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Chemical constituents of apolar fractions from fruit latex of twelve *Clusia* species (Clusiaceae)

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ABSTRACT

The apolar fractions components of fruit latex of twelve species of *Clusia* belonging to four different taxonomic sections were examined by GC-MS. The latex of *Clusia* is characterised by large amounts of sesquiterpene hydrocarbons as major constituents like germacrene D: *C. paralicola* (44.28 %), *C. criuva* subsp. *criuva* (29.03 %); *β*-caryophyllene: *C. fluminensis* (35.61 %), *C. lanceolata* (36.39 %), *C. hilariana* (58.10 %); *α-trans*-bergamontene: *C. spirictus-sanctensis* (36.30 %); *α*-bulnesene: *C. weddelliana* (25.61 %); bicyclogermacrene: *C. panapanari* (25.93 %) and *trans-β*-farnesene: *C. nemorosa* (24.63 %), while *C. grandiflora* is composed of 42.16 % monoterpene hydrocarbons. Verbenone (31.91 %) was the major component. In contrast, *C. rosea, C. grandiflora, C. lanceolata* and *C. criuva* subsp. *parviflora* are rich in 3-methylcyclohexanone (19.56 %), hexadecanol (22.72 %), *p*-anisaldehyde (23.39 %) and octadecanol (26.81 %), respectively. This study suggests considerable chemical variation among the non-polar fractions of fruit latex of the twelve *Clusia* species.

Key words: *Clusia* spp., latex, apolar fraction, *β*-caryophyllene, verbenone.

INTRODUCTION

Many plants can store a great diversity of liquids and fluids, including latex, resins, mucilage and gums in specialised cells, channels and/or intercellular cavities. The latex can be defined as a water-based suspension or emulsion of various types of small particles. Latex is accumulated either in living cells and/or specialised structures called laticifers (Lewinsohn 1991) or in intercellular secretory canals or ducts lined by epithelial cells secreting substances into the canal. The latter is the case in the close families Clusiaceae, Calophyllaceae and Hypericaceae (*Vismia*). The aqueous suspension is generally constituted by an infinity of compounds belonging to a variety of classes, including inorganic constituents. Among the secondary compounds

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* Contribution to the centenary of the Brazilian Academy of Sciences.

present in these suspensions, mainly terpenoids, fatty acids, aromatic compounds, hydrocarbons and alkaloids are found (Konno 2011, Hua et al. 2015).

The roles of laticifers or secretory canals and latex in plants are up to now not completely clear, but it is generally assumed that they serve to store nutrients or that the latex has a distribution function for these nutrients for the different plant parts (Agrawal and Konno 2009). Other functions described for latex production units are regulation of water storage and oxygen transport in plants, and especially the protection of the plant against natural enemies, more precisely, microorganisms and herbivores (Hua et al. 2017). Comparison between closely related plant groups led to the hypothesis that the presence of latex is directly related to plant survival and species richness of clades (Farrell et al. 1991). As plants with latex are observed with a higher frequency in Eudicotyledoneae, one estimates that about 40 families and more than 20,000 species are latex or resin producers (Konno 2011). Of these species, among the most important are the ones of the family Clusiaceae that can store in the latex and in the floral resin different classes of secondary compounds.

The neotropical genus *Clusia* L. (Clusiaceae) has attracted the interest of botanists for its resin producing flowers, which are collected especially by Euglossinia and Trigonini bees. Chemical research revealed that these resins are mainly composed of polyisoprenylated benzophenones (Porto et al. 2000, Anholeti et al. 2015) and have shown HIV-inhibitory activity (Wu et al. 2014).

Like all members of the family Clusiaceae, *Clusia* plants have latex in nearly all their tissues. Quantities and colour of the latex vary among the species but also between different plant organs of one plant. The latex, extracted mainly from fruits of the *Clusia* species has been used in popular medicine by ingestion for rheumatic treatment (Sanz-Biset et al. 2009) and infant oral candidiasis (Barbosa and Pinto 2003). Up to the present moment, only one study referring to the chemical composition of latex from *Clusia* has been realized, which was restricted to *C. grandiflora* species where the antimicrobial activity of two polyisoprenylated benzophenones obtained from the latex of stems was reported (Lokvam et al. 2000).

