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Chemical Composition of Oleoresins from Berries of *Schinus Molle* L. Grown in Buenos Aires Province (Argentina)

[1, 2]Gerardo M. Caballero, [1]Emilse V. Padin and [1]Maria Lucia Pollio
[1]Departamento de Ciencia y Tecnologia, Universidad Nacional de Quilmes,
Roque Saenz Pena 352 (1876) Bernal, Provincia de Buenos Aires, Argentina
[2]Instituto de Ciencias de la Salud, Universidad Nacional Arturo Jauretche,
Avenida Calchaqui 6200 (1888), Florencio Varela, Provincia de Buenos Aires, Argentina

Abstract: In this study, the chemical composition of the oleoresins prepared from fresh and 1 year stored (-18°C) berries of Argentine Aguaribay trees was established by GC-MS. The fresh oil was found to contain mainly 40% of monoterpene and sesquiterpene hydrocarbons and 34% of sesquiterpene alcohols and related compounds (caryophyllene oxide and guaiol acetate). Principal individual compounds were guaiol acetate (11.96%), δ-cadinene (8.64%) and γ-caryophyllene (7.12%). The oleoresin obtained from stored berries contained 71% of monoterpene and sesquiterpene hydrocarbons and 16.5% of sesquiterpene alcohols and related compounds. More abundant individual compounds were γ-caryophyllene (18.84%), γ-muurolene (16.23%) and bicyclogermacrene (13.54%). The observed differences in composition could be mainly attributed to dehydration of alcohols to some structurally related alkenes occurred into the stored berries. Hydrocarbon composition is further discussed in relation to the previously reported antibacterial activity of the oleoresin.

Key words: Berries, aguaribay, oleoresin, chemical composition, hydrocarbon

INTRODUCTION

In the last years, there has been a growing interest in the use of oleoresins and essential oils as functional ingredients in foods, drinks, toiletries and cosmetics because they could replace potentially harmful synthetic additives (Sacchetti *et al*., 2005). For example, it is well known the use in the flavor and fragrance industry of essential oils from lemon, mandarin and bergamot (Vekiari *et al*., 2002). From other side, studies on the biological activity of oleoresins of different spices have been mentioned in the literature since 1980 (Padin *et al*., 2007) and references cited therein and recently it has been demonstrated that the essential oil of Green Huajiao (*Zanthoxylum schinifolium*) possesses antibacterial activity against three Gram-positive bacteria and four Gram-negative bacteria to various extents in particular against *S. epidermidis*. These results show the potential of the essential oil from Green Huajiao for application as natural food preservatives (Diao *et al*., 2013). Another example is thereport that the essential oil obtained from *Curcuma zedoaria* Roscoe, known as zedoary, possesses efficient cytotoxic effects on Non-Small Cell Lung Carcinoma (NSCLC) cells and causes cell apoptosis (Chen *et al*., 2013).

In the case of the Aguaribay tree (*Schinus molle* Linn), berries, leaves sap and trunk sap are the main parts used as additives in the agricultural and food industry and for treatment of different illnesses in the pharmaceutical industry (Kramer, 1957). It has also been found that the essential oil extracted from leaves of this tree possess antimicrobial properties (Gundidza, 1993). The chemical composition of the essential oil of berries and leaves of *Schinus molle* L. has been determined in plants cultivated on different coastal locations of the Mediterranean Sea (Maffei and Chialva, 1990; Baser *et al*., 1997; Marongiu *et al*., 2004). More recently, it has been demonstrated that berries collected from Aguaribay trees located in Buenos Aires Province (Argentina) yielded an oleoresin which has antibacterial properties against different bacterial species responsible for the contamination of food products. The same publication also showed that the oleoresin antibacterial properties were quite stable since the ethanolic extract tolerates heat up to 80°C and the oleoresin retains its bactericidal properties when kept either at refrigeration temperature (4°C) or at room temperature (25°C) for 30 days (Padin *et al*., 2007). Other researchers tested the activity of the essential oil obtained from the fruit of *Schinus molle* against *S. pneumoniae* resistant to

