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Antimicrobial activity of essential oils of *Aloysia triphylla* (L`Her.) Britton from different regions of Argentina

[Actividad antimicrobiana de aceites esenciales de *Aloysia triphylla* (L`Her.) Britton procedentes de diversas regiones de Argentina]

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Abstract

Essential oils are known to exert antimicrobial activity. Differences in the chemical composition of them influence this activity. This work intends to study the variability in the chemical composition and the antimicrobial activity of essential oils obtained from plants of *A. triphylla* collected from different regions of Argentina. Essential oils were obtained by hydrodistillation and analyzed with GC-MS. The antimicrobial studies were carried out by the paper disc diffusion method. The essential oils shared common components but presented differences in the quantity and quality of the rest of them. The essential oil from La Paz showed the highest citral/limonene relation and the best antimicrobial activity. Yeasts resulted to be the most sensitive microorganisms, followed by the Gram positive bacteria. Statistical analysis showed significative differences in the antimicrobial activity. The differences in the biological activity of each essential oil could be attributed to the quantity and quality of the terpenic composition.

Keywords: Aromatic plants; *Aloysia triphylla*; Antibacterial; antifungic; terpenes.

Resumen

Los aceites esenciales poseen conocida actividad antimicrobiana. Esta actividad puede estar influenciada por la composición química de los aceites. El objetivo del presente trabajo fue estudiar la variabilidad en la composición química y la actividad antimicrobiana del aceite esencial obtenido a partir de plantas de *A. triphylla* recolectada de diferentes regiones de Argentina. Los aceites esenciales fueron obtenidos por hidrodestilación y analizados por GC-MS. Los estudios antimicrobianos se llevaron a cabo por la técnica de difusión en disco. Los aceites esenciales presentaron componentes mayoritarios comunes y presentaron diferencias en la cantidad y calidad del resto de los componentes. La mayor relación citral/limoneno y la mejor actividad antimicrobiana fue obtenida con el aceite esencial de La Paz. Las levaduras resultaron ser los microorganismos más sensibles, seguidos por las bacterias Gram positivo. El análisis estadístico mostró diferencias significativas en la actividad antimicrobiana de las distintas muestras. Las diferencias en la actividad biológica de cada aceite esencial podría ser atribuido a la cantidad y calidad de los terpenos lo constituyen.

Palabras Clave: Plantas Aromaticas; *Aloysia triphylla*; Antibacterianos; antifungicos; terpenos.

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INTRODUCTION

For centuries, indigenous plants have been used in herbal medicine for curing various diseases. The acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics have led scientific groups to investigate the antimicrobial activity of medicinal plants (Ozturk and Ercisli, 2007). In addition, a major problem in the food industry is that the microbial activity is a primary mode of deterioration of many foods. Currently there is a growing interest to use natural antibacterial compounds for the preservation of foods, as these possess a characteristic flavor and sometimes show antioxidant activity as well as antimicrobial activity (Schelz *et al*., 2006; Teixeira-Duarte *et al*., 2007).

Plants which are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, polyphenols) are generally superior in their biological activities suggesting that this strength is dependent on the diversity and quantity of such constituents (Geyid *et al*, 2005). Most of the antimicrobial activity in essential oils (EOs) appears to derive from oxygenated terpenoids as alcoholic and phenolic terpenes, while other constituents are believed to contribute little to the antimicrobial effect (Burt. 2004; Koroch *et al*. 2007.; Zygadlo and Juliani. 2000). Therefore, the determination of the compounds responsible for any biological activity would facilitate the selection of the plants for future investigation

