academicJournals

Vol. 10(7), pp. 88-92, 17 February, 2016
DOI: 10.5897/JMPR2015.6016
Article Number: 62104A957243
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Effect of frost on yield and composition of *Aloysia triphylla* essential oil

**Denise Schmidt[1], Braulio Otomar Caron[1], Daiane Prochnow[1], Carine Cocco[1], Elvis Felipe Elli[1]*, John Stolzle[1], Bruna Altissimo[1] and Berta Maria Heinzmann[2]**

[1]Graduate Program in Agronomy - Agriculture and Environment, Federal University of Santa Maria, Northern Center for Higher Education in Rio Grande do Sul - Frederico Westphalen Campus, Linha Sete de Setembro s/n, BR386 Km 40, CEP 98400-000, Frederico Westphalen, Rio Grande do sul, Brazil.
[2]Department of Industrial Pharmacy, School of Pharmacy, Federal University of Santa Maria, Roraima Avenue, 97015-900 Santa Maria, RS, Brazil.

Received 20 November, 2015; Accepted 21 January, 2016

***Aloysia triphylla,* also known as lemon verbena is a medicinal plant with aromatic leaves which produce essential oil rich in citral and has great importance for pharmaceutical and cosmetic industries. The objective of this study was to evaluate the effect of frost on the chemical composition and yield of *A. triphylla* essential oil. The experiment was conducted at the Campus of UFSM in Frederico Westphalen, Rio Grande do Sul, in July 2014. A complete randomized design with three repetitions was used where three random collections and extractions occurred before and after the occurrence of frost. The oil was obtained via hydrodistillation and analyzed by a process of gas chromatography and mass spectrometry. The total yield obtained from leaves before frost was 0.162% and 0.187% after frost. Essential oil composition was largely not affected by the presence of frost; however, of the macro components, limonene was observed to be most sensitive to frost, accounting for 14.36% of essential oil content before and 10.15% after frost.**

**Key words:** Chemical composition, secondary metabolites, low temperatures.

## INTRODUCTION

*Aloysia triphylla* is synonymous with *Aloysia citriodora* Palau, *Lippia citriodora* (Lam.) Kunth, and *L. triphylla* (L Her.) Kuntze and also known as Lemon verbena. Native to South America, it is an herb rich in essential oil with known astringent properties (Lira et al., 2013). Its leaves have great medicinal importance and have been shown to have antipyretic and antispasmodic properties, and are known to aid in digestion (El-Hawary et al., 2012). One of the chemicals of greatest interest of this essential oil is citral, due to its importance in the development of pharmaceuticals (Rojas et al., 2012).

In recent years, several studies have been conducted around the world with regards to the production of this species (Ali et al., 2011; Rojas et al., 2012; Felgines et al., 2014). Great differences in the yield and composition of essential oil distilled from the leaves of *A. triphylla* have

*Corresponding author. E-mail: elvisfelipeelli@yahoo.com Tel: (55) 9664-7464.

been noted in plants collected from different seasons of the year and in different regions of the world (Lira et al., 2013; Díaz et al., 2007).

The biosynthesis of essential oils in plants is influenced by seasonal variation, such as temperature, photoperiod, relative humidity, precipitation, and solar intensity, and can be determinants of optimal harvest times used to analyze periods of greater essential oil yield or the yield of a specific component. In the state of Rio Grande do Sul, the temperature most favorable to the growth and development of *A. triphylla* occurs between September and April where the average temperature ranges from 12 to 25°C. During winter there is a general reduction in the plant's growth rate, likely related to a decrease in average daily temperatures.

According to Paulus et al. (2013), *A. triphylla* cultivated in the slightly more northern state of Paraná began losing leaves between the months of May and June, at a gradual rate of 40%; the authors observed a total loss of leaves in July due to the occurrence of frost which made it impossible to harvest leaves for analysis.

