11

Development and Validation of Analytical Methodology and Evaluation of the Impact of Culture Conditions and Collection Associated with the Seasonality in the Production of Essential Oil of *Plectranthus amboinicus* (Lour) Spreng

Fabíola B. Carneiro[1,*], Irinaldo D. Júnior[2],
Pablo Q. Lopes[1] and Rui O. Macêdo[2]
[1]*Depto. de Ciências Farmacêuticas, Universidade Federal de Pernambuco - UFPE, - Cidade Universitária, Recife – PE,*
[2]*Laboratórios Unificados de Desenvolvimento e Ensaios de Medicamentos – LUDEM – Universidade Federal da Paraíba - UFPB,João Pessoa – PB, Brazil*

1. Introduction

The species of Plectranthus (Lamiaceae) are used in folk medicine in many parts of the world (Hedge, 1992). The genus occurs in four continents: Africa, America, Oceania and Asia and phytochemical studies reported that abietane diterpenes and triterpenoids are the most common metabolites in the genus (Albuquerque, 2000). The β-caryophyllene, a constituent of essential oil of Plectranthus amboinicus, according to Haslam (1996), can be used in traditional medicine as a remedy for the treatment of various organic illnesses. The caryophyllene showed the following properties: antiedêmico (Shimizu, 1990), fagorrepelente (Keeler et al., 1991), antiinflammatory (Shimizu, 1990), antitumor (Zheng et al., 1992), bactericidal (Kang et al., 1992), insetífugo (Jacobson et al., 1990) and spasmolytic (Duke, 1992). Some of these activities have been given to its oxide derivative (Shimizu, 1990, Zheng et al., 1992). The registration of herbal medicines (Anvisa, 2004) establishes quality control of raw material is a prerequisite for obtaining essential herbal with reproducibility of action. The development of an analytical method, the adaptation method known or the implementation of the evaluation process involves estimating its efficiency in the laboratory routine. Particular method is considered valid if its characteristics are in accordance with the prerequisites established (Brito et al, 2003). Validation is essential to determine whether methods developed are completely suitable for their intended purposes, in order to obtain reliable results that can be satisfactorily interpreted. In this way, it allows the knowledge of the limitations and reliability of the measurements made in the analysis. The main objective

* Corresponding Author

of the validation of analytical methodologies is to ensure that the method is accurate, specific, reproducible and durable within the range specified for the substance under examination, thus ensuring its credibility in routine use (USP 1994). One of the difficulties to develop herbal medicines that have these characteristics of reproducibility, batch to batch, the therapeutic action is the variability in the content of major constituents, due to the effects of seasonality in the cultivation of plants. Temporal and spatial variations in the total content as well as the relative proportions of secondary metabolites in plants occur at different levels (seasonal and daily, intraplantar, inter-and intraspecific) and, despite the existence of a genetic control, the expression may undergo changes resulting from interaction of biochemical processes, physiological, ecological and evolutionary (Lindroth et al. 1987; Hartmann, 1996). In fact, secondary metabolites represent an interface between chemistry and the environment surrounding the plants (Kutchan, 2001). The constituents of essential oil are biosynthesized mainly in glandular trichomes of leaves and floral cups (Lawrence, 1992) and depend, in addition to genetic factors, also of physiological and environmental factors (Davis et al., 2004). Whereas the amount of constituents present in plants vary considerably depending on external factors, including temperature, irrigation, solar irradiation, soil nutrients, time of collection, plant age, among others, it is necessary a detailed study of these features aiming at the quality of the raw plant, ensuring product quality and clinical effectiveness of herbal medicine. Our objectives are the development and validation of analytical methodology for the determination of β-caryophyllene in essential oil extracted from Plectranthus amboinicus (Lour) Spreng and the relationship between some variables that may be related to variations in the amount of essential oil of the species Plectranthus amboinicus (Lour.) Spreng., Lamiaceae, grown. We evaluated the influences of the following variables: incidence solar, irrigation, time of collection, stages of plant development and different types of fertilization. For this was monitored quantitatively by gas chromatography, the essential oil β-caryophyllene.

2. Materials and methods

2.1 Development and validation of analytical method

The chemical used reference (β-caryophyllene) was obtained from the manufacturer Sigma-Aldrich (USA). The fresh plant Plectranthus amboinicus (Lour) Spreng was collected in an experimental plot of the Laboratory Rabelo. The quantitative and qualitative studies of essential oils obtained from Plectranthus amboinicus (Lour) Spreng were performed on two types of detection techniques

2.1.1 Identification of volatile constituents of Plectranthus amboinicus (Lour) Spreng

The analysis of identification of volatiles were performed by gas chromatography coupled to a mass spectrometer. Was performed on a Shimadzu GC/MS-QP5050A system equipped with a capillary column DB-5 5 phenyl methylpolysiloxane (30 mx 0.25 mm, 0.10 micron). The carrier gas used was helium with a flow of 1.6 ml / min, split 1:200, injector temperature 260 ° C, initial column temperature equal to 60 º C heated at a rate of 10 ° C / min up to 280 º C. The injection volume was 1.0 µl. Time analysis 30 minutes. The mass spectrometer was operated in SCAN mode with scan range of 50-400 with an electron impact (70 eV) and the detector temperature equal to 280 º C.

2.1.2 Quantification of β-caryophyllene

Quantification studies were performed on a Shimadzu Gas Chromatograph (model GC-17A) equipped with a capillary column DB-1 dimethylpolysiloxane (30 mx 0.25 mm, 0.25 micron) and flame ionization detector (FID) was used for analysis of samples. The carrier gas was N2 with a flow of 1.3 ml / min, split 1:5, injector temperature 260 ° C, detector temperature 280 ° C, initial column temperature equal to 60 º C heated at a rate of 8 ° C / min up to 280 º C for 10 minutes remaining at this temperature. The injection volume was 1.0 µl. Analysis time of 30 minutes.