Aloysia triphylla (L`Her.) Britton, (*Aloysia citriodora* Palau,) popularly known as "cedrón", is a member of the Verbenaceae Family. It is perennial and grows widely in North and South America and also in northeast, northwest and central regions of Argentine. It is cultivated from Mexico till the South region of the continent. It is a bush with white flowers and fruits, with an intense scent lemon-like, sweet, lightly floral, and herbaceous (Barboza *y col*. 2001; Gil *et al.* 2007). This specie is used in folk medicine to treat many digestive disorders, as antiinflamatory, analgesic, antipyretic, tonic and stimulating. It shares an important place on the international herbal market due to the sensory and medicinal properties of it EOs. These attributes determine its use as a primary ingredient for infusions and nonalcoholic beverages as well as aromatic ingredient for the flavor and fragrance industries. The pharmaceutical industry uses *A. triphylla* for its carminative, antispasmodic and sedative properties. There are several scientific studies that support the use of products obtained from *A. triphylla*. It has been found good antimicrobial activity of the methanolic and ethanolic extracts, as well as it has been described antimicrobial activity in the EOs (Akroum *et al*. 2009; Demo et al. 2005; Oskay *et al*. 2005; Sartoratto *et al.* 2004). The increasing interest in this specie has largely contributed to expanding *A. triphylla* crops in Argentina, Chile, Paraguay, Europe and Africa Mediterranean regions (Gil *et al.*, 2007; Pascual *et al*., 2001, Sartoratto *et al.* 2004).

Cedrón is included in the Código Alimentario Argentino (CAA) as a corrective and coadjutant, in the section referred to vegetal condiments (Código Alimentario Argentino). "Cedrón" is recognized and described in the Farmacopea Nacional Argentina, VI Edición (FNA) as "the dried leaves with young stems, flowers and fruits of *Aloysia triphylla (L´Hérit) Britt"*. It is also described in the Pharmacopoeias of France, Spain, Mexico and Europe (Bandoni. 2000). It is included in the GRAS list (Generally Regarded as safe) and the Food and Drug Administration (FDA) has categorized it as a dietary supplement due to the wide use in America and Occidental Europe (Barboza *y col*. 2001).

Many EOs are known to exert antimicrobial activity (Schelz *et al.*, 2006, Teixeira Duarte *et al.*, 2007). Differences in the chemical composition of them related to variety, agronomic practice and processing are also likely to influence antimicrobial properties, since these factors contribute to both the profile and relative concentrations of active ingredients (Delaquis *et al.*, 2002). The EOs content is influenced by genetic material, culture conditions, environment, season, crop and post-crop processing (Gil *et al*., 2007; Hussain *et al*., 2008).

The components commonly found in *A. triphylla* EOs are: neral, geranial limonene, geranyl acetate, betacaryophyllene, *ar*-curcumene, and spathulenol. Other compounds that could be founds in specific chemotypes are carvone, cedrol, 1,8-cineol, thujone isomers and citronellal (Gil *et al*., 2007).

Due to the differences described in the chemical composition of the EOs of a particular vegetable specie, the aim of this work was to study the chemical composition of EOs obtained from samples of *A. triphylla* collected in different regions

of Argentine and the relationship with the antimicrobial activity.

MATERIALS AND METHODS

Plant material

The plants of *Aloysia triphylla* were obtained in March, 2005, from plants growing in farms (plantations) located in different regions of Argentina: from Córdoba Province: Río Primero and La Paz, from Salta Province: Las Viñas, from Mendoza Province, from San Luis Province and one sample from Paraguay Republic.

Essential oils obtention

The EOs were obtained from dried vegetable material, which was hydrodistilled in a Clevenger-like apparatus. The oil obtained was dried with anhydrous sodium sulphate and stored in the freeze until analysis (De Feo *et al.*, 1998).

Gas Chromatography-FID

The EO were analyzed with a Shimadzu GC-R1A gas chromatograph equipped with a fused silica column (30 m x 0.25 mm) coated with CBP-1. The temperature of the column was programmed from 60^0C to 240^0C at 4^0C/min. The injector and detector temperatures were at 270^0C. The gas carrier was He, at a flow rate of 1 ml/min. Peak areas were measured by electronic integration. The relative amounts of the individual components are based on the peak areas obtained, without FID response factor correction. Programmed temperature retention index of the compounds were determined relative to n-alkanes. GC analysis was still performed using a column Supelcowax-10 with the same conditions as described above (Zunino *et al*., 1998).

