

Circadian variation of essential oil from *Piper marginatum* Jacq

[Variaciones circadiana de aceite esencial de *Piper marginatum* Jacq]

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Abstract: The composition of the essential oil from the leaves of *Piper marginatum* (Piperaceae) shows circadian variation and a higher yield during high solar incidence. The essential oils samples were investigated by GC-FID and GC-MS, which allowed identification of 29 compounds. Principal component analysis (PCA) and cluster analysis (CA) showed a significant quantitative variability in the chemical composition of the studied samples as well as a correlation between the oil profiles and the collection time. Two main groups were observed, with predominance of phenylpropanoids or sesquiterpenes. The predominant chemical compounds were phenylpropanoids, having as main representative (*Z*)-asarone and (*E*)-asarone.

Keywords: *Piper marginatum*, essential oil, Piperaceae, Circadian variation

Resumen: La composición del aceite esencial de hojas de *Piper marginatum* (Piperaceae) muestra una variación circadiana y un mayor rendimiento durante la mayor incidencia solar. Las muestras de aceites esenciales se investigaron por GC-FID y GC-MS, lo que permitió la identificación de 29 compuestos. El análisis de componentes principales y el análisis de conglomerados mostraron una variación cuantitativa significativa en la composición química de las muestras estudiadas, así como una correlación entre los perfiles del aceite esencial y el tiempo de recolección. Se observaron dos grupos principales, con predominio de fenilpropanoides o sesquiterpenos. Los compuestos predominantes fueron los fenilpropanoides, (*Z*)-asarona y (*E*)-asarona.

Palabras clave: *Piper marginatum*, aceite esencial, Piperaceae, Variación circadiana

Recibido | Received: May 16, 2013

Aceptado en versión corregida | Accepted in revised form: October 20, 2013

Publicado en línea | Published online: May 30, 2014

Declaración de intereses | Declaration of interests: This work was funded by grants provided by FACEPE. The authors are indebted to the Centro de Apoio a Pesquisa (CENAPESQ).

Este artículo puede ser citado como / This article must be cited as: MM Moraes, TMG da Silva, RR da Silva, CS Ramos, CAG da Camara. 2014. Circadian variation of essential oil from *Piper marginatum* Jacq. *Bol Latinoam Caribe Plant Med Aromat* 13(3): 270 – 277.

INTRODUCTION

The Piperaceae family comprises 14 genera (Lopes et al., 1997), the most abundant being the *Piper* genus, with approximately 700 species, distributed in tropical and temperate regions in both hemispheres (Barroso, 1986). In Brazil, there are approximately 266 species, many of them occurring in remaining Atlantic Forest areas, where they are present in great abundance and diversity (Di Stasi et al., 2002). Some species of this genus, such as *P. nigrum* and *P. hispidinervium*, have great economic and medicinal importance (Barbosa et al., 2012; Parmar et al., 1997).

The bushy aromatic shrub *Piper marginatum* Jacq. abundantly grows in Central America, the Antilles and South America. In Brazil, it is particularly found around the borders of Atlantic Forest stands. It is popularly known as “malvaíscó” in northeastern Brazil (states of Pernambuco, Paraíba and Rio Grande do Norte) and as “caapeba cheirosa” or “pimenta do mato” in the northern region, especially in the Amazon. It is generally encountered at forest edges, reaching heights up to five meters (Pio-correia, 1984; Guimarães and Giordano, 2004).

The extract of *P. marginatum* leaves has been used in popular medicine to treat inflammation, snake bites and diseases of the liver and vesicles (Van Den Berg, 1982; D'Angelo et al., 1997; Maxwell and Rampersad, 1988).

Phytochemical and biological studies of *P. marginatum* have been extensively conducted, describing the presence of phenylpropanoids, flavonoids, aristolactams, prenylated 4-hydroxybenzoic acid derivatives, terpenes and phenylpropanoids (Lago, et al., 2004, Ramos and Kato, 2009, Fougbe et al., 1976, Hussain et al., 1990, Ramos et al., 1986, Tillequin et al., 1978).

Variations have been found in the chemical composition of the essential oil from *P. marginatum* leaves. Among the predominant chemical classes mentioned are both sesquiterpenes and phenylpropanoids (Autran et al., 2009; Andrade et al., 2008). This variation is attributed to the environment and climate conditions (Gobbo-Neto and Lopes, 2007).