2.1.3 Preparation of standard solution

The standard solution of 0.180 g / ml was prepared by diluting 0.20 ml of the chemical reference (β-caryophyllene) in 20 ml of hexane and an aliquot of 0.5 ml of this solution was diluted with 25 ml of hexane.

2.1.4 Preparation of sample solution

The essential oils obtained from fresh plant Plectranthus amboinicus (Lour) Spreng were analyzed in order to observe the presence of markers (β-caryophyllene) and possible interferences.

The plants were collected at 7 am, 12h and 16h and submitted separately to hydrodistillation in Clevenger apparatus, according to the parameters of the Brazilian Pharmacopoeia Fourth Edition, using approximately 60 g of plant material. The extraction time was determined by quantifying the hexane fractions obtained by clavenger at intervals of 1, 2, 3, 4, 5, 6 and 7 hours and analyzed by gas chromatography with flame ionization detector (GC / FID) for so after 7 hours of analysis was no longer observed the presence of volatile components, and thus set the time of 7 hours for the extraction of essential oils.

2.1.5 Procedure for validation of analytical methodology

The analytical validation protocol applied in the study was based on the recommendations of Resolution 899 RE, the National Agency of Sanitary Surveillance for raw materials, establishing the following parameters: The specificity of the method was demonstrated by injecting the standard solution, sample solution and the solvent (hexane). The injections were performed to demonstrate the absence of interference that may promote the elution peak of the chemical reference (β-caryophyllene). The linearity of the curve area versus concentration of β-caryophyllene (standard) was obtained in the following concentration range 0.050 mg / mL to 0.450 mg / mL. The linear curve was evaluated by linear regression analysis by the least squares of the midpoints of three (3) calibration curves authentic. Studies of repeatability and intermediate precision were performed using six replicas of dilutions of the standard solution. The repeatability was evaluated on the same day, for each sample, and intermediate precision was performed on three consecutive days, also for each sample. The studies followed the proposed method and the data were expressed as relative standard deviation (rsd). The repeatability and intermediate precision of the standard solution were analyzed at concentrations of β-caryophyllene equivalent to 0.180 mg / ml, respectively. The accuracy of the method was performed through studies of recovery of β-caryophyllene from the parent plant through the addition of a known amount of standard β-caryophyllene to extract

amboinicus Plectranthus (Lour) Spreng (Brazil, 2003) being expressed from the results found (mean concentration experimental) divided by value added (theoretical concentration) multiplied by 100, and the average recovery obtained from the analysis in triplicate. The limits of detection and quantification were obtained with data from the calibration curve of the chemical reference and mathematically calculated using the equations below:

Equation 1. Calculation for determining the limits of detection (LOD) and quantification (LQ).

$$LD = \frac{(3{,}3 \cdot S)}{I} \qquad LQ = \frac{(10 \cdot S)}{I}$$

where:

S = standard deviation of the intercept of the three standard curves;
I = average slope of the three curves.

Were performed on the strength variations related to different temperatures and flows evaluating the impact of these changes in the areas obtained. The studies were performed with dilutions of standard solution with concentration level of 0.180 mg/ml. Analyses were prepared in triplicate and the data evaluated using the relative standard deviation (RSD).

2.2 Study of seasonality

2.2.1 Plant material

The plant material [amboinicus Plectranthus (Lour.) Spreng. Lamiaceae] was grown in experimental plots on the premises of the Laboratory Rabelo, in the municipality of Cabedelo that is inserted into the drive and geoenvironmental coastal tableland has an average altitude from 50 to 100 m. In general, the soils are deep and low natural fertility. The climate is wet tropical with dry summer. The rainy season begins in the fall starting in February and ending in October, as rainfall amounts shown in Table 1. A sample of the plant material was identified, herborized and incorporated into the collection of the Agricultural Research Institute of Pernambuco. Proof: MB Costa e Silva, excicata No. 024/2006.

MONTHS	*PRECIPITATION (mm)	**TEMPERATURE (ºC)
December/2006	42,2	**29,8**
January/2007	31,7	**30,0**
February/2007	162,1	**30,0**
March/2007	194,0	**29,9**
April/2007	262,7	**29,5**
May/2007	224,7	**28,8**
June/2007	616,9	**27,4**
July/2007	127,8	**27,3**
August/2007	203,1	**27,0**
September/2007	201,2	**28,0**

* Source: EMATER. ** Source: Solar energy laboratory UFPB.

Table 1. Precipitation of rain (rainfall) and temperature.

2.2.2 Local cultivation and preparation of the substrate: Experimental groups

P. amboinicus were grown in experimental plots during the months of December 2006 to September 2007. The substrate was organic fertilizer (manure), a mineral fertilizer [NPK (nitrogen, phosphorus and potassium)] and two mineral fertilizer (NPK with limestone) under different treatments. In each experimental plot eight seedlings were grown, distributed in groups of three, according to Table 2.

	Sun	*Shadow	Organic fertilizer	NPK	NPK c/ limestone	Daily irrigation (DI)	**Alternating irrigation (AI)
Bed 1	X		X			X	
Bed 2	X		X				X
Bed 3		X	X			X	
Bed 4		X	X				X
Bed 6	X			X			X
Bed 7	X				X		X

* Shadow = Covered with shade 50% ** Irrigation alternating = Three times a week.

Table 2. Experimental groups.