Gas Chromatography-Mass Spectrometry

GC-MS analyses were performed on a Perkin Elmer Q-910 using a 30 m x 0.25 mm capillary column coated with CBP-1. The temperature of the column and the injector were the same than those from GC. The carrier gas was He, at a flow rate of 1ml/min. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices, mass spectra with those of authentic samples, by peak enrichment, with published data, mass spectra library of National Institute of Standards and Technology (NIST 3.0) and our mass spectra library which contains references mass spectra and retention indices of volatile compounds. GC-MS analysis was still performed using a column Supelcowax 10 with the same conditions as describe above (Adams. 1989).

Microorganisms

The activity of the EOs was tested against the following microorganisms: Gram positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* (milk), *Micrococcus luteus* ATCC 9341, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* (rice). Gram negative bacteria: *Escherichia coli* (water), *Proteus mirabilis* (urine), *Klebsiella pneumoniae* (composting of poultry), and *Pseudomonas aeruginosa* (water). The yeasts *Candida albicans* (mouth), *Rhodotorula sp.* (cereal) and *Hansenula sp.* (cereal) were used in order to probe antifungal activity.

Antimicrobial assays

Analysis of the antibacterial activity

The antibacterial studies were carried out according to De Pooter *et al.*, (1995). The paper disc diffusion method was used to test the antimicrobial activity. Tubes containing Triptein Soy Broth (TSB) inoculated with the microorganisms were incubated during 18 h, at 37 ºC. From these tubes ten-fold dilutions were made, until an OD ≅ 0.04 (10^6 cfu/ml) was reached. The antifungal activity was determined with the same methodology but using that dilution with an OD ≅ 0.4 (10^6 cfu/ml). The inoculumm (200µl) was spread over plates containing Mueller-Hinton Agar and a paper filter disc (6mm) was impregnated with 10µl of the EO and placed on the surface of the media. The plates were left 30 minutes at room temperature to allow the diffusion of the oil in the agar; then they were incubated at 37°C during 24 hours. After this time the inhibition zone around the disc was measured with a caliper. Discs with gentamicine (10 µg) were used as positive control.

Analysis of the antifungal activity

Antifungal experiments were performed in the same way as those with bacteria using Sabouraud Agar (SA) for the plates. Discs with anfotericine B (2 µg/ml) were used as positive controls.

Minimum inhibitory concentration assay (MIC)

The minimum inhibitory concentration (MIC) was performed according to the method previously described by De Feo et al., (1998). It was determined by two-fold dilutions of EOs in dimethyl sulfoxide (DMSO), placing 10 µl of each dilution on a filter paper disc. The EOs concentration range was from 900 mg/ml to 7.03 mg/ml. The discs were placed on the surface of a TSA plate, previously inoculated with 200 µl of each inoculumm, and left at room temperature to allow the diffusion of the oil. Then they were incubated at 37 ^{o}C during 24 h. After this time the inhibition zone around the disc was measured with a caliper. MIC was defined as the lowest concentration that inhibited visible growth. The MIC with fungus was determined in the same way as with bacteria using Sabouraud Agar (SA) in the plates. The negative control consisted in a paper disc impregnated with 10 µl of DMSO. The positive control was a disc impregnated with the antibiotic gentamicine (10 µg) for bacteria. For yeasts, anfotericine B (2 µg/ml) was used.

Statistical analysis

All the experiments were performed in duplicate and statistical analysis of the data were performed using GraphPad Prism 4.0 program. A probability value of $p<0.05$ was considered statistically significant.