The essential oil of *P. marginatum* has been extensively examined for its biological activities, such as insecticidal (Mesa et al., 2012; Coitinho et al., 2010; Coitinho et al., 2011), acaricidal (Assis et al., 2011) and antifungal (Reigada et al., 2007), as well as for its cytotoxic effects (Olivero-Verbel et al.,

2009). However, no previous studies have investigated the chemical variation of the essential oil of *P. marginatum* associated with the time of day of collecting plant parts. Therefore, the objective of this work was to assess the effect of collection time on the yield and chemical components of the essential oil obtained from the leaves of *P. marginatum*.

MATERIAL AND METHODS

Plant

Fresh *P. marginatum* Jacq. leaves were collected from a fragment of Atlantic Forest located on the campus of Federal Rural University of Pernambuco (UFRPE) in Recife, Pernambuco, Brazil. The leaves were collected in July 2009 at each two hours from 10:30 a.m. to 8:30 p.m. of next day. The plant was identified by Dr. Margareth F. de Sales of the Department of Biology of UFRPE and a voucher specimen was deposited in the Vasconcelos Sobrinho Herbarium of UFRPE with number 48210.

Chemicals

The monoterpenes (β -Pinene, linalool, α -terpineol, α -copaene and β -elemene), sesquiterpenes (β -Caryophyllene, α -cadinene, γ -cadinene, elemol, α -humulene) and phenylpropanoids (*E*-methyl isoeugenol, *Z*-asarone, *E*-asarone) used to identify the volatile components were purchased from Sigma-Aldrich, Brazil.

Isolation of the Essential Oil

The essential oils from fresh leaves (100 g) were obtained by hydrodistillation using a modified Clevenger apparatus for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept under refrigeration at 0° C until analysis. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

GC Analysis

Oil samples were analyzed using a Hewlett-Packard 5890 Series II GC apparatus equipped with a flame ionization detector and a J & W Scientific DB-5 fused silica capillary column (30 m \times 0.25 mm i.d.), with a programmed temperature of 60 to 246° C at 3° C/min. The injector and detector temperatures were 260 and 280° C, respectively. Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min; injection was in split mode (1:30) and the injection volume was 1.0

μL of a solution containing 10 mg/mL of oil in hexane.

The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. Analyses were carried out in triplicate and results were submitted to descriptive statistical analysis.

GC/MS Analysis

Qualitative GC/MS analysis was carried out using a Varian GC/MS (GC: Varian 431/GC-MS: Varian 220-MS) system operating in the EI mode at 70 eV, fitted with the same column and using the same temperature program as for the GC experiments. The carrier gas was helium, 1 mL/min flow rate, split mode (1:30), with an injected volume of 1.0 μL of a solution containing 10 mg/mL of oil in hexane.

Statistical Analysis

The clustering method based on linkage distances was used to determine similarities between the examined samples. Principal component analysis (PCA) based on the complete data set was conducted to evaluate the chemical variation of essential oil from *P. marginatum* leaves and the relationship between the day of collection of the plant material. All the analyses were performed using the Unscrambler software version 9.5 (CAMO Process AS, Norway, 1996-2007).

Identification of Compounds

The initial identification of the separated components of the essential oil was carried out by comparison with previously reported values of retention indices, obtained by co-injection of oil samples and C_{11} – C_{24} linear hydrocarbons and calculated using the Van den Dool & Kratz equation (Van den Dool and Kratz, 1963). Subsequently, the MS acquired for each component was matched with those stored in the Wiley/NBS mass spectral library of the GC–MS system and with other published mass spectral data (Adams, 2007). Monoterpenes and sesquiterpenes purchased from Sigma-Aldrich, Brazil also were used in the identifications of volatile components.

RESULTS AND DISCUSSION

The essential oils of *P. marginatum* leaves obtained by hydrodistillation showed yellowish coloration. The chemical constituents identified in these oils are listed in Table 1 in the order of elution in the DB-5 column. The range of oil yields varied according to the time of collection. The highest yield was observed during the hottest period (26.6 - 27.3° C), from the sample collected at 10:30 a.m. (yield of $0.31 \pm 0.4\%$). This was twice the yield of the sample collected during the period of lowest temperature. Also, the yield varied according to the relative air humidity (Table N° 1), with the highest yield found from the leaves gathered during the period of lowest humidity. These results were consistent with other findings in the literature regarding variations in yield depending on temperature and humidity (Evans, 1996).