The present work has the objective to investigate the chemical composition of the apolar fraction of latex from fruits of twelve *Clusia* species pertinent to four sections (*Chlamydoclusia*, *Criuva*, *Phloianthera* and *Cordylandra*).

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The latex were obtained from fruits of *Clusia* species cultivated on the "Fazenda Santa Elisa", Agronomic Institute of Campinas (IAC), Campinas - SP, Brazil. Maria do Carmo Estanislau do Amaral and Volker Bittrich have deposited voucher specimens in the Herbarium of the State University of Campinas (UEC). The latter was responsible for the identifications. The species studied and herbarium numbers are listed in Table I.

EXTRACTION OF LATEX

The exuded latex of plants was obtained from freshly cut fruits and collected onto methanol. Filtration and solvent evaporation produced methanolic extract. The triplicated yields were average and calculated based on fresh weight of the fruit. The fruits from different *Clusia* species were collected randomly.

ISOLATION OF APOLAR COMPONENT

Methanolic extract was dissolved in dichloromethane (30 mL) and filtrated on 2.0 g of silica gel 60 on glass column (Ø = 1 cm). The collected dichloromethane fraction was dried over anhydrous sodium sulphate and reduced to ca. 0.5 mL at room temperature under reduced pressure on a rotatory evaporator and stored in sealed vials at low temperature before analysis. Three replicated analyses were used for some species (*C. paralicola*, *C. fluminensis*, *C. lanceolata*, *C. criuva* subsp. *parviflora*, *C. grandiflora*, and *C. rosea*).

GAS CHROMATOGRAPHY FID (GC-FID) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

Analyses were carried out using a HP-5990/5970 system equipped with a flame ionization detector (FID) and J&W Scientific non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm); column temperatures were programmed from 60 to 240 °C at 3 °C min^{-1} for integrating purposes.

GC-FID ANALYSIS

Injector and detector temperatures were 220 and 285 °C respectively. Hydrogen was used as carrier gas at a flow rate 1.16 ml min^{-1} in the split mode (1:30), with an injection volume, 1.0 µL solution of about 10 mg of latex in 1mL of dichloromethane. The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. The retention indices were obtained by co-injecting the oil sample with a C_{11}-C_{24} linear hydrocarbon mixture (retention index from 900 to 1099 range was obtained by extrapolation).

GC-MS ANALYSIS

The carrier gas was helium and the temperature program was the same as that for GC experiments. The mass spectra were taken at 70 eV with a scanning speed of 0.84 scan s^{-1} from *m/z* 40 to 550.

The latex were analysed by GC and GC-MS, and identification was made on the basis of standard compound co-injection and comparison of retention indices (Van den Dool and Kratz 1963) as well as by computerised matching of the acquired mass spectra with those stored in wiley / NBS mass spectral library of the GC-MS data system and other published mass spectra (Adams 2007).

RESULTS AND DISCUSSION

The latex isolated from the fruits of different *Clusia* species showed colours from white to yellowish green with characteristic citric odours. The yields (calculated based on fresh fruits weight) of fruits latex of *Clusia* species are shown in Table I as well as the colour and herbarium number.

This investigation allowed the identification of more than 70 components in the dichloromethane fractions of the late latexes from different *Clusia* species. In the mean 15 compounds were identified in each species, which are represented in more than 99 % of the fruits latex. In general, even species with the same taxonomic section, showed different composition of the dichloromethane fraction of the latex. With the objective of getting a better visualization of the molecules comprising the apolar latex constituents of the fruits from the different studied species, the identified compounds were allocated in three different compound groups in accordance with the criteria established by Knudsen et al. (1993), which are fatty acid derivatives, isoprenoids and benzenoids (Table II).