RESULTS

The chemical composition of the EOs from *Aloysia triphylla* collected from Rio Primero, La Paz, Las Viñas, Mendoza, San Luis and Paraguay has been investigated by means of gas chromatographic techniques. The EOs average yield in all the samples was 0.4% (w/v) and the components which were commonly found in all the EOs samples were: limonene, neral, geranial, spathulenol and caryophyllene oxide, with intrinsic variations in the quantity and quality of the resting terpenes in each sample (Table 1). The samples analyzed showed that the EOs from Mendoza had the biggest proportion of neral and geranial, followed by La Paz. Las Viñas had the biggest proportion of limonene and carvone, while neral and geranial were in the least proportion. Rio Primero EOs was the only one presenting camphor and borneol and the biggest proportion of α-thujone. The EOs from Paraguay had spathulenol and caryophyllene oxide while San Luis had D germacrene and biciclogermacrene (Table1).

The relation between major terpenic components of an EO has been proposed as a criterion to identify chemotypes (Muñoz-Collazos *et. al*., 1993). In this work the rate between citral (neral + geranial) and limonene has been calculated. The EOs from La Paz showed the highest citral/limonene relation (16.9) and Las Viñas the least relation (0.73). The rest of the EOs relations were located between both of them (Table 1).

The antimicrobial activity of the EOs was assayed against Gram positive bacteria, Gram negative bacteria and yeasts. The yeasts resulted to be the most sensitive microorganisms to the effect of the EOs, followed by the Gram positive bacteria and lastly the Gram negative ones. It is interesting to note that the three yeasts were inhibited by all the EOs, with average inhibition zones diameters of 22 mm. (Table 2)

The EOs from La Paz showed the best antimicrobial activity, inhibiting the growth of all tested microorganisms, except *P. aeruginosa*. The inhibition zones obtained with this oil for *B. cereus* (38mm), *M. luteus* (33mm) and *C. albicans* are remarkable. Mendoza`s EOs presented good inhibition activity against microorganisms with average diameters of 29 mm against *B. cereus.* The EOs from Las Viñas, Paraguay and San Luis showed varied antimicrobial activity, with inhibition zones of 20 mm, 21 mm and 15 mm for *B. cereus*, respectively. (Table 2)

Previously, it had been reported good antimicrobial activity for the EOs obtained from Río Primero (Demo, *et al.* 2005). Taking into consideration these previous studies, La Paz and Rio Primero, both located in Córdoba Province, showed the best inhibition spectrum of all the oily samples. However there were differences in their chemical composition.

B. cereus, S. aureus and *M. luteus* were the most susceptible Gram positive bacteria to the EOs action. The Gram negative bacteria *E. coli* and *K. pneumoniae* were inhibited by the EOs from La Paz and Las Viñas.

Table 1: Components identified in the essential oils of *A. triphylla* (%) colleced in 6 different places. In bold data accounting for the main differences in composition.