The chemical composition analysis of essential oil samples allowed the identification of 29 compounds. The essential oil did not show qualitative variations in chemical composition, but there were quantitative variations of compounds according to the collection time (Figure N° 1). The samples showed high concentrations of phenylpropanoids (in the range of 35.6 ± 0.6 at $54.1 \pm 0.1\%$) and sesquiterpenes (in the range of 41.7 ± 0.2 at $56.8 \pm 0.3\%$). On the other hand, there were low concentrations of monoterpenes (in the range of 0.07 ± 0.0 at $7.0 \pm 0.0\%$).

The main compounds identified in the all samples were two phenylpropanoids, (*Z*)-asarone and its isomer (*E*)-asarone. (*Z*)-asarone was the main compound in the leaves collected at 10:30 a.m., with a percentage of $33.8 \pm 0.2\%$. In turn, (*E*)-asarone showed the highest percentage ($20.6 \pm 0.2\%$) at 4:30 p.m.

The major sesquiterpene identified was α -acoradiene. The highest yields were obtained from the leaves collected at 12:30 a.m. and 8:30 p.m., with 11.2 ± 0.2 and $11.6 \pm 0.1\%$, respectively. The β -acoradiene isomer α -acoradiene was also identified in the sample, however in lower percent.

Table N° 1
Chemical composition and yield of leaf essential oils of *P. marginatum*. Temperature and relative humidity of collection site