Curiously, the five benzenoid compounds identified (benzaldehyde, acetophenone, methyl benzoate, ethyl benzoate and *p*-anisaldehyde), were never found simultaneously in the investigated species. Nevertheless, comparison of the latex composition of these *Clusia* species shows that, although not being the compound class with the major quantity, benzenoid compounds are characterised (quantity > 0.1 %) in practically all species, with exception of *C. panapanari* and *C. spirictu-sanctensis* of the section *Cordylandra* (Table II). The benzenoid compounds present at more than 1 % concentration were acetophenone, section *Cordylandra* (*C. weddelliana*, 3.83 %), section *Chlamydoclusia* (*C. rosea*, 10.49 %), section

TABLE I
Herbarium number, colour and percentage of fruits latex from *Clusia* species collected in the Fazenda Santa Elisa of the Agronomic Institute of Campinas - SP, Brazil.

Taxonomic sections	*Clusia* species	% Fruit latex	Colour	Herbarium number
Chlamydoclusia	*C. nemorosa* G. Mey.	0.87	yellowish green	#95/150
	C. rosea Jacq.	1.14	yellowish green	#95/154
	C. grandiflora Splitg.	0.12	white	#95/153
Criuva	*C. criuva* Cambess	3.97	yellowish green	#97/247
	C. parviflora Engl. Nom. Illeg	3.03	yellowish green	#97/7
Phloyanthera	*C. hilariana* Schltdl.	0.66	white	#97/248
	C. lanceolata Cambess.	0.56	yellowish green	#96/27
Cordylandra	*C. paralicola* G. Mariz	1.29	yellowish green	#97/5
	C. weddelliana Planch & Triana	0.46	yellowish green	#2001/57
	C. fluminensis Planch & Triana	1.06	white	#2001/54
	C. panapanari (Aubl.) Choisy	0.67	yellowish green	#95/156
	C. spiritu-sanctensis G.Mariz	0.60	white	#95/185a

Criuva (*C. criuva* subsp. *parviflora*, 3.11 % and *C. criuva* subsp. *criuva*, 2.73 %); ethyl benzoate, only present in section *Criuva* (*C. criuva* subsp. *parviflora*, 4.53 % and *C. criuva* subsp. *criuva*, 2.34) and *p*-anisaldehyde, in *Chlamydoclusia* section (*C. grandiflora*, 1.21 %), Section *Criuva* (*C. criuva* subsp. *criuva*, 1.11 %) and section *Phloianthera* (*C. lanceolata*, 23.39 %). Aromatic esters, aromatic aldehydes, aromatic ketone and fatty acid derivatives have been reported to be part of floral scents for *Clusia* species. However, with the exception of acetophenone, ethyl benzoate, methyl benzoate, *p*-anisaldehyde and benzaldehyde, found in apolar fractions of fruits latex were also identified in the floral essential oil from the same *Clusia* species here investigated (Nogueira et al. 2001).

By the organisation of the latex constituents in the above-mentioned three groups, independently from taxonomic section, the predominance of compounds from the isoprenoid class became evident. Outstanding are the species of section *Cordylandra*, where isoprenoid concentration of 91.73 - 99.19 % were found. In the sections *Chlamydoclusia* and *Phloianthera*, only the species *C. nemorosa* (99.43 %) and *C. hilariana* (96.92 %) show isoprenoid percentages higher than 90 %. The other species show the respective value in the range of 47.50 - 82.53 %. With exception of *C. grandiflora*, which is comprised by 42.16 % monoterpene hydrocarbons with verbenone (31.91 %) as major component, *C. rosea* and *C. criuva* subsp. *parviflora* with 3-methylcyclohexanone (19.56 %) and octadecanol (26.81 %) as major constituents, all the other species are characterised by the presence of sesquiterpenes as major components. For example, in *C. paralicola* (44.28 %) and *C. criuva* subsp. *criuva* (29.03 %), the germacrene D is more abundant. Other sesquiterpenes that were present in high amounts were *β*-caryophyllene in *C. fluminensis* (35.61 %), *C lanceolata* (36.39 %) and *C. hilariana* (58.1 %); *α*-*trans*-bergamontene in *C. spirictus-sanctensis* (36.30 %); *α*-bulnesene in *C. weddelliana* (25.61 %); bicyclogermancrene in *C. panapanari* (25.93 %) and trans-*β*-farnesene in *C. nemorosa* (24.63 %) (Table II).

TABLE II
Percentage of chemical constituents of apolar fractions from fruits latex of twelve *Clusia* species belonging to four taxonomic sections.