2.2.3 Collection of plant material

It was regarded as the start date of the experiment 12 December 2006, samples were collected in two vegetative cycles DAP (days after planting or plant age) according to the dates of Table 3. In each treatment were collected aerial parts of six plants to obtain a homogeneous sample, at 7, 12 and 16

	DAYS AFTER PLANTING (dap)			
	60	**90**	**120**	**150**
Vegetative cycle 1	12 a 23/02/07	12 a 23/03/07	16 a 2 7/04/07	14 a 25/05/07
Vegetative cycle 2	11 a 22/06/07	16 a 27/07/07	13 a 24/08/07	10 a 21/09/07

Table 3. Colection period.

2.2.4 Essential oil extraction and determination of extraction time

The collected material was submitted separately to hydrodistillation in Clevenger apparatus, according to the parameters of the Brazilian Pharmacopoeia Fourth Edition, using approximately 60 g of plant material. The extraction time was determined by quantifying the hexane fractions obtained by Clevenger at intervals of 1 h and analyzed by gas chromatography with flame ionization detector (GC / FID) for both after 7 h of analysis was no longer observed the presence of volatile components, and thus set the time of 7 h for extraction of essential oils.

2.2.5 Analysis of essential oil

2.2.5.1 Identification of essential oils

The analyses of identification of volatiles were performed by gas chromatography coupled to a mass spectrometer. Was performed on a Shimadzu GC/MS-QP5050A system equipped with a capillary column DB-1 dimethylpolysiloxane (30 mx 0.25 mm, 0.25 micron). The carrier gas used was helium with a flow of 1.6 mL/min, split 1:200, injector temperature 260 ° C, and initial column temperature equal to 60 º C heated at a rate of 10 ° C/min up to 280 º C. The injection volume was 1.0 µl. The mass spectrometer was operated in SCAN mode with scan range of 50 to 400 with an electron impact (70 eV) and the detector temperature equal to 280 º C.

2.2.5.2 Quantification of β-caryophyllene

Quantification studies were performed on a Shimadzu Gas Chromatograph (model GC-17A) equipped with a capillary column DB-1 dimethylpolysiloxane (30 m x 0.25 mm, 0.25 micron) and flame ionization detector (FID) was used for analysis of samples. The carrier gas was N2 with a flow of 1.3 mL/min, split 1:5, injector temperature 260 ° C, detector temperature 280 ° C, initial column temperature equal to 60 º C heated at a rate of 8 ° C / min remaining up to 280 º C for 10 min at this temperature. The injection volume was 1.0 mL.

3. Results and e discussion

3.1 Validation

Validation testing

The solutions of the samples and blank (placebo) were analyzed by the proposed method. No interference of other constituents was observed in time corresponding to the peak marker β-caryophyllene. The data area and retention time of peak marker showed no co-eluting peaks, indicating that the method is specific. The identification of peaks was based on comparison of retention time of standard and sample, and comparing their mass spectra obtained under the same conditions and with the mass spectrum of the instrument library (Wiley, 6th Edition for Class-5000 1999, 229,119 spectra). After optimization of analytical conditions, the parameters selectivity, linearity, precision, accuracy, limits of detection and quantification and robustness were evaluated. Linearity was tested with a diluted solution in hexane, concentration of 0.050 mg / mL to 0.450 mg / mL of β-caryophyllene, performing three replicates authentic as described in Table 4. The results shown graphically in Figure 1 showed linearity in the track, where the correlation coefficient should not be less than 0.99.

Conc. de β-caryophyllene (µg/ml)	Avarege* ± dp**	dpr(%)***
0,45	201926,6 ± 2736,0	1,35
0,23	98561,0 ±1169,5	1,19
0,18	71092,6 ±1415,3	1,99
0,09	41221,3 ±797,1	1,93
0,05	18300,6 ±1812,6	9,90

*Average, ** Coefficient of variation, ***Relative standard deviation

Table 4. Linearity of the method from a dilute solution in hexane their respective areas, medium and three replicates authentic.

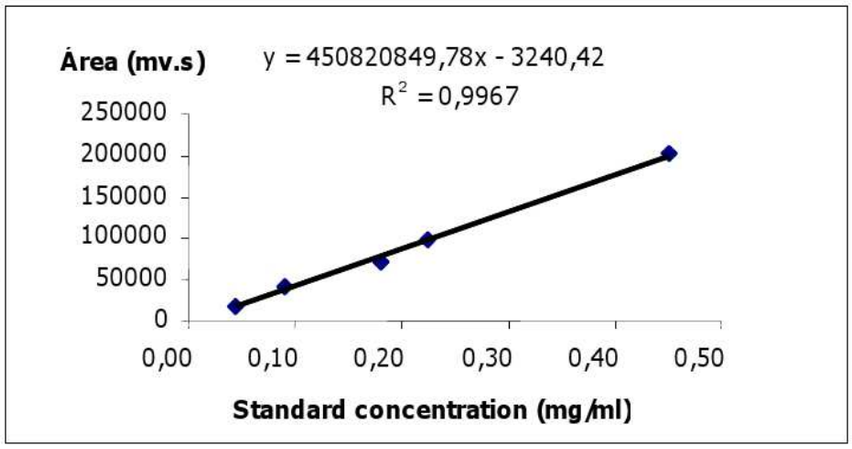


Fig. 1. Equation of the linear calibration of β-caryophyllene (y = a + bx), which shows the relative peak area (Y) and the standard concentration (X).

The repeatability (intra-run precision) was evaluated from injections in six times as Brazil (2003) where the concentration used was 0.180 mg / ml, representing the midpoint of the range specified by the procedure (0.050 mg / mL to 0.450 mg / mL), whose results are shown in Table 5, and expressed as mean (Xm), relative standard deviation (RSD).

Repeatability	**Área of the peaks of the sampls**	**Concentration (μg/ml)**
1	70803	0,1803
2	70768	0,1802
3	71438	0,1819
4	71260	0,1814
5	70577	0,1897
6	70093	0,1785
Média	70823,2	0,1804
dpr (%)*	0,68	0,67

*Relative standard deviation

Table 5. Test of repeatability (precision intra-run) with the average values of peak areas.