Leaves damaged by frost can display lower rates of respiration, the inhibition of protein synthesis, and a degradation of proteins which disrupts the plasma membrane during cooling (Taiz and Zeiger, 2013). Some studies claim that when subjected to stress conditions, the plants produce a greater amount of essential oil (Abreu and Mazzafera, 2005). The frequency and duration of these stress periods often results in more serious consequences which can threaten the overall health of a plant. The chemical composition of essential oil is genetically determined; however several abiotic factors such as light, temperature, and water availability can significantly modify the production of secondary metabolites. For species of *A. triphylla*, production and composition of essential oil appears to be due to climatic conditions, especially temperature and radiation (Paulus et al., 2013). The effect of frost on essential oil composition and production is relatively unknown.

The identification of limiting climatic conditions for essential oil production is an important aspect for systems of commercial production. This study aimed to evaluate the effect of frost on the composition and yield of essential oil production, as well as identify major compounds in the essential oil of *A. triphylla*.

## MATERIALS AND METHODS

The experiment was conducted in fields belonging to the Federal University of Santa Maria, Campus of Frederico Westphalen, in Rio Grande do Sul, Brazil, latitude 27° 23'26" S; longitude 53° 25'43", altitude 4613 m in the city of Frederico Westphalen. The climate, according to Koppen climate classification is Cfa, with distinct seasons throughout the year and mean temperatures for the hottest month exceeding 22°C and for the coldest month near 3°C, with the possible formation of frost during the winter (mainly in July and August). Rainfall is well distributed over the months of the year, and the local soil is a typical dystrophic Red Latosol.

The seedlings were originally grown from cuttings 15 to 20 cm long, which were taken from healthy branches of mature plants; a 1000 ppm dose of indole butyric acid was applied to these cuttings. The cuttings were then placed to root in trays with 96 seedling tubes of medium size, which were filled with a mixture of commercial substrate and vermiculite in a 1:1 ratio and placed in a protected environment with sprinkler irrigation until the full development of the root system within the core of the plant.

After the preparation of seedlings, transplanting occurred in previously prepared plant beds. Each experimental unit was composed of an *A. triphylla* plant, with 0.8 m of space between plants and 1.0 m between rows. The transplant was performed on November 23, 2011. The plants were hand weeded and provided water through drip irrigation.

The experiment used a complete randomized design which resulted in three collections from different plants before frost, and three collections from additional plants after frost; each collection resulted in an independent process of hydrodistillation. The collection of samples was performed in July 2014, which is considered the coldest month of the year in the region. Samples were collected in different weather conditions: the first in a typical winter day with low temperatures (the average temperature was 4.6°C) but without the occurrence of frost, and the second collection after the occurrence of frost (average temperature for the day was 2.6°C).

In order to assess the effect of frost on the yield (%) and composition of the essential oil, the date of collection for the two groups (before and after the presence of frost) was based on a forecast from the previous day and the probability of occurrence of frost (on July 23$^{rd}$). After the frost, additional samples of plant material were unable to be taken as a result of the total leaf senescence due to the formation of frost.

The air temperature values were obtained from the Climatological Station INMET (National Institute of Meteorology) located 50 m from the experiment, the station is linked to the Agroclimatology Laboratory of the Federal University of Santa Maria. For collection the day before frost, mean maximum temperature was 5.2°C and the mean minimum temperature was 4.2°C with a minimum of 2.2°C; for collection after the presence of frost, the mean maximum was 3.5°C and the mean minimum was 1.9°C with a minimum recorded temperature of -1.8°C.

The essential oil was obtained from fresh material (leaves) by hydrodistillation, as accepted in the literature (Argyropoulou, et al., 2007, Gomes et al., 2006). Three samples consisting of 200 g of fresh leaves were used in the calculation of the total yield; the leaves were removed from the apical, middle and basal branches and chopped into fractions of approximately 1 cm. The collection of plant material took place at 14:00 h in order to let the leaves dry after the morning frost and dew. The extraction time was 3 h (Schwerz et al., 2015). After obtaining the essential oil, the yield (%) was measured and calculated by the formula: % T = oil mass (g) / 200 g x 100. Three extractions were performed for the calculation of each total yield (before and after frost).