			Taxonomic sections											
			Cordylandra					Chlamydoclusia			Criuva		Phloianthera	
Compounds	[a]RI	[b]RI	*C. paralicola*	*C. weddelliana*	*C. fluminensis*	*C. spiritu-sanctensis*	*C. panapanari*	*C. nemorosa*	*C. rosea*	*C. grandiflora*	*C. parviflora*	*C. criuva*	*C. lanceolata*	*C. hilariana*
Fatty acid derivatives														
2-Methyl butyl acetate	883	881	0.51	-	-	-	-	-	2.42	-	-	5.55	-	-
Undecane	1100	1100	-	-	-	-	-	-	9.70	0.29	-	1.79	-	-
Dodecane	1200	1200	-	-	-	-	-	-	-	-	-	-	-	0.20
Tridecane	1301	1300	-	-	-	0.43	-	-	-	-	-	-	-	0.42
Tetradecane	1400	1400	-	-	7.82	-	-	-	-	-	-	-	-	1.11
Hexadecane	1600	1600	-	-	-	-	-	-	-	-	-	-	-	0.80
Octadecene	1796	1790	-	-	-	-	-	-	-	-	4.41	-	-	-
Hexadecanol	1882	1875	-	-	-	-	3.72	-	10.19	22.72	1.94	-	-	-
Octadecanol	2081	2077	-	-	-	-	-	-	-	-	26.81	-	-	-
Octadecanol acetate	2212	2209	-	-	-	-	-	-	-	-	3.55	-	-	-
	Subtotal		**0.51**	**-**	7.82	0.43	3.72	-	22.31	23.01	36.71	7.34	-	2.53
Isoprenoids														
Camphene	951	954	-	-	-	-	-	-	-	-	-	2.93	-	0.30
β-Pinene	981	979	-	-	-	-	-	-	-	-	-	4.08	-	-
α-Terpinene	1015	1017	-	-	-	-	-	-	-	-	-	-	-	0.12
Verbenone	1201	1205	-	-	-	-	-	-	-	31.91	-	-	-	-
cis-Ocimenone	1234	1229	-	-	-	-	-	-	-	8.84	-	-	-	-
trans-Ocimenone	1238	1238	-	-	-	-	-	-	-	1.41	-	-	-	-
δ-Elemene	1340	1338	1.21	-	-	1.35	1.93	-	-	-	-	-	-	-
α-Cubebene	1354	1348	0.54	-	-	-	-	-	-	-	-	-	-	0.54
α-Ylangene	1375	1375	-	-	-	-	-	-	-	-	-	1.45	0.73	-
α-Copaene	1380	1376	1.83	-	8.69	1.41	-	4.32	-	1.30	-	15.58	10.39	1.72
β-Elemene	1394	1390	8.51	1.70	-	2.54	2.28	-	-	-	-	-	-	-
Cyperene	1397	1398	-	-	-	-	-	-	-	-	-	-	3.33	-
α-Gurjunene	1412	1409	-	0.89	-	-	-	-	-	-	-	-	-	-
α-Santalene	1416	1417	-	-	-	-	-	-	4.08	-	-	-	-	-

TABLE II (continuation)

Compounds	aRI	bRI	Cordylandra: *C. paralicola*	Cordylandra: *C. weddelliana*	Cordylandra: *C. fluminensis*	Cordylandra: *C. spiritu-sanctensis*	Cordylandra: *C. panapanari*	Chlamydoclusia: *C. nemorosa*	Chlamydoclusia: *C. rosea*	Chlamydoclusia: *C. grandiflora*	Criuva: *C. parviflora*	Criuva: *C. criuva*	Phloianthera: *C. lanceolata*	Phloianthera: *C. hilariana*
β-Caryophyllene	1418	1419	2.08	24.05	35.61	0.55	-	21.10	-	2.39	4.12	1.38	36.39	58.11
β-Gurjunene	1435	1433	3.19	7.73	-	-	-	-	-	2.22	-	-	-	-
(E)-α-Bergamontene	1438	1434	-	-	-	36.30	5.52	-	12.67	-	-	4.42	-	-
Aromadendrene	1442	1441	6.25	-	-	-	1.79	-	-	-	-	-	-	-
α-Humulene	1457	1454	-	5.28	2.11	0.98	2.19	-	1.35	0.27	-	-	7.81	18.27
(E)-β-Farnesene	1458	1456	-	-	-	-	-	24.63	-	-	-	-	-	-
Allo-aromadendrene	1460	1460	-	-	-	-	-	-	-	-	11.08	-	-	-
dihydro-Aromadendrane	1463	1462	-	-	-	2.33	-	-	-	-	-	-	-	-
γ-Muurolene	1479	1479	-	-	-	-	13.42	18.71	-	2.53	-	-	3.81	-
ar-Curcumene	1483	1480	-	-	-	-	-	-	5.20	-	-	-	-	-
Germancrene D	1486	1485	44.28	-	8.78	-	10.16	5.50	-	-	12.88	29.03	-	1.85
β-Selinene	1489	1490	10.98	4.44	12.44	-	-	-	3.46	0.97	-	-	1.17	-
Valencene	1492	1496	-	-	-	-	-	-	-	-	-	-	1.26	-
α-Selinene	1494	1498	-	4.35	6.56	-	-	-	-	1.02	-	-	-	-
Bicyclogermancrene	1495	1500	-	-	-	3.14	25.93	-	-	-	-	-	-	-
α-Muurolene	1499	1500	-	-	-	-	-	2.80	-	-	-	5.59	-	2.52
trans-β-Guaiene	1504	1502	-	-	2.18	-	-	-	-	-	-	-	-	-
β-Bisabolene	1506	1505	-	-	-	-	-	-	1.98	-	-	-	-	-
α-Bulnesene	1510	1509	-	25.61	-	-	-	-	-	-	-	-	-	-
γ-Cadinene	1515	1513	-	-	-	5.49	10.84	13.87	-	0.74	3.79	tr	1.40	2.02
δ-Cadinene	1526	1523	12.14	12.34	-	15.30	11.04	-	3.52	0.83	-	5.10		1.69
trans-γ-Bisabolene	1536	1531	-	-	-	23.66	-	-	-	-	-	-	-	-
trans-Cadina-1,4-diene	1537	1534	-	1.53	-	-	-	-	-	0.42	-	-	0.98	-
α-Cadinene	1539	1538	-	-	-	tr	3.64	1.04	-	-	-	9.01	-	0.73
α-Calacorene	1542	1545	-	-	-	-	-	-	8.28	0.30	4.40	-	-	-
(*E*)-Nerolidol	1567	1563	-	3.41	-	-	-	-	-	-	-	-	-	-

Taxonomic sections: Cordylandra, Chlamydoclusia, Criuva, Phloianthera.

TABLE II (continuation)