Collection time (h)	00:30	02:30	04:30	06:30	08:30	10:30			
Yield	0.16±0.0	0.15±0.1	0.17±0.2	0.17±0.2	0.18±0.2	0.31±0.4			
Temperature	24.1	23.5	24.2	22.5	26.2	26.6			
Relative humidity	79%	80%	73%	90%	83%	83%			
Compounds	^a IR	^b IR						Identification	
<i>β</i> -Pinene	974	979	0.03±0.0	0.02±0.0	1.02±0.0	0.05±0.0	0.28±0.0	0.01±0.0	RI, MS, CI
<i>δ</i> -3-Carene	1008	1014	0.01±0.0	0.01±0.0	2.60±0.1	0.10±0.0	0.63±0.0	0.01±0.0	RI, MS
Sylvestrene	1025	1022	0.01±0.0	0.01±0.0	2.38±0.0	0.26±0.0	0.69±0.0	0.01±0.0	RI, MS
<i>β</i> -Z-Ocimene	1044	1047	0.04±0.0	0.01±0.0	1.67±0.0	0.02±0.0	0.03±0.0	0.01±0.0	RI, MS
Linalool	1095	1098	0.01±0.0	0.01±0.0	0.06±0.0	0.01±0.0	0.04±0.0	0.01±0.0	RI, MS, CI
Isopentyl isovalerate	1102	1106	0.04±0.0	0.06±0.0	0.19±0.0	0.02±0.0	0.02±0.0	0.01±0.0	RI, MS
<i>α</i> -terpineol	1186	1189	0.02±0.0	0.02±0.0	0.05±0.0	0.02±0.0	0.01±0.0	0.01±0.0	RI, MS, CI
<i>δ</i> -Elemene	1335	1338	0.74±0.0	0.77±0.0	1.65±0.0	0.92±0.0	0.67±0.0	0.02±0.0	RI, MS
Isolodene	1374	1378	2.05±0.0	0.96±0.0	1.20±0.0	2.27±0.0	1.24±0.0	0.04±0.0	RI, MS
<i>α</i> -Copaene	1374	1379	0.26±0.0	0.28±0.0	0.21±0.0	0.25±0.0	0.26±0.0	0.92±0.0	RI, MS, CI
<i>β</i> -Elemene	1389	1392	2.08±0.0	2.36±0.0	2.03±0.0	2.11±0.0	2.19±0.0	1.40±0.1	RI, MS, CI
<i>β</i> -Caryophyllene	1417	1421	5.06±0.0	5.13±0.1	5.33±0.2	5.16±0.2	5.66±0.1	5.17±0.7	RI, MS, CI
<i>α</i> -Guaiene	1437	1440	1.27±0.0	0.84±0.0	0.10±0.0	1.33±0.0	0.94±0.0	0.10±0.0	RI, MS
<i>α</i> -Humulene	1452	1456	0.84±0.0	5.93±0.3	0.96±0.0	1.05±0.2	0.93±0.1	0.12±0.0	RI, MS, CI
<i>α</i> -Acoradiene	1464	1464	6.78±0.1	4.97±0.0	7.39±0.1	6.69±0.1	5.24±0.0	3.89±0.0	RI, MS
<i>β</i> -Acoradiene	1469	1471	0.42±0.0	1.09±0.0	3.81±0.1	1.24±0.0	0.76±0.0	4.16±0.0	RI, MS
<i>γ</i> -Himachalene	1481	1485	1.16±0.0	0.37±0.0	2.68±0.0	0.65±0.0	0.05±0.0	1.28±0.0	RI, MS
<i>E</i> -Methyl isoeugenol	1492	1496	0.12±0.0	1.64±0.0	1.99±0.0	0.07±0.0	0.48±0.0	0.60±0.0	RI, MS, CI
<i>cis</i> - <i>β</i> -Guaiane	1495	1495	0.39±0.0	1.26±0.0	0.33±0.0	1.23±0.0	0.57±0.0	0.09±0.0	RI, MS
Bicyclogermacrene	1500	1501	6.74±0.1	5.69±0.0	1.04±0.0	5.30±0.1	6.70±0.1	4.10±0.1	RI, MS
<i>trans</i> - <i>β</i> -Guaiane	1502	1506	1.08±0.0	1.15±0.0	6.43±0.1	0.83±0.0	0.75±0.0	1.17±0.0	RI, MS
<i>γ</i> -Cadinene	1513	1515	0.67±0.0	0.28±0.0	1.01±0.0	0.33±0.0	0.21±0.0	0.73±0.0	RI, MS
<i>α</i> -Cadinene	1537	1540	0.34±0.0	0.05±0.0	0.08±0.0	0.04±0.0	0.04±0.0	0.32±0.0	RI, MS, CI
Elemol	1548	1551	0.04±0.0	2.86±0.1	2.21±0.0	3.46±0.0	2.62±0.0	0.02±0.0	RI, MS, CI
Elemicin	1555	1559	3.77±0.1	7.85±0.1	3.38±0.0	5.77±0.1	6.13±0.1	3.02±0.0	RI, MS
Ledol	1602	1605	7.19±0.1	4.79±0.0	3.01±0.1	2.80±0.2	4.50±0.1	1.20±0.0	RI, MS
<i>Z</i> -Asarone	1616	1620	28.03±0.2	22.86±0.6	20.82±0.1	22.11±0.5	26.09±0.2	33.80±0.2	RI, MS, CI
Patchouli alcohol	1656	1670	11.52±0.2	14.56±0.2	12.95±0.2	14.15±0.3	12.70±0.5	17.43±0.2	RI, MS, CI
<i>E</i> -Asarone	1675	1677	16.89±0.5	12.13±0.1	10.91±0.1	18.92±0.3	17.49±0.2	16.72±0.1	RI, MS, CI
Total			97.59±0.3	97.96±0.0	97.48±0.0	97.16±0.3	97.92±0.5	96.36±0.6	
Monoterpenes			0.16±0.0	0.12±0.0	6.96±0.0	0.48±0.0	1.71±0.0	0.07±0.0	
Sesquiterpenes			48.62±0.5	53.34±0.5	53.41±0.2	49.81±0.3	46.03±0.6	42.16±0.6	
Phenylpropanoids			48.81±0.6	44.48±0.5	37.11±0.2	46.87±0.3	50.19±0.4	54.13±0.1	
<i>β</i> -Pinene	974	979	0.02±0.0	1.68±0.0	1.64±0.0	0.31±0.0	0.01±0.0	0.02±0.0	RI, MS, CI
<i>δ</i> -3-Carene	008	1014	0.01±0.0	1.21±0.0	1.23±0.0	0.80±0.0	0.01±0.0	0.01±0.0	RI, MS
Sylvestrene	025	1022	0.03±0.0	1.83±0.0	1.79±0.0	2.81±0.0	0.01±0.0	0.01±0.0	RI, MS
<i>β</i> -Z-Ocimene	044	1047	0.03±0.0	0.09±0.0	0.08±0.0	0.43±0.0	0.01±0.0	0.01±0.0	RI, MS
Linalool	095	1098	0.01±0.0	0.04±0.0	0.04±0.0	0.07±0.0	0.01±0.0	0.01±0.0	RI, MS, CI
Isopentyl isovalerate	102	1106	0.09±0.0	0.01±0.0	0.01±0.0	0.19±0.0	0.02±0.0	0.06±0.0	RI, MS
<i>α</i> -terpineol	186	1189	0.02±0.0	0.01±0.0	0.02±0.0	0.07±0.0	0.01±0.0	0.02±0.0	RI, MS, CI
<i>δ</i> -Elemene	335	1338	0.51±0.0	0.78±0.0	0.76±0.0	2.33±0.0	1.11±0.0	0.79±0.0	RI, MS
Isolodene	374	1378	0.48±0.0	1.83±0.0	1.80±0.0	2.09±0.0	1.30±0.0	0.98±0.0	RI, MS
<i>α</i> -Copaene	374	1379	0.11±0.0	0.19±0.0	0.22±0.0	0.32±0.0	0.30±0.0	0.28±0.0	RI, MS, CI
<i>β</i> -Elemene	389	1392	1.07±0.0	1.83±0.0	1.80±0.0	2.27±0.1	2.59±0.1	2.35±0.0	RI, MS, CI
<i>β</i> -Caryophyllene	417	1421	4.20±0.1	4.86±0.0	4.90±0.2	7.87±0.1	6.45±0.1	5.19±0.0	RI, MS, CI
<i>α</i> -Guaiene	437	1440	0.44±0.0	0.95±0.0	0.93±0.0	0.06±0.0	1.17±0.0	0.87±0.0	RI, MS
<i>α</i> -Humulene	452	1456	1.80±0.7	0.40±0.0	0.79±0.0	1.06±0.0	4.64±0.1	5.88±0.1	RI, MS, CI
<i>α</i> -Acoradiene	464	1464	11.19±0.2	5.13±0.1	5.01±0.1	8.87±0.1	11.58±0.1	4.92±0.0	RI, MS