The chemical composition of the essential oil was determined with a gas chromatograph-mass spectrometry system: Agilent Technologies 6890N GC-FID equipped with a capillary column DB-5 (30 x 0.25 mm; 00:25 mm film thickness) and connected to a FID detector. The temperatures of the detector and the injector were adjusted to 280°C. Helium gas was used at a flow rate of 1.3 ml min$^{-1}$. The temperature program was from 50 to 300°C at a rate of 5°C min$^{-1}$. One microliter of essential oil was added to the chromatograph.

The percent area of the compound was calculated based on the areas of the GC peaks without the use of correction factors. In addition to GC-MS results, essential oil chemical compounds were also identified by comparing retention indexes (RI) using a homologous series of n-alkanes (C7-C26). Mass spectra were then compared with the Wiley 275 L mass spectral library and literature (Adams, 2009). The results were submitted to analysis of variance

**Table 1.** Chemical composition and yield of essential oil (%) for leaves of *Aloysia triphylla* before and after the occurrence of frost in Frederico Westphalen – RS, 2014.

| Peak | [a]Constituent | [b]IK Reference | Treatments | | | |
|---|---|---|---|---|---|---|
| | | | Before frost | | After frost | |
| | | | [c]IK calculate | Area (%) | [c]IK calculate | Area (%) |
| | ***Others*** | | | ***4.51*** | | ***6.69*** |
| 1 | 1-Octen-3-ol | 974 | 982 | 0.74 | 982 | 0.68 |
| 2 | 6-Metil-5-hepten-2-ona | 986 | 991 | 3.77 | 991 | 6.01 |
| | ***Monoterpene hydrocarbons*** | | | ***16.36*** | | ***12.68*** |
| 3 | Limoneno | 1028 | 1027 | 14.36 | 1027 | 10.16 |
| 4 | β-Ocimeno | 1053 | 1049 | 2.00 | 1048 | 2.52 |
| | ***Oxygenated monoterpenes*** | | | ***57.33*** | | ***53.70*** |
| 5 | Linalol | 1097 | 1101 | 0.56 | 1101 | 0.51 |
| 6 | α-Ciclocitral | 1102 | 1103 | 0.81 | 1103 | 0.79 |
| 7 | *Z*-*p*-Menten-2,8-dienol | 1122 | 1122 | 0.53 | - | - |
| 8 | *E*-Verbenol | 1144 | 1139 | 2.19 | 1139 | 1.96 |
| 9 | *E*-Crisantemal | * | 1150 | 0.88 | 1151 | 1.18 |
| 10 | *Z*-Isocitral | 1164 | 1167 | 2.21 | 1166 | 2.11 |
| 11 | *E*-Isocitral | 1180 | 1184 | 4.43 | 1184 | 3.83 |
| 12 | *Z*-Geraniol | 1230 | 1230 | 1.05 | 1230 | 1.19 |
| 13 | β-Citral (Neral) | 1240 | 1242 | 15.89 | 1242 | 13.26 |
| 14 | *E* –Geraniol | 1260 | 1256 | 0.81 | 1256 | 1.05 |
| 15 | α-Citral | 1270 | 1272 | 27.21 | 1271 | 26.77 |
| 16 | Acetato de geranila | 1381 | 1385 | 0.76 | 1385 | 1.05 |
| | ***Sesquiterpene hydrocarbons*** | | | ***7.37*** | | ***7.52*** |
| 17 | α-Cedreno | 1412 | 1413 | 0.41 | 1412 | 0.35 |
| 18 | β-Cariofileno | 1420 | 1420 | 4.08 | 1421 | 3.1 |
| 19 | Elixeno | 1492 | 1498 | 1.48 | 1498 | 1.76 |
| 20 | β-Curcumeno | 1516 | 1513 | 0.91 | 1514 | 1.18 |
| 21 | δ-Cadineno | 1524 | 1524 | 0.49 | 1525 | 1.13 |
| | ***Oxygenated sesquiterpenes*** | | | ***5.57*** | | ***8.86*** |
| 22 | *E*-Nerolidol | 1563 | 1565 | 0.99 | 1565 | 2.32 |
| 23 | Espatulenol | 1580 | 1579 | 2.05 | 1579 | 2.61 |
| 24 | Óxido de cariofileno | 1584 | 1585 | 1.22 | 1585 | 1.55 |
| 25 | Helifolen-12-al A | 1593 | 1594 | 0.34 | 1594 | 0.59 |
| 26 | δ- Cadinol | 1646 | 1643 | 0.97 | 1643 | 1.79 |
| | Total identified (%) | | | 91.14 | | 89.45 |
| | Yield (%) | - | | 0.162 | | 0.187 |

