flowering period of the species to attract its natural pollinators, bees and small insects. As previously noted, among the periods analysed, there was no significant variation in essential oil production in the species, with a trend toward increased production found only in the dry season or in the chemical composition of its oil. It is evident that there was considerable variation among the three periods independent of the rainy or dry period. It is possible that the essential oil of *B. salicifolius* plays a major role in the interaction between the species and other biological agents, as opposed to adverse weather conditions. A great similarity between the species was observed in the ^{1}H NMR spectra of the three periods. Because it is related to a type of natural product, ^{13}C analysis reveals a larger number of compounds and its chemical shift range is larger than that found with the ^{1}H analysis.

In the ^{13}C NMR spectra from *P. myrsinites*, the presence of ester was observed in all periods, unlike the ketone carbonyl functional group, which was found only in July. This result is important because the presence of ketones greatly influences the aromatic aspect of the essential oils and is greater in the oil extracted during July than in that extracted during the other months. Aliphatic, oxygenated and olefinic carbons were also observed and confirmed in the ^{1}H NMR spectra (Table 5, 6).

Some of the major compounds detected with GC/MS were also found in ^{13}C NMR spectra, verifying their presence. Through, qualitative analysis of the ^{13}C NMR spectra, it was evident that the March extract had fewer compounds and lower intensity peaks than the July extract, which exhibited the highest number of different carbons. The November extract had an intermediate quantity.

One possible explanation for this result is that the flowering period occurs from November to December and the fruiting period occurs from November to February. One of the functions of essential oils is the attraction of pollinators/dispersers and it is possible that more intense production of these compounds may act as an attractant during that period. With the end of the fruiting period in March, production of these compounds becomes less intense until July, when the climatic conditions become less favourable for plant physiology and a new peak in compound production occurs. According to the records, quantitative and qualitative production is even more intense during the dry period than during the early flowering and fruiting periods.

In the ^{1}H and ^{13}C NMR spectra for *P. myrsinites* in all periods, the presence of the monoterpene linalool was observed, as reported by Castelo *et al.* (2010). Comparing the spectra of the species with those from synthetic and pure linalool it was possible to identify the characteristic peaks of the detected compounds, especially in the olefinic regions (Table 7).

The ^{1}H NMR spectroscopic analysis indicated the presence of linalool but this compound was not detected by chromatography among the five major compounds. Competition with other sources, particularly cultivated sources, may hamper the incorporation of the product obtained from this species in the market. However, compound extraction from these species can be enhanced by the use of extracts from the natural environment that generate less damage to the environment, which is highly valued by the market. The Brazilian rosewood

Table 7: The ^{1}H and ^{13}C NMR chemical shifts of compounds obtained from linalool and *Psidium myrsinites*

Linalool		*P. myrsinites*	
δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1.27 (s)	17.3	1.27	17.2; 17.3
1.52-1.58 (m)	22.5	1.55-1.58	22.4; 22.6
1.60 (s)	25.4	1.60	25.2; 25.4; 25.5
1.67 (d, 1,2 Hz)	27.3	1.67	27.2; 27.3
1.96-2.07 (m)	41.9	1.96-2.07	41.9
5.05 (dd, J = 1.2 and 10.8 Hz)	111.3	5.03	111.3
5.12 (th, J = 1.5 and 7.2 Hz)	124.2	5.12	124.3
5.22 (CH, dd, J = 1.5 and 15 Hz)	144.8	5.22	144.8
5.90 (CH, dd, J = 10.8 and 17.4 Hz)	5.90; 5.91		
-	73.0	-	73.1
-	131.1	-	131.2

D: Doublet, dd: double doublet, m: Multiplet, th: Heptet-triplet, s: Singlet

(*Aniba rosaeodora* Duck) is the most used and known natural source of linalool but it is under great pressure due to unmanaged extraction and new sources would be important to ensure the survival of the species. Some native plants, such as Amazonian sacaca (*Croton cajucara* Benth) have been analysed for the presence of linalool (Chaves *et al*., 2006); however, *P. myrsinites* could become a new source for the product in a new region, as the demand for new sources is currently concentrated in northern Brazil. This creates the possibility that other communities could compete within the market, stimulating diverse production sources and placing value on the social and environmental aspects of production.