Components	**Rio Primero**	**La Paz**	**Las Viñas**	**Paraguay**	**Mendoza**	**San Luis**
α thujene	0,8	tr	0,6	tr	tr	tr
α pinene	1,2	0,3	1,5	tr	tr	tr
camphene	0,6	-	0,9	-	-	-
6-metil-5-hepten-2-one	-	tr	1,7	tr	tr	tr
myrcene	1,7	tr	0,9	tr	tr	tr
p-cymene	0,4	tr	tr	tr	tr	tr
limonene	**6,9**	**2,9**	**21,3**	**19,1**	**14,2**	**17,9**
cis ocimene	0,5	tr	1,2	tr	tr	tr
γ terpinene	0,7	tr	0,9	tr	tr	tr
sabinene hydrate	0,5	-	tr	-	-	-
camphenilone	0,7	tr	2,2	tr	tr	tr
linalool	0,3	0,5	2,2	0,6	tr	0,3
α thujone	13,1	0,5	1,7	0,6	tr	0,2
2,2 dimetil-3,4 octadienal	0,4	1,3	-	0,5	tr	0,4
camphenol (6)	0,3	-	tr	-	-	-
dihydrolinalool	0,1	-	tr	-	-	-
cis verbenol	0,2	-	tr	-	-	-
citronellal	0,1	tr	1,1	tr	tr	tr
menthone	0,3	-	-	-	-	-
isoborneol	-	tr	0,8	tr	tr	tr
terpin-4-ol	0,3	-	tr	-	-	-
α terpineol	0,5	-	2,4	-	-	-
trans carveol	0,5	-	3,3	-	-	-
cis carveol	0,4	-	1,1	-	-	-
(E) ocimenone	0,4	0,5	tr	0,8	tr	0,4
neral	**18,7**	**20**	**12,4**	**15,5**	**31,5**	**13**
carvone	1,2	-	13,1	-	-	-
carvotanacetone	0,1	-	-	-	-	-
geranial	**21,3**	**29,2**	**3,3**	**19,5**	**22,6**	**18,5**
camphor	**4,1**	-	-	-	-	-
borneol	**1,2**	-	-	-	-	-
α copaene	0,8	0,5	tr	0,3	tr	0,8
β bourbonene	1	1	0,6	0,8	tr	0,9
β cubebene	0,2	tr	4,2	tr	tr	tr
α cedrene	3,2	0,9	tr	2,8	tr	3,2
(E) caryophyllene	0,4	0,6	tr	0,7	tr	0,4
α humulene	0,6	1,1	tr	0,5	tr	0,6
Cisdihydroαterpineol	-	-	tr	-	-	-
curcumene (ar)	0,1	tr	3,3	tr	tr	tr
germacrene D	4,3	2,3	tr	5,3	tr	6,9
α zingiberene	0,4	0,5	tr	0,3	tr	0,4
bicyclogermacrene	3,8	4,2	tr	6,8	3,9	7,2
cubebol	0,1	0,9	tr	0,7	tr	1,3
β curcumene	0,1	0,4	0,5	tr	tr	0,8
δ cadinene	0,2	0,3	4,2	tr	3,2	0,2
(E)nerolidol	0,5	0,6	tr	1,6	9,6	0,9
spathulenol	**0,9**	**8,9**	**6,6**	**11,1**	**4,4**	**10,1**
cariophyllene oxide	**1**	**7**	**6,9**	**10,5**	**4,5**	**10**
globulol	0,8	0,3	tr	tr	tr	0,3
viridiflorol	0,5	2,5	tr	0,6	tr	1,1
guaiol	0,3	0,3	0,8	tr	tr	0,2
Total	**95,3**	**87,5**	**99,7**	**98,6**	**93,9**	**96**
Markers						
Citral (Neral + geranial)	40	49,2	15,7	35	54,1	31,5
Citral:Limoneno	5,8	16,9	0,73	1,83	3,8	1,76

Table 2: Antimicrobial activity of EOs of *A. triphylla.* Inhibition zones in (mm)

MO	La Paz		Las Viñas		Paraguay		Mendoza		San Luis		P(<0.05)
	$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ	
S. aureus	20	5.95	9	6.3	17	0.7	10	5.42	NI	0	0.0001
S. epidermidis	22	2.83	NI	0	13	3.53	11	2.82	9	2.12	0.0001
M. luteus	33	1.41	8	0.95	11	0.7	14	0.7	23	4.24	0.0001
E. faecalis	13.5	5.53	NI	0	NI	0	NI	0	NI	0	0.0001
B. cereus	38	11.2	20	11.54	21	5.65	29	6.38	15	0.7	0.0265
E. coli	8	4.33	7	7.68	NI	0	9	1.74	NI	0	0.0208
K. pneumoniae	10	0	4	4.92	NI	0	NI	0	NI	0	0.0169
P. mirabilis	10	0.7	NI	0	NI	0	4	4.94	NI	0	0.0001
P. aeruginosa	NI	-	NI	-	NI	-	NI	-	NI	-	-
C. albicans	39	18.18	14	4.76	9	12.72	27	8.81	9	12.02	0.0101
Hansenula sp	16	3.53	20	0	22	0	20	0	NI	0	0.0001
Rhodotorula sp	34	7.07	10	0.7	11	0	22	2.12	17	1.41	0.0054