β -Acoradiene	469	1471	1.00±0.0	0.87±0.0	0.81±0.0	3.18±0.0	1.33±0.0	1.12±0.0	RI, MS
γ -Himachalene	481	1485	0.09±0.0	0.08±0.0	0.10±0.0	1.62±0.0	0.12±0.0	0.35±0.0	RI, MS
<i>E</i> -Methyl isoeugenol	492	1496	0.86±0.0	0.53±0.0	0.49±0.0	0.56±0.0	1.82±0.0	1.62±0.0	RI, MS, CI
<i>cis</i> - β -Guaiene	495	1495	0.43±0.0	0.18±0.0	0.21±0.0	0.35±0.0	0.98±0.0	1.24±0.0	RI, MS
Bicyclogermacrene	500	1501	2.46±0.0	6.07±0.1	6.10±0.2	6.04±0.0	7.83±0.1	5.61±0.0	RI, MS
<i>trans</i> - β -Guaiene	502	1506	0.46±0.0	0.52±0.0	0.51±0.0	1.08±0.0	1.18±0.0	1.13±0.0	RI, MS
γ -Cadinene	513	1515	0.13±0.0	0.25±0.0	0.20±0.0	0.05±0.0	0.31±0.0	0.28±0.0	RI, MS
α -Cadinene	537	1540	0.05±0.0	0.06±0.0	0.02±0.0	1.43±0.0	0.05±0.0	0.05±0.0	RI, MS, CI
Elemol	548	1551	2.20±0.2	2.70±0.0	2.77±0.1	6.30±0.1	2.45±0.0	2.96±0.0	RI, MS, CI
Elemicin	555	1559	5.29±0.0	4.94±0.1	4.82±0.1	2.10±0.0	7.86±0.1	7.76±0.1	RI, MS
Ledol	602	1605	5.92±0.1	2.96±0.0	3.30±0.0	0.28±0.0	5.77±1.0	4.71±0.0	RI, MS
<i>Z</i> -Asarone	616	1620	29.20±0.6	24.76±0.3	23.57±0.9	22.15±0.2	20.09±0.5	22.99±0.4	RI, MS, CI
Patchouli alcohol	656	1670	13.06±0.2	11.91±0.1	11.60±0.2	11.58±0.1	7.29±0.1	14.53±0.3	RI, MS, CI
<i>E</i> -Asarone	675	1677	15.89±0.6	20.54±0.3	20.57±0.2	10.74±0.3	11.86±0.4	12.19±0.7	RI, MS, CI
Total			97.07±1.0	97.20±0.1	96.09±0.0	96.99±0.1	98.16±0.2	97.94±0.0	
Monoterpenes			0.21±0.0	4.87±0.0	4.81±0.1	4.67±0.0	0.08±0.0	0.14±0.0	
Sesquiterpenes			15.62±1.0	41.56±0.2	41.83±0.8	56.78±0.3	56.46±0.6	53.24±0.6	
Phenylpropanoids			11.24±0.1	50.77±0.2	49.45±0.9	35.55±0.5	41.62±0.4	44.56±0.6	

^aRetention indices calculated from retention times in relation to those of the series n-alkanes on a 30 m DB-5 capillary column; ^blinear retention indices from the literature; RI: retention index; MS: mass spectrum; CI: co-injection with authentic standards.

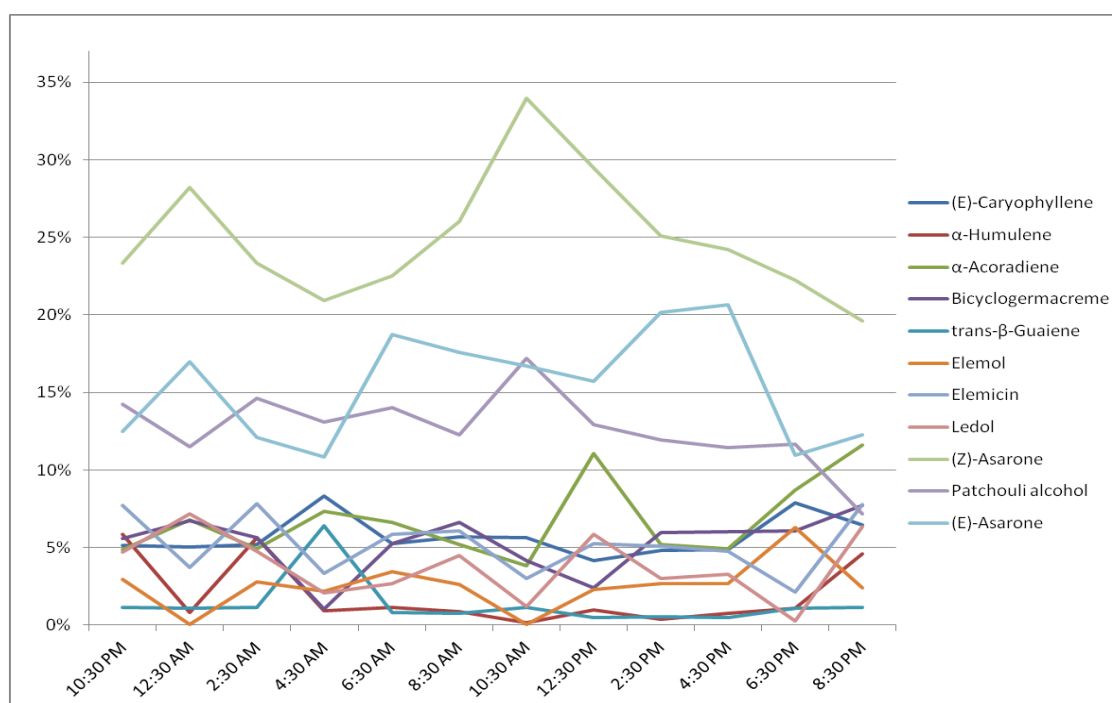


Figure N° 1

Quantitative variations of compound main identified in the leaf essential oil from *P. marginatum* during day.

Other sesquiterpenes, such as β -caryophyllene, bicyclogermacrene and α -copaene, also were identified in significant quantity in the essential oil, with percentages ranging from $4.2 \pm 0.1\%$ to $7.9 \pm 0.1\%$, $1.0 \pm 0.0\%$ to $7.8 \pm 0.1\%$ and from $0.1 \pm 0.0\%$ to $0.92 \pm 0.0\%$ respectively. These three sesquiterpenes have been reported previously in the

essential oil in different specimens of *P. marginatum* (Ramos et al., 1986; Andrade et al., 2008; Autran et al., 2009; Olivero-Verbel et al., 2009).

Only small amounts of monoterpenes were found in the oils. The compounds β -pinene, δ -3-carene and sylvestrene were present in the highest percentages, but none were higher than 3%.

In previous studies, other compounds such as safrole, 3,4-(methylenedioxy) propiophenone, *p*-mentha-1(7), 8-diene, myristicin, (*E*)- β -ocimene, γ -terpinene, (*E*)-anethole, (*E*)-isoosmorhizole coveacin, α -phellandrene, isoelemicine and limonene were identified in the essential oil from leaves of *P. marginatum* in significant percentages (Ramos et al., 1986; Andrade et al., 2008; Olivero-Verbel et al., 2009) but it were not detected here.

A dendrogram illustrating the results of CA calculations is shown in Figure N° 2. Taking into consideration the linkage distances, two important clusters were formed. The first group, named A, showed predominance of samples that showed the

highest percentage of phenylpropanoids while group B samples showed predominance of sesquiterpenes. In group A, the oil from the material collected at 10:30 a.m. contained a high quantity of phenylpropanoid (*Z*)-asarone, which was not observed in the other samples of this group. In turn, in group B the sample collected at 8:30 p.m. presented high concentrations of α -acoradiene, bicyclogermacrene, α -humulene and elemicin, while the sample collected at 6:30 p.m. contained higher concentrations of the sesquiterpenes α -acoradiene, β -acoradiene, elemol, (β)-caryophyllene and trans- β -guaiene.