Tests of intermediate precision (inter-run precision) were performed on different days and the values shown in Table 6.

Parâmetros	**Conc. of β-caryophyllene in essential oils of Plectranthus amboinicus (μ g / ml)**		
	Day 03	Day 02	Day 03
Médias*	0,1759	0,1751	0,1737
*Dpr***	0,65	1,19	1,31

*N=3 **Relative standard deviation

Table 6. Assessment of intermediate precision expressed on different days at concentrations of β-caryophyllene.

Intermediate precision expresses the effects of variations between different conditions. The value of standard deviation below 5% indicates that the method has an acceptable level of accuracy.

The recovery of the matrix plant through the addition of a known amount of standard β-caryophyllene to extract amboinicus Plectranthus (Lour) Spreng. The mean recovery performed in triplicate with the results shown in Table 7 indicates that the method has an acceptable level of accuracy.

Samples*	Concentration		Recovery (%)
	Added (mg/ml)	Recovered (mg/ml)	
1	0,35	0,32±0,00031	91,42±4,5
2	0,18	0,17±0,00007	94,44±4,12
3	0,06	0,05±0,00002	83,33±4,00

Table 7. Results of the analysis accuracy.

By measuring the linearity, the data obtained by area, we calculated the limit of detection and quantification using the equations:

$$LD = \frac{(3{,}3 \cdot S)}{I} \qquad LQ = \frac{(10 \cdot S)}{I}$$

where:
S = standard deviation of the intercept of the three standard curves;
I = average slope of the three curves.

Having obtained the values 0.00004 mg/ml and the detection limit of 0.00012 mg/ml for the limit of quantification. These results demonstrate that the proposed method is sensitive enough to detect and quantify the sample. Studies of robustness were evaluated by the recovery results obtained after the change of the analytical parameters showed that it is possible to consider the robust method for these parameters because the data showed an accuracy of the method in the range 90 to 100%, according to the table 8.

Parameters	Retention time (min.) ± dp*	Average content (%) ± dp*	dpr (%)**
Temperature			
detector	11,6±0,20	99,32 ± 777,35	1,13
injector	11,5±0,12	98,88 ± 882,85	1,28
flow through the column	11,7±0,05	100,27 ± 429,82	0,62

* Standard Deviation ** Relative standard deviation.

Table 8. Results of the robustness.

3.1.1 Performance equipment

3.1.1.1 System Suitability

Validation using the software Class -5000 (SHIMADZU, Japan) were calculated area and retention time according to the USP-24. The standard deviation (SD) of replicate injections of standard solution was calculated for the peak areas of the marker. The system suitability data are shown in Table 9 and indicate an acceptable level with a standard deviation less than 2%.

Injections	Area	Retention time
1	78807	9,786
2	75724	9,404
3	77134	9,579
4	76887	9,548
5	78583	9,759
Media	77427	9,615
dpr (%)*	1,65	1,64

** Standard deviation

Table 9. Suitability of the chromatographic system for the β-caryophyllene.

3.2 Seasonality

3.2.1 Vegetative cycle 1

In the period from February to May, corresponding to a growth cycle according to Figure 2, one can deduce that the highest concentration of β-caryophyllene as a function of

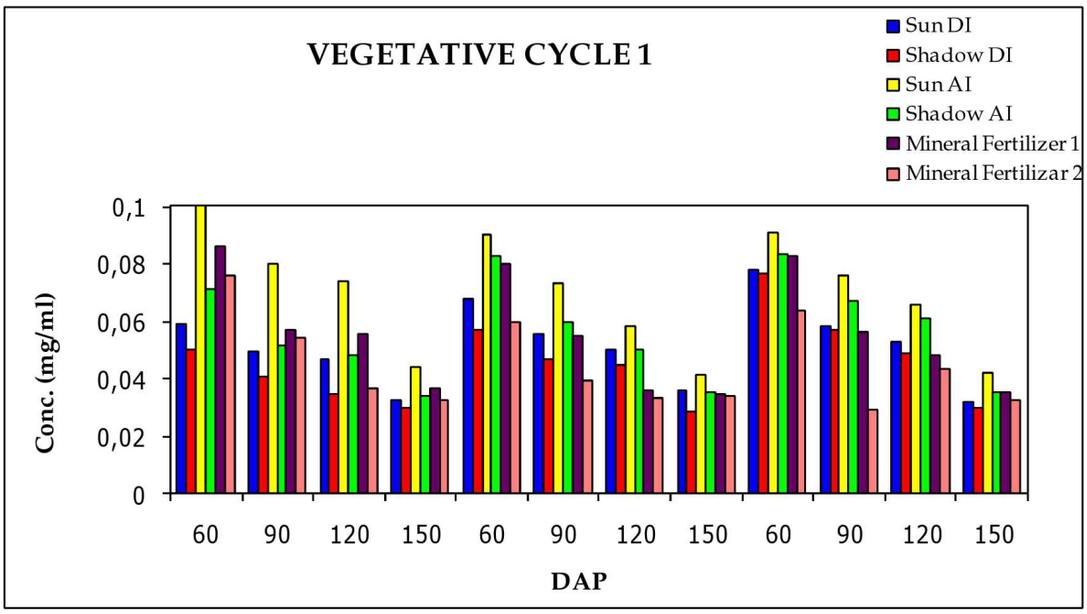


Fig. 2. Study of the seasonality of β-caryophyllene (Vegetative Cycle 1). Concentration (mg / mL) x time of collection X DAP (days after planting).

experimental conditions was provided with irrigation alternating sun followed by shade conditions with irrigation alternating sun with daily irrigation, a mineral fertilizer, mineral fertilizer with irrigation and shade 2 daily, respectively. Relating the best experimental condition (alternating sun with irrigation) in terms of DAP shows that the highest concentration of β-caryophyllene occurred at 60 DAP followed by a descending manner for 90, 120 and 150 DAP, respectively. This fact is also observed in other conditions. In relation to the different collection times (7, 12 and 16 h), the data show that the schedule with the highest concentration of β-caryophyllene related to experimental condition was the best of 7h with a concentration of (0.1005 mg/mL) 60 DAP (0.0805 mg/mL) 90 DAP (0.0742 mg/mL) and 120 DAP (0.0444 mg/mL) 150 DAP, followed by 16 and 12 p.m., respectively.