NI: No inhibition; (-): Not done;

Table 3: Minimun Inhibitory Concentration of the EOs of *A. triphylla* (mg/ml)

MO	Place/EOs Concentration (900-7 mg/ml)				
	La Paz	**Las Viñas**	**Paraguay**	**Mendoza**	**San Luis**
S. aureus	28.1	NI	900	56.25	NI
S. epidermidis	28.1	NI	900	112.5	225
M. luteus	7	900	450	225	900
E. faecalis	56.25	NI	NI	NI	NI
B. cereus	7	900	7.03	7.03	56.25
E. coli	900	NI	NI	NI	NI
K. pneumoniae	900	900	NI	NI	NI
P. mirabilis	450	NI	NI	NI	NI
P. aeruginosa	NI	NI	NI	NI	NI
C. albicans	28.1	7	56.25	56.25	14
Hansenula sp	7	56.25	7	225	NI
Rhodotorula sp	7	900	7	28.1	112.5

The yeasts were the most sensitive microorganisms, being inhibited by al the EOs. La Paz EOs showed the biggest inhibition zones, with diameters of 39 mm against *C. albicans* and 34 mm against *Rhodotorula sp*, while San Luis showed the smallest one. The others EOs samples showed different degrees of inhibition activity against the yeasts.

In order to analize if the differences found in the antimicrobial activity could be attributed to the origin and composition of the EOs, the statistical analysis was performed. This analysis showed significative differences in the antimicrobial activity of the EOs from all the places collected. These variability was present in all the tested microorganisms (Table 2).

For Gram positive bacteria the best values were obtained with La Paz EOs with values of 28.1 mg/ml for *S. aureus* and *S. epidermidis* and 7 mg/ml for *M. luteus* and *B. cereus* (Table 3). Gram negative bacteria were inhibited by pure compounds with the exception of *P. mirabilis* that presented a CIM of 450 mg/ml with La Paz EOs.

The best MIC values for *C. albicans* were obtained with the EOs of Las Viñas (MIC: 7 mg/ml) and the best MIC values for *Rhodotorula sp* and *Hansenula sp* were obtained with La Paz and Paraguay EOs (MIC: 7 mg/ml) (Table 3).

DISCUSSION

Differences in the content and composition of the EOs of *A. triphylla* have been reported previously. In these reports, the EOs content ranged between 0.2 and 1% on dry weight (Gil *et al.*, 2007, Sartoratto, *et al*. 2004). In this study, the average yield obtained with the EOs samples (0.4% (w/v)) was included between these values.

There were chemical differences in the quantity and quality of the EOs obtained from *A. triphylla* collected from different places. However, all the EOs samples shared the terpenic components limonene, neral, geranial, espathulenol and cariophyllene oxide, which were described by other authors as the characteristically constituents of *A. triphylla* EOs (Gil *et al*., 2007; Pascual *et al*., 2001; Stashenko *y col*., 2003). Each EO sample showed particular features in the rest of the constituents with variability in the composition of each one. The EOs content in vegetable species is influenced by genetic material, culture conditions, environment, season, extraction methods, crop and post-crop processing (Gil *et al*., 2007; Hussain *et al*., 2008; Tampieri *et al*.; 2005). The culture conditions, kind of soil and climate were particular for each one of the samples collected. Río Primero and La Paz are located in the central region of Argentina, Mendoza and San Luis are located in the central-west of Argentina while Las Viñas and Paraguay are in the north. Previous studies on *A. triphylla* have reported that the production and composition of the EOs vary according to the part of the plant, the stage of development and the harvesting locations of the plant (Gil *et al*., 2007). Other investigations support the fact that the differences in the quantity of the terpenes identified in an EO obtained from a vegetable collected in the same zone are due to the environment (Karaman, 2006; Merle *et al*., 2004). It was found variability in the terpenic composition of *Ocimun basilicum* EOs collected in different seasons, concluding that the growing season was affecting the chemical content (Hussain *et al*., 2008). It is worth to mention that it was analyzed an EO sample from La Paz collected the following year (2006) in order to analyze if variations in the chemical composition related to the harvesting year happened and it was found the same terpenic composition as in 2005 with variations in the quantity of them. (Data not shown)