[a]Constituent listed in order of elution from DB-5 column. [b]Identification based on Kovats Index (IK); [c]identification based on comparison of mass spectra; * Kovats index not reported.

using the computational program 'Statistical Analysis System' (SAS, 2003).

## RESULTS

In the essential oil extracted for leaves of *A. triphylla*, 26 constituents were detected identified from GC-MS analysis and listed by elution time (Table 1). Limonene, citral, and neral were the dominant chemical constituents of the essential oil making up 57.56% of the oil before frost and 50.19% of the oil after frost. Oxygenated monoterpenes made up the majority of the essential oil with 57.33% composition before frost and 53.70% after frost.

The major compounds identified in the essential oil were citral, neral, and limonene: citral varied between 27.21% before and 26.77% after frost, neral between 15.89% before and 13.26% after frost, and Limonene 14.36% before and 10.15% after frost. The analysis of variance showed no significance between the before and after frost test samples.

## DISCUSSION

The essential oil yield of 0.162 and 0.187% observed before and after the formation of frost (Table 1), respectively, is in agreement with results found by Paulus et al. (2013) who found lower essential oil content of *A. triphylla* during the winter, ranging from 0.07 to 0.27%. Taiz and Zeiger (2013) argue that a reduction in temperature and other weather elements can reduce the primary and secondary metabolism of the plant, causing cellular stress, and a lower rate of essential oil synthesis. According to Correa (1994), under stress conditions there is an increase in the production of trichomes on the leaves, with an inverse relationship in essential oil production.

The main visual observation was of tissue necrosis and subsequent senescence and death of leaves, as observed on the 23rd of July, 2014 where temperatures ranged from 0.9 to -1.8°C for a total of eight hours; this characterizes the species as sensitive to frost.

The essential oil storage of Lamiaceae family plants usually occurs in peltate glandular trichomes which are located both on the adaxial and abaxial leaf faces (Gattuso et al., 2008). As the essential oil does not freeze, it is likely that the cells of these trichomes are not as strongly affected. The cells contain concentrations of oil and low concentration of water; this helps to keep the structure of their biomembranes intact in the presence of frost. Similarly, as noted in this study, frost only had an effect on leaf necrosis and senescence; it had no influence on the yield and composition of the oil, likely because of its storage in trichomes, allowing its later extraction.

The commercial importance of essential oil of *A. triphylla* depends on the percentage neral among other constituents, as well as a low percentage of other undesirable components such as nerol and geraniol, which are oxidative forms of neral and geranial and affect overall composition (Tabatavaie and Nazari, 2007). These oxidative components (nerol and geraniol) were found in low amounts in the essential oil described in this study.

The results of this study demonstrate the importance of crop management on a commercial scale during the winter. Under conditions of low temperatures and frost it is common for leaf necrosis and senescence to occur. As the results showed, it is possible to harvest and extract oil after the presence of frost; however, within two days was the observed natural rate of ensuing leaf senescence. Thus it is possible to optimize the extraction of essential oil, without a decrease in strength and composition, after the occurrence of frost.

## Conclusions

The yield and overall chemical composition of essential oil from *A. triphylla* are not significantly, by analysis of variance, influenced by the occurrence of frost; however, of the macro components, limonene was observed to be most sensitive to frost, accounting for 14.36% of essential oil content before and 10.15% after frost.

## Conflict of Interests

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENTS

The authors are grateful to the National Council for Scientific and Technological Development (CNPq – Brazil) for the financial support.