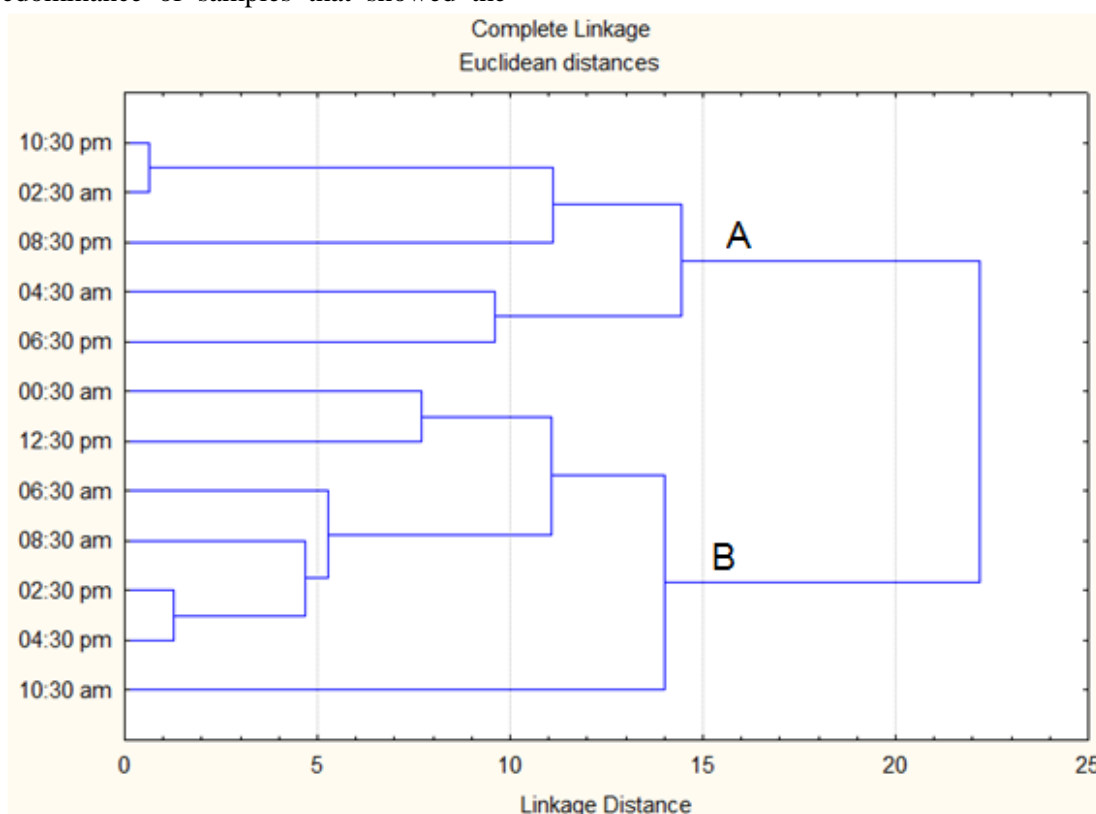


Figure N° 2
Dendrogram obtained by cluster analysis of the *P. marginatum* leaf oil samples

The results of PCA calculations show that the first principal component (PC1) explained 50% of the variability studied, and the second component (PC2) 17%. In other words, the plot described 67% of the variance in the data. Based on this, the PCA results are best visualized in a two-dimensional plot, PC1 vs PC2, in which the chemical variation of studied

essential oil during the day creates clear clusters. The interpretation of PCA calculations shown graphically in Figure N° 3 fully confirms the results obtained by means of CA.

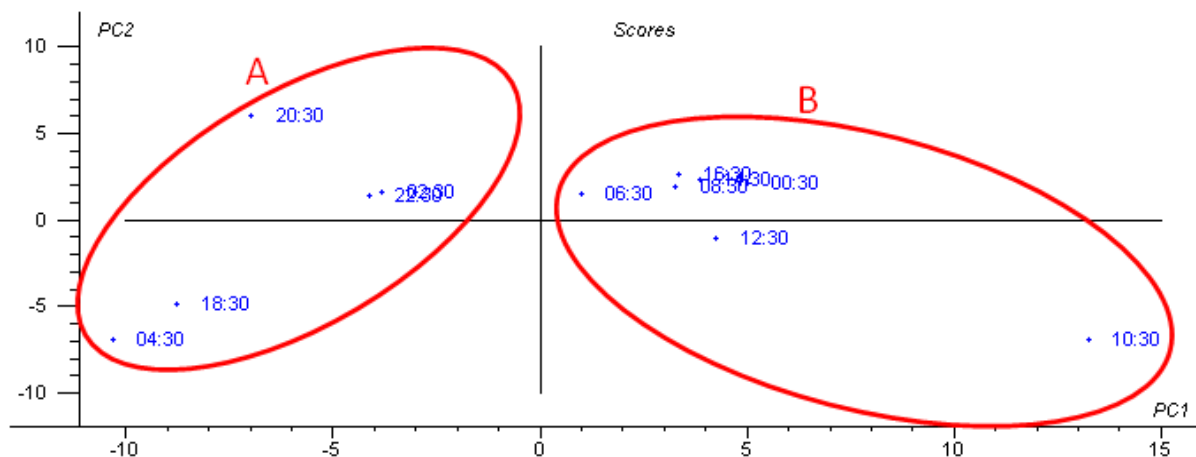


Figure N° 3
Principal component analysis of the *P. marginatum* leaf oil samples

CONCLUSION

The study of the circadian rhythm of *P. marginatum* in producing essential oil indicated that the chemical composition varied quantitatively, mainly between the two predominant chemical classes of compounds: the phenylpropanoids and sesquiterpenes. This significant quantitative variation in the essential oil composition, mainly involving the phenylpropanoids, is relevant from ecological perspective, considering that the phenylpropanoids are associated with the chemical response of plants induced by abiotic and biotic stress as well as antibacterial activity (Ramos *et al.*, 2012; Silva *et al.*, 2012; Barbosa *et al.*, 2012). Moreover, the change of the chemical profile can significantly affect the biological activities. In summary, the analysis of the plant response in terms of chemical class showed that at certain times there was preferential production of specific compounds.

ACKNOWLEDGEMENTS

This work was funded by grants provided by FACEPE. The authors are indebted to the Centro de Apoio a Pesquisa (CENAPESQ), UFRPE, for the laboratory facilities.

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