3.2.2 Vegetative cycle 2

In the period from June to September corresponding to the second growing season, according to Figure 3, shows that the highest concentration of β-caryophyllene as a function of experimental conditions was provided a mineral fertilizer [nitrogen, phosphorus and potassium (NPK)] followed by two conditions mineral fertilizer (NPK with lime), alternating with irrigation sun, sun with daily irrigation, shade and shadow alternated with irrigation with daily irrigation respectively. Relating the best experimental condition (a mineral fertilizer) as a function of days after planting (DAP) shows that the highest

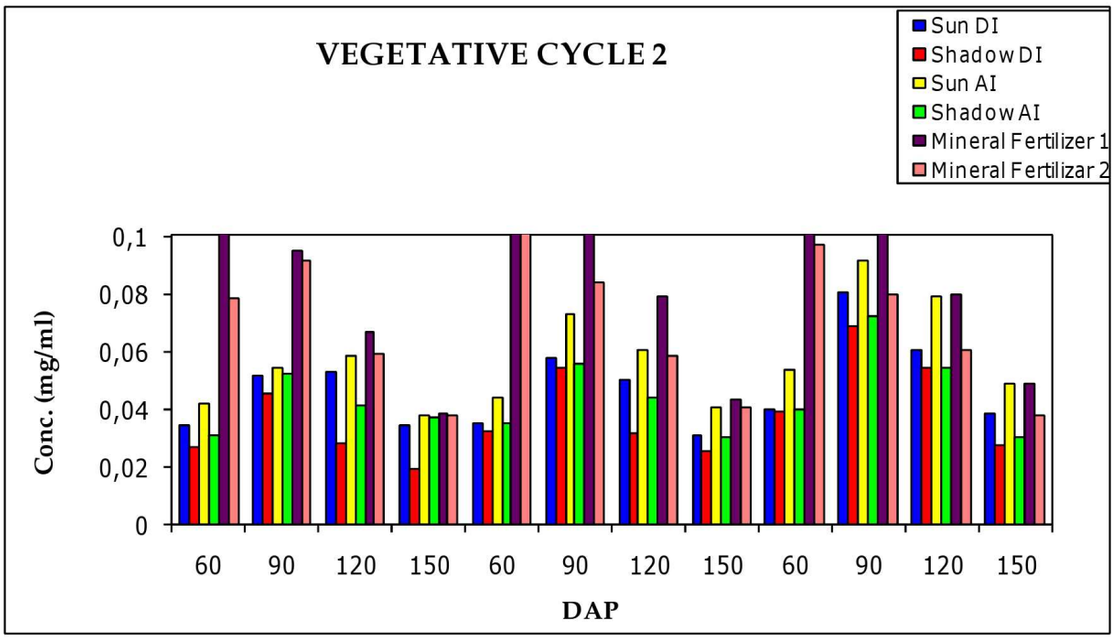


Fig. 3. Graph of the seasonal study of β-caryophyllene (Vegetative Cycle 2). Concentration (mg / mL) x time of collection X DAP (days after planting).

concentration of β-caryophyllene occurred at 60 DAP followed by a descending manner for 90, 120 and 150 DAP, respectively. This fact is also observed in mineral fertilizer condition 2. In other conditions can be observed that the highest concentration of β-caryophyllene occurred at 90 DAP followed in decreasing order by 120, 60 and 150 DAP, respectively. In relation to the different collection times (7, 12 and 16 h), the data show that the schedule with the highest concentration of β-caryophyllene related to better the condition of the experiment was 16 h at concentrations (0.1175 mg/mL) 60 DAP (0.1133 mg/mL) 90 DAP (0.0799 mg/mL) and 120 DAP (0.0493 mg/mL) 150 DAP, followed by the time 12 and 7 p.m., respectively.

Figure 4 lists the concentration of β-caryophyllene condition of the sun in different irrigation alternating cycles of vegetation to rainfall in terms of DAP, the data confirm that the production of essential oil yield has increased in the months of low rainfall and low income in months of high precipitation. It is observed that a higher concentration of rainfall in June (vegetative cycle 2/60DAP), with an index of 616.9 mm, recorded the lowest yield of essential oil (0.0541 mg/mL), confirming that the production of essential oil is reduced in the presence of excess water.