Corresponding Author: Gerardo M. Caballero, Departamento de Ciencia y Tecnologia, Universidad Nacional de Quilmes,
Roque Saenz Pena 352 (1876) Bernal, Provincia de Buenos Aires, Argentina

conventional antibiotics and concluded that the global spread of the use of *S. molle* for the treatment of respiratory infections is justified. The fraction showing antimicrobial activity consisted of four monoterpenes and one sesquiterpene as principal components. Performing bioassay-guided fractionation, they could identify δ-cadinene as the main active constituent of the essential oil fraction showing antimicrobial activity (Perez-Lopez *et al*., 2011).

The present study continues the research on antibacterial properties of Aguaribay oleoresin (Padin *et al*., 2007). It was undertaken to establish the chemical composition of the oleoresins prepared from berries of Argentine Aguaribay trees, analyzing its GC-MS data and to present preliminary results of oleoresin composition stability as a function of berries storage time.

MATERIALS AND METHODS

Preparation of oleoresins: Aguaribay berries were collected from trees located on public streets of Florencio Varela District, Province of Buenos Aires, Argentina. For the preparation of the first oleoresin sample, 10 g of freshly collected berries were grounded and shaken with 100 mL ethanol 96% at 40°C for 48 h (40 cycles min^{-1}). The insoluble material was filtered off with filter study (Whatman No. 4) and the solution was evaporated to dryness at 40°C under reduced pressure (rotavap HeidolphLaborata 4000) (Padin *et al*., 2007). A second oleoresin sample was prepared from grounded berries kept frozen at -18°C for 1 year and processed in the same way than the first sample.

GC-MS analysis: The first sample was analyzed with a Shimadzu QP 5000-GC 17A Gas Chromatograph-quadrupole Mass Spectrometer (GC-MS). It was equipped with a split-splitless injector and an Ultra 2 fused silica column (5% phenyl-methylpolysiloxane, 25×0.20 mm i.d., film thickness 0.25 μm). GC conditions used were: programmed heating from 60-290°C at 3°C min^{-1} and then kept constant at 290°C for 5 min. The injector was maintained at 250°C. Helium was the carrier gas at 0.95 mL min^{-1}; the sample (1 μL) was injected in split mode (10:1). Interface temperature was 280°C. MS conditions were as follows: ionization energy 70 eV; electronic ionization ion source temperature, 250°C; scan rate 2 scans sec^{-1}; mass range, 40-700 μ. The sample was run diluted in dichloromethane with a dilution ratio of 1:100.

The second sample was analyzed with a Thermo Scientific DSQ II-Trace GC Ultra Gas Chromatograph-quadrupole Mass Spectrometer (GC-MS). The GC column employed and GC and MS conditions were analogous to those employed with the first sample.

RESULTS AND DISCUSSION

In the Table 1, chromatographic results are expressed as area percentages calculated without any response factor and reported as a function of Retention Indices (RI). Identifications were made by matching mass spectrum and retention index of each compound with those reported in the literature (Maffei and Chialva, 1990; Baser *et al*., 1997; Marongiu *et al*., 2004; www.pherobase.com/database).

Table 1 shows percentage composition of oleoresin 1 and 2. The first oil is made up of nearly 40% of monoterpene and sesquiterpene hydrocarbons, 34% of sesquiterpene alcohols and related compounds (caryophyllene oxide and guaiol acetate) and 17% of other compounds such as palmitic and oleic acids,

Table 1: Percentage composition of oleoresin from berries of *Schinus molle*

RI	Compounds	Sample 1 (%)	Sample 2 (%)
933	α-pinene	-	0.13
994	β-myrcene	0.96	2.53
1005	α-phellandrene	1.12	0.54
1035	Limonene+β-phellandrene	5.19	1.52
1126	Methyl octanoate	-	1.14
1337	δ-elemene	-	0.69
1376	α-copaene	0.66	0.96
1398	3-cedrene	0.73	0.40
1404	γ-caryophyllene	7.12	18.84
1418	β-caryophyllene	-	1.78
1438	α-humulene	1.12	1.47
1439	Aromadendrene	1.42	1.94
1444	γ-muurolene	0.42	16.23
1452	β-selinene	0.85	-
1457	9-epi-(E)-caryophyllene	1.17	-
1459	Bicyclogermacrene	-	13.54
1463	α-muurolene	1.99	-
1471	E-β-farnesene	0.61	-
1476	α-cadinene	4.16	-
1480	Germacrene D	-	3.47
1517	γ-selinene	0.60	-
1522	α-cadinene	8.64	4.88
1532	1,4-cadinadiene	0.53	-
1534	δ-cadinene	0.63	0.40
1536	cis-α-bisabolene	0.37	-
1547	Elemol	0.54	-
1556	Germacrene B	1.12	0.58
1569	Palustrol	0.44	-
1573	Caryophyllene oxide	0.81	1.51
1580	Globulol	4.96	-
1588	Epiglobulol	0.51	-
1627	α-cadinol	2.79	-
1633	Cubenol	-	0.89
1633	epi-α-muurolol (δ-muurolol)	1.17	-
1636	α-cadinol	1.70	8.66
1640	epi-α-cadinol (T-cadinol)	1.50	-
1724	Guiaol acetate	11.96	1.21
1917	Hexadecanoic acid	0.60	1.51
1968	Ethyl hexadecanoate	1.33	0.18
2010	Octadecanal	8.28	1.97
2010	(Z)-9-octadecenal	3.50	1.05
2039	1-octadecanol	2.86	0.66
2188	Ethyl octadecanoate	0.51	0.08

free or as ethyl esters (artifacts of the extraction procedure), 1-octadecanol, octadecanal and (Z)-9-octadecenal. Main individual compounds are guaiol acetate (11.96%), δ-cadinene (8.64%) and γ-caryophyllene (7.12%). Some compounds present in the oleoresin could not be completely identified but they show similar mass spectra and retention indices as those of sesquiterpene alcohols. This group of "unknown sesquiterpenic alcohols" of molecular formula $C_{15}H_{26}O$ accounts for 7.83% of the total oleoresin composition. There are also present other unidentified minor compounds (area<0.35%) whose mass spectra were not registered and as a group sum up to 9.3% of the total.

These results clearly show that oleoresin composition is markedly different from essential oil composition of *Schinus molle* berries which is explained by the different extraction methods employed for preparation of both type of oils (alcohol extraction for the former, steam distillation or hydro distillation for the latter). For example, the oil of berries collected from plants cultivated on Ligurian coasts, obtained by steam distillation-extraction with a mixture of diethyl ether and hexane (5:1) was composed mainly of monoterpene and sesquiterpene hydrocarbons (93.59%), being α-phellandrene (55.42%) and β-phellandrene (15.39%) the main ones (Maffei and Chialva, 1990). In another region of the Mediterranean Sea, more specifically in the coasts of the Aegean Sea (Izmir, Turkey), the oil obtained by hydro distillation of berries from cultivated trees yielded a big fraction of monoterpene and sesquiterpene hydrocarbons (75.38%), being α-phellandrene (22.11%), β-phellandrene (10.37%), limonene (9.59%) and α-cadinol (5.57%) the main individual components (Baser *et al.*, 1997).

The second oleoresin sample is found to contain 71% of monoterpene and sesquiterpene hydrocarbons, 16.5% of sesquiterpene alcohols and related compounds and 5.5% of other compounds such as palmitic and oleic acids, free or as ethyl esters (artifacts of the extraction procedure), 1-octadecanol, octadecanal and (Z)-9-octadecenal. Main individual compounds are γ-caryophyllene (18.84%), γ-muurolene (16.23%) and bicyclogermacrene (13.54%). In this sample, the group of "unknown sesquiterpenic alcohols" of molecular formula $C_{15}H_{26}O$ accounts for 5.46% of the total oleoresin composition. And the group of unidentified minor compounds (area<0.35%) whose mass spectra were not registered, sum up to 5.79% of the total.

It can be noticed that the most striking difference between oleoresins 1 and 2 composition is the higher percentage composition of monoterpene and sesquiterpene hydrocarbons along with the lower percentage composition of sesquiterpenic alcohols (identified plus unidentified) and related compounds, in sample 2.

The percentage values for hydrocarbons and alcohols in both samples are 40 and 42%, respectively for sample 1, 71 and 22%, respectively for sample 2. A great part of this difference could be attributed to dehydration of alcohols to some structurally related alkenes occurred into the stored berries. In particular, γ-muurolene percentage is much higher in sample 2 (16.23 vs. 0.42%). At the same time, most γ-muurolene structurally related sequiterpenic alcohols are not detected in sample 2 (α-cadinol, T-cadinol and T-muurolol). This fact could be tentatively, though reasonably, explained assuming dehydration of T-muurolol and dehydration followed by isomerization of α-cadinol and T-cadinol, to yield γ-muurolene. The possible isomerization of cadinene to γ-muurolene is sustained by the percentage composition change observed for γ-cadinene which goes from 4.16% in sample 1 to 0% in sample 2. These alleged biotransformations are depicted in Fig. 1.

Another expected difference between sample 1 and 2 is the lower percentage of guaiol acetate in the latter with respect to the former (1.21 and 11.96%, respectively). As shown in Fig. 2 this difference could be due to hydrolysis of the ester in sample 2 to the corresponding alcohol, followed by its dehydration to an exo-alkene. This alkene could next isomerize and suffer a ring opening reaction that would led to bicyclogermacrene, a structurally related sesquiterpene which was not observed in sample 1 but whose percentage composition in sample 2 is 13.54%.

A remarkable exception to the observed tendency of lower sesquiterpenic alcohol percentage in sample 2 is δ-cadinol. It can be observed that δ-cadinol percentage in sample 2 is 8.66% whereas in sample 1 is just 1.70%. It seems that the increasing percentage of δ-cadinol has occurred in part at the expense of the structurally related sesquiterpene hydrocarbon δ-cadinene, whose percentage composition change from 8.64% in sample 1 to 4.88% in sample 2. However, no reasonable explanation to these results can yet be proposed.

It is worth mention the results observed for a particular group of mono sesquiterpene hydrocarbons. As was briefly cited in the introduction section, it was demonstrated that a fraction of *S. molle* essential oil shows antimicrobial activity against *S. pneumoniae*. The principal components of this fraction were identified as β-myrcene, p-cymene, δ-cadinene, α-phellandrene and limonene (Perez-Lopez *et al.*, 2011). The oleoresins prepared for this study contain all this hydrocarbons except p-cymene. The fresh oleoresin demonstrated antibacterial activity against a wide range

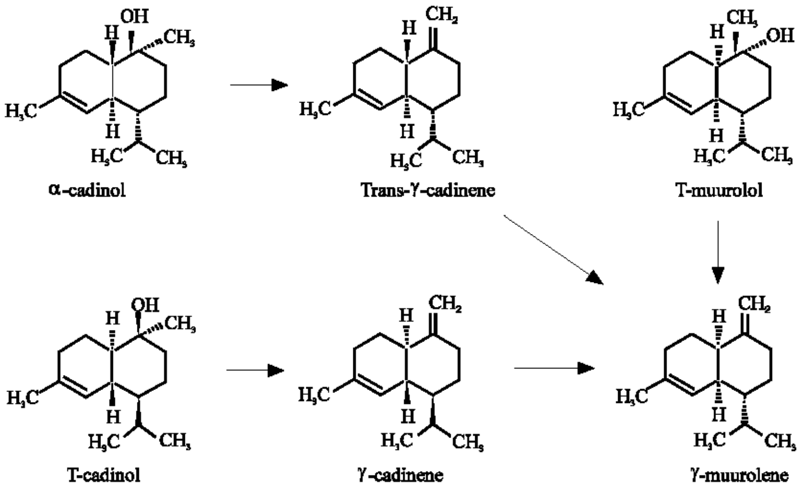

Fig. 1: Biotransformations of sesquiterpenic alcohols to γ-muurolene

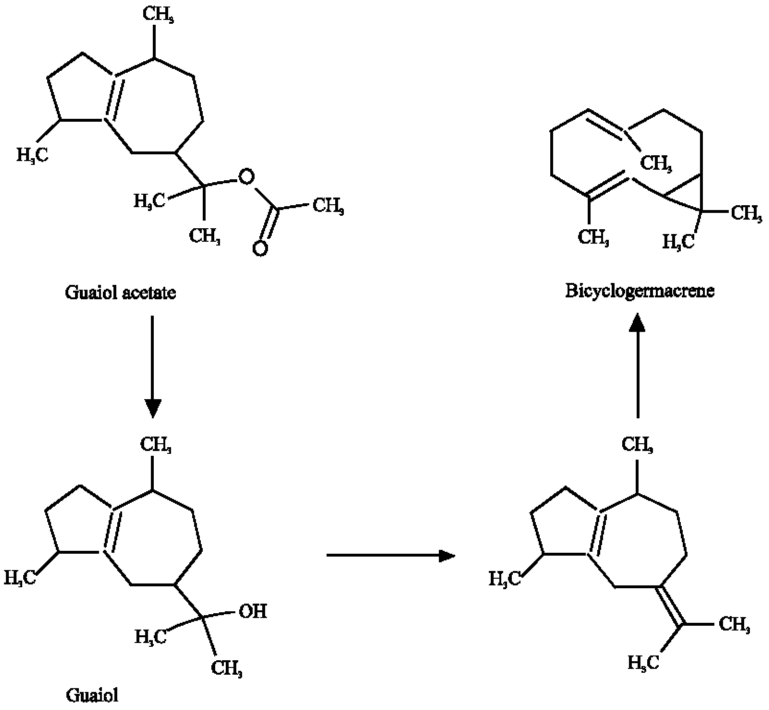

Fig. 2: Biotransformations of guaiol acetate to bicylogerrmacrene

of Gram-positive and Gram-negative bacteria but *S. pneumoniae* was not included in the test (Padin *et al*., 2007) and no study was performed yet to establish the identity of the compounds responsible for the antimicrobial activity. However, in view of the results obtained with *S. pneumoniae*, it is highly probable that this same four hydrocarbons present in the oleoresins might be the main active components. Then, the composition of this group should be carefully assessed.

It can be observed that the percentage composition of the group formed by β-myrcene, α-phellandrene and limonene (plus β-phellandrene) in the oleoresins is 7.27% for sample 1 and 4.59% for sample 2. Moreover, β-cadinene which was found to be the principal active constituent of the essential oil fraction of *S. molle*, changed its percentage composition in the oleoresin from 8.64-4.88% after 1 year of berries storage at -18°C.

CONCLUSION

These preliminary results could suggest that preparation of oleoresins for uses as antibiotic has to start from berries freshly collected to ensure maximal bactericidal activity due to the lower percentage composition of the supposed active hydrocarbons. However, it is also observed that the global percentage of hydrocarbons rise with time of berries storage. So, the door is opened for future detailed studies in order to establish the identity of the compounds of oleoresin from Aguaribay berries responsible for antibacterial activity and the variability of oleoresin composition and antibiotic efficacy with time.

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