			Taxonomic sections											
			Cordylandra					Chlamydoclusia			Criuva		Phloianthera	
Compounds	**[a]RI**	**[b]RI**	*C. paralicola*	*C. weddelliana*	*C. fluminensis*	*C. spiritu-sanctensis*	*C. panapanari*	*C. nemorosa*	*C. rosea*	*C. grandiflora*	*C. parviflora*	*C. criuva*	*C. lanceolata*	*C. hilariana*
Spathulenol	1573	1578	-	-	6.37	4.81	2.53	4.59	6.72	11.71	-	1.67	-	-
Caryophyllene oxide	1580	1583	-	-	3.45	-	-	-	-	6.86	11.07	-	8.75	9.05
Globulol	1587	1590	-	-	-	-	-	-	-	-	3.96	-	-	-
β-Copaen-4α-ol	1589	1590	-	-	-	-	-	-	-	0.44	-	1.16	-	-
Guaiol	1596	1600	-	-	-	-	-	-	-	-	-	1.13	-	-
Khusimone	1599	1604	-	-	-	-	tr	-	-	-	-	-	-	-
1,10-di-epi-Cubenol	1617	1619	-	-	-	0.55	-	-	-	-	-	-	-	-
10-epi-γ-Eudesmol	1619	1623	-	4.17	5.54	-	-	-	-	-	-	-	-	-
1-epi-Cubenol	1630	1628	-	-	-	-	-	1.32	-	-	-	-	-	-
epi-α-Cadinol	1638	1640	-	-	-	-	-	1.55	0.31	-	-	-	-	-
epi-α-Muurolol	1642	1642	4.16	-	-	-	3.12	-	-	-	3.96	-	-	-
Cubenol	1645	1646	-	-	-	0.78	1.04	-	-	0.21	-	-	-	-
α-Cadinol	1656	1654	-	-	-	-	-	-	-	1.01	-	-	-	-
Khusinol	1677	1680	tr	-	-	-	-	-	-	-	-	-	-	-
14-hydroxi-α-Muurolene	1778	1780	3.17	-	-	-	-	-	-	-	-	-	-	-
	Subtotal		**98.34**	95.50	91.73	99.19	95.43	99.43	47.57	75.38	55.26	82.53	76.02	96.92
Benzenoids														
Benzaldehyde	963	960	-	-	-	-	-	-	-	-	-	3.46	-	-
Acetophenone	1067	1065	0.20	3.83	tr	-	-	0.52	10.49	tr	3.11	2.73	0.58	-
Methyl benzoate	1094	1090	0.33	-	-	-	-	-	-	-	-	-	-	0.46
Ethyl benzoate	1172	1173	-	-	-	-	-	-	-	-	4.53	2.54	-	-
p-Anisaldehyde	1255	1250	0.58	-	0.36	tr	-	-	-	1.21	-	1.11	23.39	-
	Subtotal		**1.11**	**3.83**	**0.36**	**tr**	**-**	**0.52**	**10.49**	**1.21**	**7.64**	**9.84**	23.97	0.46
Others														
3-Methylcyclohexanone	951	952	-	-	-	-	-	-	19.56	-	-	-	-	-
	Subtotal		**-**	**-**	**-**	**-**	**-**	**-**	**19.56**	**-**	**-**	**-**	**-**	**-**
	Total		**99.96**	**99.33**	**99.91**	**99.62**	**99.15**	**99.95**	**99.93**	**99.67**	**99.61**	**99.71**	**99.99**	**99.91**

[a]Retention indices calculated from retention times in relation to those of a series of n-alkanes on a 30m DB-5 capillary column. [b]Retention indices from the literature. tr = trace <0.1 %.

The main constituents of latex present in the different *Clusia* species were monoterpenes and sesquiterpenes and as the major percentile was observed verbenone (31.91 %) in *C. grandiflora* and β-caryophyllene (58.11 %) in *C. hilariana*. In relation to β-caryophyllene as a major constituent of apolar fraction of species from section *Phloianthera*, these findings are consistent with those reported by Guimarães et al. (2013) for the major constituent of essential oil from leaves of *Clusia lanceolata* (43.20 - 56.40 %) and by Fernandes et al. (2016) for essential oil of flowers of *Clusia hilariana* (37.1 - 49.0 %), who found β-caryophyllene also as major constituent.

The constituents of the apolar fraction of fruit latex of *Clusia* species have been investigated for the first time and showed in all species, independent of the taxonomic section, the presence of fatty acid derivatives, benzenoids and isoprenoids. Our study shows qualitative and quantitative differences in the chemical compositions for the studied *Clusia* species, even between species belonging to the same taxonomic group. As an explanation, one can exclude differences in the habitat as all plants, from which the fruits were collected, are cultivated in the same small area of Fazenda Sta. Elisa in Campinas, São Paulo state. Thus, a genetic basis of the chemical differences must be assumed. Only in few cases, the chemical data show correlation with phylogenetic relationship of the studied species as supported by morphological and DNA sequence data (Gustafsson et al. 2007). There are no data yet about differences in the latex chemistry within the same species and the possibility of an infraspecific variation cannot be excluded. It seems plausible that the latex a least partly serves to protect the developing seeds from herbivores, until the fruits (still carnose) open to expose the seeds to birds for dispersal. The very dense canals in the pericarp support this idea. However, the specific role of the observed latex constituents in such a supposed protection is unknown, and thus the efficiency of the different components to deter possible attacks. Nevertheless, during many visits to the plants cultivated in Campinas, we never observed immature fruits damaged by herbivores; apparently, the chemical defense is efficient.

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