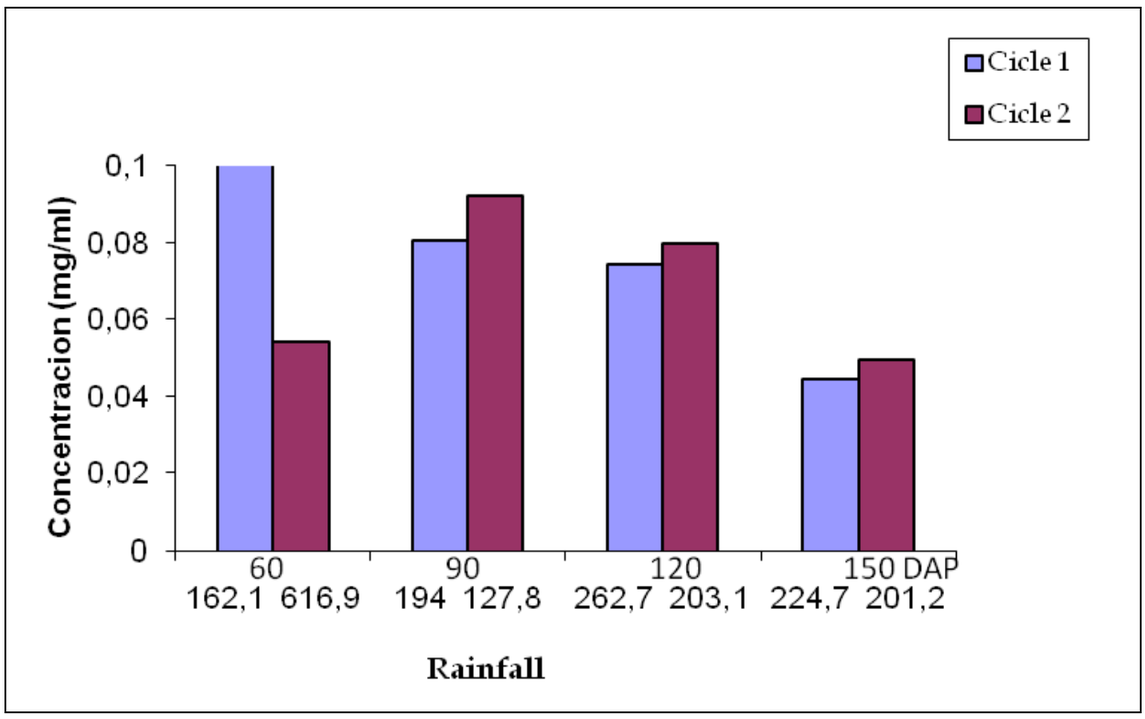


Fig. 4. Concentration (mg / mL) x rainfall in terms of DAP (days after planting).

According to Salisbury & Ross (1991) and Bazaaz et al. (1987), critical physiological factors, such as photosynthesis, stomatal behavior, mobilization of reserves, leaf expansion and growth can be altered by water stress and consequently lead to changes in secondary metabolism.

The effects of rain on the vegetation should be considered in relation to the annual rate, its distribution throughout the year, its effect on humidity and its effect together with the ability to absorb water from the soil (Evans, 1996). Examples of the influence of rainfall on the production of secondary metabolites are the positive correlation of some components of the essential oil of Santolina rosmarinifolia (Pala-Paul et al., 2001) and the negative correlation between the production of saponins, as in Phytolacca dodecandra lemmatoxina with precipitation levels (Ndamba et al., 1994). The effect of drought on the concentration of metabolites is sometimes dependent on the degree of water stress and the period in which, while short-term effects seem to lead to increased production, while the long term the opposite effect is observed (Waterman & Mole, 1989; Horner, 1990, Mattson & Haack, 1987; Waterman & Mole, 1994, Medina et al., 1986).

The age and plant development, as well as different plant organs, are also of considerable importance and may influence not only the total amount of metabolites produced, but also the relative proportions of the mixture (Bowers & Stamp, 1993; Hendriks, 1997 , Evans, 1996, Jenks et al. 1996; Kasperbauer & Wilkinson, 1972). It is also known that younger tissues generally have higher rates of biosynthetic metabolites (Hartmann, 1996), such as essential oils (Hall & Langenheim, 1986, Gershenzon et al., 1989), sesquiterpene lactones (Spring & Bienert, 1987) phenolic acids (Koeppe et al., 1970), alkaloids (Hoft et al., 1998), flavonoids and stilbenes (Slimestad, 1998). Duriyaprapan et al. (1986) and Tuomi et al. (1991) also argue that the concentration of secondary metabolites used for defense plant concentration tends to reverse the growth rates, and then there is deviation of the primary metabolism of compounds (sugars, proteins, lipids) for the production of secondary metabolites, such as terpenoids. Martins & Santos (1995) mentioned that, according to the active ingredient of the plant, there are times when the concentration of these principles is greater. In the mornings it is recommended to crop plants with essential oils and alkaloids, and in the afternoon, plant glucosides. It was noted, for example, a variation of more than 80% in the concentration of eugenol in essential oil of basil (Ocimum gratissimum), which reaches a maximum around noon time that is responsible for 98% of essential oil in contrast to a concentration of 11% around 17h (Silva et al., 1999). Coniina levels are higher in Conium maculatum when collections are made in the morning to dusk (Suwal & Fairbairn, 1961). The total content of taxanes in Taxus media was lower in the morning, increasing during the day and peaking in the late evening (ElSohly et al., 1997). Regarding the second growing season is observed that these results strengthen the hypothesis that between June and September, with milder temperatures, soils with a mineral fertilizer (NPK) and two mineral fertilizer (NPK with limestone) provide better absorption of nutrients than organic fertilizers. According to Evans (1996) to track changes that occur yearly, monthly and daily temperature is a factor that exerts the greatest influence on plant development, thus affecting the production of secondary metabolites. In general, the formation of volatile oils appears to increase at higher temperatures, although very hot day lead to an excessive loss of these metabolites.