### REFERENCES

Abreu IN, Mazzafera P (2005). Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. Plant Physiol. Biochem. 43(3):241-248.

Adams RP (2009). Identification of essential oil components by gas chromatography/mass spectrometry, 4th Edition Illinois: Allured Business Media. P. 804.

Ali HFM, El-Beltagi HS, Nasr NF (2011). Evaluation of antioxidant and antimicrobial activity of *Aloysia triphylla*. Eletronic J. Environ. Agric. Food Chem. 10(11):3044-3053.

Argyropoulou C, Daferera D, Tarantilis PA, Fasseas C, Polissiou M (2007). Chemical Composition of the Essential Oil from Leaves of *Lippia Citriodora* H.B.K. (Verbenaceae) at two developmental Stages." Biochem. Syst. Ecol. 35(12):831-837.

Correa JC (1994). Cultivo de plantas medicinais, condimentares e aromáticas. Jaboticabal: FUNEP. P. 162.

Díaz O, Duran DCG, Martínez JR, Stashenko EE (2007). Estúdio comparativo de la composición química de los aceites esenciales de *Aloysia triphylla* L'Her Britton cultivadas en diferentes regiones da Colômbia. Sci. Technica 13(33):351-353.

El-Hawary SS, Yousif MF, Motaal AAA, Abd Hameed LM (2012). Bioactivities, phenolic compounds and in-vitro propagation of *Lippia citriodora* Kunth cultivated in Egypt. Bull. Fac. Pharm. Cairo Univ. 50(1):1-6.

Felgines C, Fraisse D, Besson C, Vasson MP, Texier O (2014). Bioavailability of lemon verbena (*Aloysia triphylla*) polyphenols in rats: impact of colonic inflammation. Br. J. Nutr. 111(10):1773-1781.

Gattuso S, Van Baren CM, Gil A, Bandoni A, Ferraro G, Gattuso M (2008). Morpho-histological and quantitative parameters in the characterization of lemon verbena *(Aloysia citriodora* palau) from Argentina. Bol. Latinoam. Caribe Plantas Med. Aromat. 7(4):190-198.

Gomes PCS, Oliveira HRC, Vicente AMS Ferreira MF (2006). Production, transformation and essential oils composition of leaves and stems of lemon verbena *Aloysia triphylla* (L'Herit.) Britton grown in Portugal. Rev. Bras. Plantas Med. 8:130-135.

Lira PDL, Van Baren CM, López S, Molina AM, Heit C, Viturro C, Lampasona MP, Catalán CA, Bandoni AL (2013). Northwestern Argentina: a Center of genetic diversity of lemon verbena (*Aloysia citriodora* Palau, Verbenaceae). Chem. Biodivers. 10(2):251-261. http://dx.doi.org/10.1002/cbdv.201100416

Paulus D, Valmorbida R, Toffoli E, Nava GA (2013). Teor e composição química de óleo essencial de cidró em função da sazonalidade e horário de colheita. Hortic. Bras. 31(2):203-209. http://dx.doi.org/10.1590/S0102-05362013000200005

Rojas J, Palacios O, Ronceros S (2012). Efecto del aceite esencial de *Aloysia triphylla* Britton (cedrón) sobre el trypanosoma cruzi en ratones. Rev. Peru. Med. Exp. Salud Publica 29(1):61-68. http://dx.doi.org/10.1590/S1726-46342012000100009

Schwerz L, Caron BO, Manfron PA, Schmidt D, Elli EF (2015). Biomassa e teor de óleo essencial em Aloysia triphylla (l'hérit) Britton submetida a diferentes níveis de reposição hídrica e à variação sazonal das condições ambientais. Rev. Bras. Plantas Med. 17(4):631-641.

Tabatavaie SJ, Nazari J (2007). Influence of nutrient concentrations and NaCl salinity on the growth, photosynthesis and essential oil content of peppermint and lemon verbena. Turk. J. Agric. For. 31(1):245-253.

Taiz L, Zeiger E (2013). Fisiologia Vegetal. 5th ed. Porto Alegre: Artmed. P. 820.