Citral and limonene were the major components identified in these EOs. It has been reported antimicrobial activity of both of them (Demo *y col*. 2001, Di Pasqua *et al*., 2006; Wolken *et al*., 2002). The individual activity of citral and limonene could be suggesting that the relationship between them could be determining the antimicrobial activity. This justifies the study of the rate between them and the possible relation between this value and the antimicrobial activity of the EOs. In this work the EO of *A. triphylla* from La Paz showed the biggest rate citral/limonene and the best antimicrobial activity, while Las Viñas EO showed the least terpenic relation and antimicrobial activity (Table 1 and 2). These results are suggesting that higher rates between both compounds could be determining a better antimicrobial ability and a broader microbial spectrum. Some authors suggested that the compounds present in the greatest proportions are not necessarily responsible for the greatest share of the total activity. The data on the activity of the essential oils, in some cases are not compatible with those of the pure constituents in higher percentages Thus, the involvement of the less abundant constituents should be considered (Cimanga *et al*., 2002; Tampieri *et al*., 2005; Zygadlo and Juliani. 2000).

Mono and sesquiterpenes and the mixture between them in the oil, could constitute a barrier to microbial infections (Cowan. 1999; Lambert *et al*., 2001; Tan *et al.*, 1999; Vataru Nakamura *et al*., 2004). The biotic and abiotic factors (environment, specie, chemotype) of the places where specie are collected have influence on the quantity and quality of the terpenic composition of the EOs. Consequently, differences in the EOs yielding and in the biological activity are observed, being active against bacteria and fungi or only one of them (De Pooter *et al*., 1995; Hess *et al*., 2007; Zygadlo and Juliani. 2000). A study made with *O. basilicum* EOs with regard to seasonal variations, showed changes in the antimicrobial activity, attributing these variations to the different chemical composition of the oils. Some earlier reports showed that the changes in chemical composition of an EOs directly affected their biological activities (Hussain *et al*., 2008). The differences in the biological activity of each *A. triphylla* EOs could be attributed to the quantity and quality of the terpenic composition and the possible associations between them. In addition, it could be deduced that the antimicrobial activity is not only dependent on the quality and quantity of the EOs but, on the particular sensibility of each particular strain.

The inhibitory activity against microorganisms responsible for human and plant diseases of EOs from *A. triphylla* has been described in other investigations (Demo *et al*., 2005; Pascual *et al*., 2001; Sartoratto, *et al.* 2004). What is more, Sartoratto, *et al*. 2004, described MIC values of *A. tryphilla* (L´Hér.) Britton lower than chloramphenicol, when it was used as the positive control antibiotic, showing the antimicrobial potential of this EO. (Sartoratto, *et al*. 2004) But, a comparison of the chemical composition and the antimicrobial activity of the EOs obtained from *A. triphylla*

collected in different regions, and the relation between this two variables, have not been previously reported.

It is difficult to compare results with others reported in the literature because of the naturally varying composition of EOs even in the same species due to the presence of chemotypes, different harvest times, different extraction methods, etc. Furthermore, it is important to consider the different microbiological tests utilized and the different sensitivities of the strains (Tampieri *et al*., 2005). The information described here clearly shows the influence of the chemical composition of this EO on the antimicrobial activity. This is also suggesting that the genotypical composition of this specie should be taken into consideration, in order to obtain the ideal terpenic composition with the best antimicrobial activity.

The production of EOs and their utilization as potential therapeutic agents and natural food preservants could be of economical value. However, further investigations to establish how components interact to provide the biological activities are needed (Hussain *et al*., 2008).

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