Koeppe et al. (1970) demonstrated in tobacco leaves (Nicotiana tabacum), an increase of four to five times the content of scopolamine, chlorogenic acid and its isomers (antioxidant compounds) after submission to low temperatures. It should be noted that the application of organic fertilizer is intended to improve the physical properties (density, aeration and drainage, water retention), chemical (nutrient supply, remediation of toxic substances, pH index) and physicochemical (adsorption of nutrients, cation exchange capacity) of soil. The organic fertilizer involved in water retention and regulation of soil temperature (Manlio, 2006). The organic manure should not be considered the main source of nitrogen, phosphorus and potassium from the soil-plant system, although it contains these macronutrients, but not enough to meet the needs of the plant (Roberto et al., 2007). Mineral fertilizers are suppliers of plant nutrients, although they do not provide improvements to the physical properties of soil, only by improving the supply of chemical nutrients. Another important factor related to increased yield of essential oil in mineral fertilizer is related to loss of nitrogen by volatilization as ammonia, being lower in the second growing season probably due to lower temperatures (Table 2). According to Marschner (1995) and Malavolta et al. (1989), deficiency of nitrogen in the plant is involved in the reduction of growth as a result of the functions that the nutrient plays in the plant. Typically, nitrogen is the nutrient required by most cultures, since the molecular structure acts as the amino acids, proteins, enzymes, pigments and byproducts. Correa (1994) states that the completion of organic manure with mineral fertilizer is able to guarantee an optimal concentration of active ingredients in the plant. The first mineral fertilizer obtained higher rates than the two mineral fertilizers, because, according to Oliveira et al. (2005) the amount of essential oil produced is negatively influenced by liming (lime application). The highest yields of oil were obtained in mineral and mixed treatments (organic fertilizer with NPK) without liming. Among the treatments recommended fertilization without liming mixed by combining high yield of essential oil.

4. Conclusion

In this study we can conclude that this chromatographic method developed and validated, was sensitive, accurate, reproducible, robust and linear, can be used, therefore, to evaluate the concentration of β-caryophyllene in extracts obtained from plant Plectranthus amboinicus (Lour) Spreng. The settings allow you to evaluate the relative content of β-caryophyllene in the essential oil from aerial parts of Plectranthus amboinicus (Lour.) Spreng., Lamiaceae, it is observed that in the months of lowest rainfall has been a higher oil yield essential, and the months of heaviest rainfall showed lower yields. The assessment of the amount of essential oil in different collection times showed that in the months of lowest rainfall the best time of collection was at 7 pm where the plant had a greater retention of oil, followed by 16 and 12 h, respectively. In the rainy season, the best time of collection was at 16 h where the plant had a greater retention of oil, followed by 12 and 7h, respectively.

The results presented here strengthen the hypothesis that the organic fertilizer in the summer had the best performance in relation to essential oil yield because of its characteristic term regulatory and water retention. At high temperatures the mineral fertilizer is less efficient compared to lower temperatures.

5. References

Albuquerque RL 2000. Contribuição ao estudo químico de plantas medicinais do Brasil: *Plectranthus barbatus* Andr. E *Plectranthus amboinicus* (Lour) Spreng. Fortaleza, 166 p. Dissertação de Mestrado, Universidade Federal do Ceará

Anvisa 2004. Resolução da Diretoria Colegiada da Anvisa n° 48, 16 de março

Bazaaz F, Chiariello N, Coley P, Pitelka L 1978. Herbal medicine in Jordan with special emphasis on commonly used herbs. *Bioscience 37*: 58

Bowers MD, Stamp NE 1993. Commonly used herbal medicines in the United States: a review. *Ecology 74*: 54-57

Brito, N.M.; Amarante Júnior, P.; Polose, L.; Ribeiro, M.L. Validação De Métodos analíticos: Estratégia e discussão. R. Ecotoxicol. E Meio Ambiente, Curitiba, V.13, p. 129-143, Jan./Dez. 2003

Corrêa JC, Sharma RD 1994. Produtividade do algodoeiro herbáceo em plantio direto no Cerrado com rotação de culturas. *Pesq Agropec Bras 39*: 41-46

Duke JA 1992 Handbook of Biologically Active Phytochemicals and their Activities, *CRC Press: Boca Raton*

Duriyaprapan S, Kessler RC, Foster C, Norlock FE, Calkins DR 1986. The effect of temperature on growth, oil yeld and oil quality of Japanese mint. *Ann Bot-London 58*: 729-736

ElSohly HN, Croom EM, Kopycki WJ, Joshi AS, McChesney JD 1997. The medicinal plants in our time. *Phytochem Anal 8*: 124-127

Evans WC 1996. Trease and Evans' Pharmacognosy. *14. ed. London:* WB Saunders Company.

Fairbairn JW, Suwal PN 1961. Bioactive natural products. *Phytochemistry 1*: 38-41

Freitas MS, Souza KCB, Resende UP 2004. Produção e qualidade de óleos essenciais de *Mentha arvensis* em resposta à inoculação de fungos micorrízicos arbusculares. *Pesq. Agropecu Bras 39*: 887-894

Gershenzon J, Maffei M, Croteau R 1989. Some aspects of toxic contaminants in herbal medicines. *Plant Physiol 89*: 1351-1352

Hall GD, Langenheim JH 1986. Bioactive natural products. *Biochem Syst Ecol 14*: 61-64

Hartmann T 1996. Global harmonization of herbal health claims. *Ent Exp Appl 80*: 177-179

Haslam E 1996. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J Nat Prod 59*: 205-215

Hedge IC 1992. Country borage (*Coleus amboinicus* Lour.): a potent flavoring and medicinal plant. *Adv Lab Sci 6*: 7-17

Hendriks H, Anderson-Wildeboer Y, Engels G, Bos R, Woerdenbag H J 1997. Malay ethno-medico botany in Machang. *Planta Med 63*: 356 369

Höft M, Verpoorte R, Beck E 1998. Screening of medicinal plants for induction of somatic segregation activity. *Planta Med 64*: 148-152

Horner JD 1990. Kannada District in Karnataka, India; plants in treatment of skin diseases. *Biochem Syst Ecol 18*: 211-217

Jacobson M 1990. Glossary of Plant-Derived Insect Deterrents, *CRC Press: Boca Raton*