Infrared (IR): The functional groups observed in the ^{1}H and ^{13}C NMR spectra were confirmed by the IR technique, excluding the acyl ester groups, which were poorly defined despite an experimental period (128 transients) that was sufficient to detect them. Because the ^{13}C NMR technique detects the best features of organic groups, the presence of acyl groups may still be considered. The presence of ketone carbonyl at 1708 cm^{-1}, although less prominent in the IR spectrum, was confirmed in July for *P. myrsinites*.

CONCLUSION

Through, chemical analyses, it may conclude that the chemical profile of the essential oil produced by *B. salicifolius* is formed by a complex array of compounds and this oil could potentially be used in various industries, especially pharmaceuticals. As the quantitative variation was not significant, this oil may be extracted during different times of the year. The essential oil produced by *P. myrsinites* has potential applications, as observed by linalool. This species presents a chemical profile rich in volatile substances and by evaluating the qualitative and quantitative data together, the dry season proved the most optimal for extracting its oil.

ACKNOWLEDGMENTS

The authors are grateful for the CAPES, for the Scholarship grant to the first author and for CNPq and FINEP which provided financial support to purchase equipments.

REFERENCES

Alsafar, M.S. and Y.M. Al-Hassan, 2009. Effect of nitrogen and phosphorus fertilizers on growth and oil yield of indigenous mint (*Mentha longifolia* L.). Biotechnology, 8: 380-384.

Appezzato-da-Gloria, B. and S.M. Carmello-Guerreiro, 2003. Vegetal Anatomy. 1st Edn., Vicosa: UFV Press, Brazil.

Arthur, R.H.M. and E.O. Wilson, 1967. The Theory of Island Biogeography. Priceton University Press, Princeton, New Jersey.

Ayanoglu, F., M. Arslan and A. Hatay, 2005. Effects of harvesting stages, harvesting hours and drying methods on essential oil content of lemon balm grown in Eastern Mediterranean. Int. J. Bot., 1: 138-142.

Azizi, K. and D. Kahrizi, 2008. Effect of nitrogen levels, plant density and climate on yield quantity and quality in cumin (*Cuminum cyminum* L.) under the conditions of Iran. Asian J. Plant Sci., 7: 710-716.

Brant, R.S., J.E.B.P. Pinto, S.K.V. Bertolucci and C.J.B. Albuquerque, 2008. Essential oil content of *Aloysia triphylla* (L'Her.) Britton in function of seasonal variation. Rev. Bras. Pl. Med., 10: 83-88.

Castelo, A.V.M., C.H.S.D. Menezzi and I.S. Resck, 2010. Yield and spectroscopic analysis (1H; 13C NMR; IR) of essential oil from four plants of the Brazilian Savannah. Cerne, 16: 573-584.

Chaves, F.C.M., H.R. Bizzo, P.C.S. Angelo, J.J.B.N. Xavier and A.F.S. Sobrinho, 2006. Yield and chemical composition of the essential oil from the leaves of two varieties of sacaca (*Croton cajucara* Benth). Rev. Bras. Pl. Med., 8: 117-119.

Eiten, G., 1972. The cerrado vegetation of Brazil. Rev. Bras. Bot., 38: 201-341.

Evans, W.C., 1996. Trease and Evans Pharmacognosy. 14th Edn., WB. Saunders Co. Ltd., London, ISBN: 0-7020-1899-6, pp: 248.

Ferri, M.G., 1944. Transpiration of permanent Cerrado plants. Bol. Fac. Fil. Cien. Letras USP-41, Sao Paulo, 4: 159-224.

Franco, A.C. and U. Luttge, 2002. Midday depression in savanna trees: Coordinated adjustments in photochemical efficiency, photorespiration, CO_2 assimilation and water use efficiency. Oecologia, 131: 356-365.

Freitas, M.O., S.M. de Morais and E.R. Silveira, 2002. Volatile constituents of *Psidium myrsinoides* O. Berg. J. Essent. Oil Res., 14: 364-365.

Gobbo-Neto, L. and N.P. Lopes, 2007. Medicinal plants: Factors of influence on the content of secondary metabolites. Quim. Nova, 30: 374-381.

Henriques, R.P.D., 2005. Influence of History, Soil and Fire on the Distribution and Dynamics of Cerrado Phytophysionomies. In: Ecology, Biodiversity and Conservation MMA, Scariot, A., J.C. Sousa-Silva and J.M. Felfili (Eds.). Cerrado, Brasilia, pp: 75-79.

Joly, A.B., 2002. Botany: Introduction to Vegetal Taxonomy. 13th Edn., Companhia Editorial Nacional Press, Sao Paulo, Brazil.

Kutchan, T.M., 2001. Ecological arsenal and developmental dispatcher the paradigm of secondary metabolism. Plant Physiol., 125: 58-60.

Limberger, R.P., M.E.G. Sobral, J.A.S. Zuanazzi, P.R.H. Moreno, E.E.S. Schapoval and A.T. Henriques, 2001. Biological activities and essential oil composition of leaves of *Blepharocalyx salicifolius*. Pharm. Biol., 39: 308-311.

Marques, T.P., 2007. Subsidies to the recovery of riparian forest formations of Araucaria Forest of Parana State, from the use of non-wood forest products species. MSc. Thesis, Federal University of Parana (UFPA).

Moreira, J.J.S., C.S. Pereira, E.M. Ethur, E.C.S. Machado, A.F. Morel and W.A. Konig, 1999. Volatile constituents composition of *Blepharocalyx salicifolius*. J. Essent. Oil Res., 11: 45-48.

Mosayebi, M., G. Amin, H. Arzani, H. Azarnivand, M. Maleki and A. Shafaghat, 2008. Effect of habitat on essential oil of *Achillea filipendulina* L. in Iran. Asian J. Plant Sci., 7: 779-781.

Okoh, O.O., A.A. Sadimenko and A.J. Afolayan, 2007. The effects of age on the yield and composition of the essential oils of *Calendula officinalis*. J. Applied Sci., 7: 3806-3810.

Ozguven, M., F. Ayanoglu and A. Ozel, 2006. Effects of nitrogen rates and cutting times on the essential oil yield and components of *Origanum syriacum* L. Var. bevanii. J. Agron., 5: 101-105.

Pirzad, A., H. Alyari, M.R. Shakiba, S. Zehtab-Salmasi and A. Mohammadi, 2006. Essential oil content and composition of German chamomile (*Matricaria chamomilla* L.) at different irrigation regimes. J. Agron., 5: 451-455.

Raven, P.H., R.F. Evert and S. Eichhon, 1996. Vegetal Biology. 6th Edn., Editora Guanabara Koogan, Brazil.

Salisbury, F.B. and C.W. Ross, 1991. Plant Physiology. Wordworth Publishing Com., California, USA., Pages: 682.

Scherer, L.M., 2007. Citronela the java (*Cymbopogon winterianus* Jowitt): Effect of season and vegetal hormones on the *in vitro* multiplication and yield of essential oil. MSc. Thesis, Western State University of Parana, Marechal Candido Rondon, Brazil.

Silva-Junior, M.C., 2005. 100 Trees of Cerrado: Field Guide. 1st Edn., Rede Sementes do Cerrado Press, Brazil.

Silverstein, R.M., F.X. Webster and D.J. Kiemle, 2005. Spectrometric Identification of Organic Compounds. 7th Edn., John Wiley and Sons, New York.

Souza, A., 2009. Variability of volatile oils of myrtaceae species from the atlantic forest. Ph.D. Thesis, University of Sao Paulo (USP).

Stewart, D., 2006. The Chemistry of Essential Oils Made Simple: God's Love Manifest in Molecules. 2nd Edn., Care Publications, Marble Hill, MO., USA.

Supanjani, A.R.M. Tawaha, M.S. Yang and K.D. Lee, 2005. Calcium effects on yield, mineral uptake and terpene components of hydroponic *Chrysanthemum coronarium* L. Int. J. Bot., 1: 196-200.

Taiz, L. and E. Zeiger, 2004. Vegetal Fisiology. 3rd Edn., Sinauer Associates, Sunderland.

Telci, I. and N. Sahbaz, 2005. Determination of agronomic and essential oil properties of peppermint (*Mentha piperita* L.) in various ages of plantation. J. Agron., 4: 103-108.

Tucker, A.O., M.J. Maciarello and L.R. Landrum, 1993. Volatile leaf oils of American Myrtaceae I. *Blepharocalyx cruckshankii* (Hook and Arn.) niedenzu of chile and *B. salicifolius* (Humb., Bonpl. and Kunth) berg of Argentina. J. Essent. Oil Res., 5: 333-335.

Verma, R.K., R.S. Verma, A. Chauhan, A. Singh, Laiq-ur Rahman L. Rahman and A. Kalra, 2011. Biomass yield, essential oil yield and oil quality of rose-scented geranium (*Pelargonium graveolens* L.), intercropped with vegetables. Int. J. Agric. Res., 6: 830-839.