CHARACTERIZATION OF THE CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM *Annona emarginata* (Schltdl.) H. Rainer 'terra-fria' AND *Annona squamosa* L.[1]

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ABSTRACT - The objective of this study was to characterize the chemical composition of the essential oil from the leaves of *Annona emarginata* (Schltdl.) H. Rainer 'terra-fria' and *Annona squamosa* L. The species were grown in a greenhouse for 18 months, which nutrient solution was applied weekly; the plants were then harvested and the leaves dried to extract the essential oil. The essential oil was analyzed by gas chromatography and mass spectrometry to study its chemical profiles. Eleven substances were found in the essential oil of *A. emarginata,* primarily (*E*)-caryophyllene (29.29%), (*Z*)-caryophyllene (16.86%), γ-muurolene (7.54%), α-pinene (13.86%), and tricyclene (10.04%). Ten substances were detected in the oil from *A. squamosa*, primarily (*E*)-caryophyllene (28.71%), (*Z*)-caryophyllene (14.46%), α-humulene (4.41%)**,** camphene (18.10%), α-pinene (7.37%), β-pinene (8.71%), and longifolene (5.64%). Six substances were common to both species: (*E*)-caryophyllene, (*Z*)-caryophyllene, α-humulene, camphene, α-pinene, and β-pinene.
Index terms: Annonaceae, secondary metabolites, terpenes, pharmacological potential.

CARACTERIZAÇÃO DO PERFIL QUÍMICO DO ÓLEO ESSENCIAL DE *Annona emarginata* (Schltdl.) H. Rainer 'terra-fria'e *Annona squamosa* L.

RESUMO-O objetivo do presente estudo foi caracterizar a composição química do óleo essencial das folhas de *Annona emarginata* (Schltdl.) H. Rainer 'terra-fria' e *Annona squamosa* L. As espécies foram cultivadas em casa de vegetação, com aplicação semanal de solução nutritiva até completar 18 meses, quando se realizaram a colheita e a secagem das folhas para a extração do óleo essencial, analisado por cromatografia gasosa acoplada à espectrometria de massas, para estudo dos perfis químicos. Onze substâncias majoritárias foram encontradas no óleo essencial de *A. emarginata*: (*E*)-cariofileno-29,29%; (*Z*)-cariofileno-16,86%; γ-muroleno-7,54%; α-pineno-13,86%; tricicleno-10,04%. No óleo de *A. squamosa,* 10 substâncias foram detectadas, entre elas: (*E*)-cariofileno-28.71%; (*Z*)-cariofileno-14,46%; α-humuleno-4,41%; canfeno-18,10%; α-pineno-7,37%, e β-pineno-8,71%; longifoleno-5,64%; majoritárias. (*E*)-cariofileno; (*Z*)-cariofileno; α-humuleno; canfeno; α-pineno, e β-pineno foram as seis substâncias comuns às duas espécies.
Termos para indexação: Annonaceae, metabólitos secundários, terpenos, potencial farmacológico.

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INTRODUCTION

Annona is an important genus of the Annonaceae family, primarily because of its edible fruits. The genus *Cananga* (Annonaceae) includes species with industrially important essential oils (CHATROU et al., 2012).

Recent taxonomic studies using molecular techniques have reorganized the species and genera of the Annonaceae family. Several genera have been included in other genera (SURVESWARAN et al., 2010; ZHOU et al., 2010; COUVREUR et al., 2012). These reorganizations include the proposal of Rainer (2007) that the genus *Rollinia* A. St.-Hil. be treated as a synonym of the genus *Annona*. However, the scientific literature often refers to *Rollinia* A. St.-Hil. In the present study, *Rollinia* will be treated as a synonym of *Annona*.

Previous studies have shown that the species of the Annonaceae family exhibit a great diversity of substances produced by their secondary metabolism. These compounds include aromatic substances, phenolic acids, steroids, alkaloids, acetogenins, and essential oils (LEBOEUF et al., 1982; LIMA, 2007; REIS, 2011).

The term essential oil refers to a group of aroma-bearing substances resulting from secondary metabolism (SPERRY, 2000). These substances are often produced in secretory structures, such as glandular cells and oleiferous canals, or in other specific structures (SIMÕES; MARIOT, 2003). These chemicals include, e.g., terpenes (monoterpenes and sesquiterpenes), phenylpropanoids, compounds containing nitrogen and sulfur, and aromatic compounds (BAKKALI et al., 2008). The essential oils have cytotoxic and phytotoxic effects (BAKKALI et al., 2008), which function to protect the plants against damage caused by arthropods; in addition, they are potentially very useful in medicine (LIMA et al., 2003; POTZERNHEIM et al., 2006; REGNAULT-ROGER et al., 2012). During the development of plants, secondary metabolism may be affected by numerous environmental factors, e.g., radiation, temperature, humidity, wind, and soil characteristics (WANG et al., 2012). According to Kamatou et al. (2008), several environmental conditions throughout the year affect the production, concentration, and biological activity of essential oils by plants. In nature, essential oils in plants serve important defensive functions, including bactericidal activity against Gram-positive and Gram-negative forms, antiviral and antifungal activity, insecticidal functions, and defense against herbivores. These compounds are also important for attracting dispersers of pollen and seeds and for repelling undesirable organisms (BAKKALI et al., 2008; RIVOAL et al., 2010). Several studies have been conducted to demonstrate the potential of essential oils in the pharmaceutical industry based on the functions of these oils in plant defense (EDRIS, 2007).

A number of studies have been performed in view of the pharmacological potential of the species of the Annonaceae family (OCAMPO; OCAMPO, 2006). The essential oil of *A. coreacea* Mart. has been found to exhibit antiprotozoan activity (SIQUEIRA et al., 2011). Muganza et al. (2012) conducted ethnopharmacological reviews of 33 plant species, including three species belonging to the Annonaceae family. These three species – *Anonidium mannii* Engl. & Diels, *Enantia chlorantha* Oliv., and *Isolona hexaloba* Engl. & Diels – were found to show anti-inflammatory activity affecting the blood vessels; antiprotozoan activity; and antimalarial activity and activity against headaches and appetite loss, respectively. Lima et al. (2012) have studied *Annona cornifolia* A. St.-Hil. and have noted that this species is a promising source of drugs for treating cancer because of its antioxidant and cytotoxic potential. Costa et al. (2009) have described the antimicrobial and antimalarial activities of *A. foetida* Mart. The biological activity cited for these three species of Annonaceae has been attributed to the effects of essential oils (COSTA et al., 2009; SIQUEIRA et al., 2011; LIMA et al., 2012).

The species *A. emarginata* ‘terra-fria’ is economically important because it is the most used rootstock in Brazil for cultivating atemoya, a plant whose fruit has great economic potential because of its organoleptic characteristics (TOKUNAGA, 2005). *A. emarginata* is highly resistant to stem borers, pests, and disease (KAVATI; WATANABE, 2010). The ecological resilience of the species in nature (rusticity) allows it to adapt to different nutritional conditions (BARON et al. 2013). In the Annonaceae family, essential oils occur not only in the leaves but also in other parts of the plant, such as the bark and roots, as previously shown by Boyom et al. (2011) and Muganza et al. (2012). *A. squamosa* (custard apple) and *A. cherimola* Mill. (cherimoya) are the species from which the atemoya hybrid originated. The potential biological activity of *A. squamosa* L. has been investigated. Seffrin et al. (2010) studied the seed extract of this species and found that it showed larvicidal activity against *Trichoplusia ni*. Other studies performed with the essential oil of the bark of *A. squamosa* L. have demonstrated its activity against Gram-positive bacteria. The author of such study has related this activity to the high percentage of caryophyllene oxide (29.38%) contained in the preparation. This form of biological activity has previously been described in the literature for such component (CHAVAN et al., 2006). The essential oil extracted from the leaves, bark, and stems of *A. squamosa* has been found to contain α-pinene, limonene, β-cubebene, β-caryophyllene, spathulenol, caryophyllene oxide, and α-cadinol as major components (THANG et al., 2012).

Although several studies on the Annonaceae family can be found in the literature, there are few reports about the chemical composition of the essential oil of the leaves of *A. emarginata* 'terra-fria' and *A. squamosa*. These species are not only important to atemoya cultivation but may also show pharmacological activity of economic interest.

The goal of this study was to characterize the chemical composition of the essential oil extracted from leaves of *A. emarginata* 'terra-fria' and *A. squamosa*.

MATERIALS AND METHODS

This study was conducted in a greenhouse with controlled temperature and humidity, located in the experimental area of the *Instituto de Biociências*, *Universidade Estadual Paulista, UNESP* - Câmpus Botucatu, *Departamento de Botânica*, 48°24'35'' W, 22°49'10'' S, 850 m above sea level.

Specimens of *A. emarginata* 'terra-fria' and *A. squamosa* (custard apple) were obtained by germinating seeds from São Bento do Sapucaí, 45°44′11″ W, 22°41′18″ S, 874 m above sea level. The seeds were sown in expanded polystyrene trays without dividers. During early development, the seedlings were transplanted to 10-L pots filled with a mixture of red earth, medium-textured vermiculite, and coconut fiber. The young plants then received weekly applications of Yogen® 3 nutrient solution containing 25% N, 6.5% P, 6.7% K, 0.053% Zn, 0.02% Co, 0.077% Mn, and 0.022% B (YOORIN®), as well as calcium nitrate, $Ca(NO_3)_2$ (HOAGLAND; ARNON, 1950). The nutrients provided were sufficient to ensure vegetative growth until 18 months after the transplantation to the pots, when leaves were collected for oil extraction.

The leaves of 10 *A. emarginata* 'terra-fria' plants and 15 *A. squamosa* plants were collected to obtain sufficient dry mass for essential oil extraction. The leaves were collected in the morning from 10:00 to 12:00 and then placed in a greenhouse (drying chamber) with forced-air heaters at 40ºC for 48 hours until a constant dry mass was obtained. After the completion of drying, 80 g of the dry mass of the leaves was hydrodistilled in a Clevenger-type apparatus for 2 hours to extract the essential oils. The oils were separated from the aqueous phase with the addition of solvent dichloromethane (0.5 mL, Merck, chromatographic grade), and the solution was stored in amber glass bottles and kept in a freezer at -20ºC prior to chemical analysis.

The chemical analysis of the essential oils was performed with gas chromatography coupled with electron-ionization mass spectrometry (70 eV) (GC-MS, Shimadzu, QP-5000). An OV-5 capillary column (Ohio Valley Specialty Chemical, Inc.; 30.0 m x 0.25 mm x 0.25 μm) was used with the following operating characteristics: injector at 240ºC, detector at 230ºC, split injection (1/20), injection volume: 1 μL of solution, ramp 60ºC at 240ºC, 3ºC /min. The substances were identified by comparing their mass spectra with the GC-MS (Nist. 62 lib.) system database based on retention indices (ADAMS, 2007). The retention indices were obtained by injecting a standard mixture of hydrocarbons (C_9-C_{24}) with the same chromatography conditions described above and then applying the equation of Van Dool & Kratz (1963).

RESULTS AND DISCUSSION

Eleven substances were identified in the essential oil of the leaves of *A. emarginata* 'terra-fria', and 10 components were identified in the oil of *A. squamosa*, as shown in Table 1. The major substances in the oil of both species were sesquiterpenes and monoterpenes. These results agree with the findings for *A. glabra* L., *A. squamosa* L., *A. muricata* L., and *A. reticulata* L. reported by Thang et al. (2012).

The essential oil of *A. emarginata* 'terra-fria' included five principal constituents: (*E*)-caryophyllene (29.29%), (*Z*)-caryophyllene (16.86%), γ-muurolene (7.54%), α-pinene (13.86%), and tricyclene (10.04%) (Table 1). Five principal constituents were identified in the oil of *A. squamosa* L.: (*E*)-caryophyllene (28.71%), (*Z*)-caryophyllene (14.46%), camphene (18.10%), α-pinene (7.37%), and β-pinene (8.71%) (Table 1). Accordingly, six components present in the oils of *A. emarginata* 'terra-fria' and *A. squamosa* L. should be highlighted: (*E*)-caryophyllene, (*Z*)-caryophyllene, α-humulene, camphene, α-pinene, and β-pinene. Among the six components common to the two species, three are major components in both: (*E*)-caryophyllene, (*Z*)-caryophyllene, and α-pinene.

Note that the percentages of (*E*)-caryophyllene and (*Z*)-caryophyllene were highly similar in both oils.

A previous evaluation of the composition of the essential oil of *Rollinia leptopetala* R. E. Fries showed that the following major components were present: cis-4-tujanol (17.37%), α-terpineol (8.42%), germacrene D (7.72%), bicyclogermacrene (22.47%), and *trans*-caryophyllene (6.63%) (synonymous with (*E*)-caryophyllene) (COSTA et al., 2008). A comparison of the composition of the essential oil of this species with that of the essential oil of *A. emarginata* 'terra-fria' shows that limonene, α-pinene, sabinene, *trans*-caryophyllene, and α-humulene were present in both oils. A comparison of the composition of the essential oil of *R. leptopetala* studied by Costa et al. (2008) with that of the essential oil of *A. squamosa* L. shows that β-elemene, α-humulene, α-pinene, and *trans*-caryophyllene are common to both species. These observations show that α-pinene, *trans*-caryophyllene, and α-humulene occur in the essential oils of the three species *R. leptopetala* R. E. Fries, *A. emarginata* 'terra-fria', and *A squamosa*. Of these constituents, *trans*-caryophyllene is a major component and shows anti-inflammatory activity (FERNANDES et al., 2007).

Thang et al. (2012) evaluated the essential oils of *A. glabra* L., *A. squamosa* L., *A. muricata* L., and *A. reticulata* L., all grown in Vietnam. The authors found 48 components in the essential oil of *A. squamosa* L., among which α-pinene, β-pinene, α-humulene, γ-himachalene, camphene, and β-elemene were also found in the essential oil of *A. squamosa* L. in the present study. It was also found (*E*)-caryophyllene, (*Z*)-caryophyllene, α-humulene, γ-himachalene, δ-elemene, camphene, α-pinene, β-pinene, β-elemene, and longifolene in the essential oil of *A. squamosa* L. These results show that the essential oil of *A. squamosa* grown in Vietnam had a greater number of components than that of the plants of this species grown in Brazil in the present study. The comparison between the results from Brazil and Vietnam highlights that the major components of the essential oil of this species may vary, due to, e.g., to climatic conditions, soil, nutrition, and water availability (MORAIS, 2009). Although the composition of the essential oil of *A. squamosa* grown under different conditions may vary, α-pinene and α-humulene were found in both studies cited and have also been observed in studies of other plant species (CYSNE et al., 2005; KAMATOU et al., 2008; ZEBELO et al., 2012).

Among the components identified in the essential oil of both species of Annonaceae evaluated in this study, *trans*-caryophyllene has also been isolated from the essential oil of *Cordia verbenacea* and showed anti-inflammatory activity, offering an attractive alternative for treating inflammation (FERNANDES et al., 2007). Limonene may be used as a flavor and fragrance in food preparation, to reduce the fat content of chocolate and in the biological control of insects (ROBALO et al., 2007). β-elemene has been shown to inhibit the growth of glioblastoma multiforme cells, found in a common and aggressive type of brain tumor (YOA et al., 2008). The substances α-pinene and β-pinene have bactericidal activity (LEITE et al., 2007). Camphene is the precursor of theosemicarbazide, which has fungicidal activity (YAMAGUCHI et al., 2009). Therefore, this study identified essential oil components with known biological activity and additional components whose activity should be evaluated.

TABLE 1- Chemical composition (%) of the essential oils of *Annona emarginata* (Schltdl.) H. Rainer 'terra-fria' variety and *Annona squamosa* L.

Substances	*Annona emarginata* (Schltdl.) H. Rainer (%)	*Annona squamosa* L. (%)	RI *	RI **
Monoterpene				
Triciclene	10.04	-	926	926
α-Pinene	13.86	7.37	933	939
Camphene	1.33	18.10	947	953
Sabinene	1.96	-	971	976
β-Pinene	1.83	8.71	976	980
Ortho cimene	1.93	-	1021	1022
Limonene	1.07	-	1027	1031
Sesquiterpene				
δ-Elemene	-	1.15	1337	1339
β-Elemene	-	1.69	1391	1391
Longifolene	-	5.64	1399	1402
(*Z*)-Caryophyllene	16.86	14.46	1405	1404
(*E*)-Caryophyllene	29.29	28.71	1418	1418
α-Humulene	3.06	4.41	1445	1454
γ-Himachalene	-	2.96	1476	1476
γ-Muurolene	7.54%	-	1475	1477
Monoterpene	32.02%	34.18%	-	-
Sesquiterpene	56.75%	59.02%	-	-
TOTAL	88.77%	93.2	-	-

(*) ri – retention index of the sample (**) ri – retention index of the literature (adams, 2007).

CONCLUSIONS

The analysis of the chemical constituents of the essential oils of the species *A. emarginata* 'terra fria' and *A. squamosa* identified monoterpenes and sesquiterpenes. Six substances were common to both species: α-humulene, (*Z*)-caryophyllene, (*E*)-caryophyllene, camphene, α-pinene, and β-pinene. These two *Annona* species shared more than 50% of the same components of their essential oils.

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