Jenks MA, Tuttle HA, Feldmann KA 1996. The vegetable material medica of western India. *Phytochemistry 42*: 29-33

Kang R, Helms R, Stout MJ, Jaber H, Nakatsu T 1992. Vietnamese culinary herbs in the United States. *J Agric Food Chem 40*: 2328-2332

Keeler RF, Tu AT 1991. Toxicological of Plant and Fungal Compounds; Handbook of Natural Toxins; *Marcel Dekker: Nova York*,c p. 665

Koeppe DE, Rohrbaugh LM, Rice EL, Wender SH 1970. Pharmacological screening of plant decoctions commonly used in Cuban folk medicine. *Plant Physiol 23*: 258-261

Kutchan TM 2001. Herbal mixtures in the traditional medicine of Eastern Cuba. *Plant Physiol 125*: 58-62

Lawrence BM 1992. Chemical components of Labiatae oils and their exploitation. In: Harley RM, Reynolds T (eds.). *Advances in Labiatae science*. Kew: Royal Botanic Gardens, p. 399-436

Lindroth RL, Hsia MTS, Scriber JM 1987. Tropical Plants. *Biochem Syst Ecol 15*: 681-682

Malavolta E, Vitti, GC, Oliveira SA 1989. Avaliação do estado nutricional das plantas: princípios e aplicações. *Piracicaba,* POTAFOS, 201p

Manlio SF 2006. Nutrição Mineral de Plantas. Viçosa, MG: *Sociedade Brasileira de Ciência do Solo.* p. 89-114

Marschner, H. 1995. Mineral Nutrition of Higher Plants. *Academic Press, London*, UK

Martins ER, Santos RHS 1995. Plantas medicinais: uma alternativa terapêutica de baixo custo. Viçosa: *UFV Imprensa Universitária*

Mattson WJ, Haack RA 1987. Amazonian uses of some plants growing in India. *Bioscience 37*: 110-112

Medina E, Olivares E, Diaz M 1986. Herbal mixtures in the traditional medicine of Eastern Cuba. *Oecologia 70*: 441-443

Ndamba J, Lemmich E, Mølgaard P 1994. African traditional medicine. A dictionary of plant use and applications. *Phytochemistry 35*: 95-98

Oliveira TK, Carvalho GJ, Moraes RNS 2005 Plantas de cobertura e seus efeitos sobre o feijoeiro em plantio direto. *Pesq Agropec Bras 37*: 1079-1087

Palá-Paúl J, Pérez-Alonso MJ, Velasco-Negueruela A, Palá-Paúl R, Sanz J, Conejero F 2001. Plants as source of drugs. *Biochem Syst Ecol 29*: 663-665

Roberto CS, Francisco EAB, Câmara GMS, 2007. Estado nutricional e produção do capim-pé-de-galinha e da soja cultivada em sucessão em sistema antecipado de adubação. *Bragantia 66*: 259-266

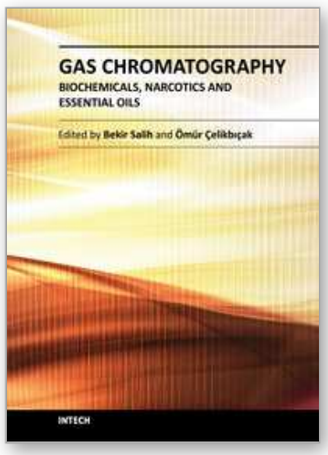
Salisbury FB, Ross CW 1991. Inhibition of the growth of cariogenic bacteria *in vitro* by plant flavanones. *Plant Physiol 2*: 71-75

Shimizu M 1990. Quantity estimation of some contaminants in commonly used medicinal plants. *Chem Pharm Bull 38*:2283-2287

Silva MGV, Craveiro AA, Matos FJA, Machado MIL, Alencar JW 1999. Efeito antioxidante de extrato fluido de *Plectranthus amboinicus. Fitoterapia 70*: 32-34

Slimestad R 1998. Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. *Biochem Syst Ecol 26*: 225-229

United States Pharmacopeia 23ª Ed. Rockville: United States. Pharmacopeial convention Inc, 1994. 2391p



Gas Chromatography - Biochemicals, Narcotics and Essential Oils
Edited by Dr. Bekir Salih

ISBN 978-953-51-0295-3
Hard cover, 236 pages
Publisher InTech
Published online 09, March, 2012
Published in print edition March, 2012

Gas Chromatography involves the study of various vaporizable molecules in chemistry and the other related research fields. This analytical method has a number of features and advantages that make it an extremely valuable tool for the identification, quantification and structural elucidation of organic molecules. This book provides detailed gas chromatography information to applications of biochemicals, narcotics and essential oils. The details of the applications were briefly handled by the authors to increase their comprehensibility and feasibility. This guide should be certainly valuable to the novice, as well as to the experienced gas chromatography user who may not have the enough experience about the specific applications covered in this book. We believe this book will prove useful in most laboratories where modern gas chromatography is practiced.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Fabíola B. Carneiro, Irinaldo D. Júnior, Pablo Q. Lopes and Rui O. Macêdo (2012). Development and Validation of Analytical Methodology and Evaluation of the Impact of Culture Conditions and Collection Associated with the Seasonality in the Production of Essential Oil of Plectranthus amboinicus (Lour) Spreng, Gas Chromatography - Biochemicals, Narcotics and Essential Oils, Dr. Bekir Salih (Ed.), ISBN: 978-953-51-0295-3, InTech, Available from: http://www.intechopen.com/books/gas-chromatography-biochemicals-narcotics-and-essential-oils/variation-in-the-amount-of-caryophyllene-in-essential-oil-of-plectranthus-amboinicus-lour-spreng-lam

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen