Revista de la Sociedad Química de México, Vol. **47***, Núm. 2 (2003) 139-142*

Investigación

Chemical Composition and Antimicrobial Activity of the Essential Oils from *Annona cherimola* (Annonaceae)

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Recibido el 25 de febrero del 2003; aceptado el 23 de mayo del 2003

This paper is dedicated to Professor Alfonso Romo de Vivar

Abstract. The chemical composition of the essential oils obtained by steam distillation of the fresh leaves, flowers and fruits from *Annona cherimola* was analyzed by means of Gas Chromatography-Mass Spectrometry (GC/MS). Sixty constituents were identified from the oils. While bicyclogermacrene, *trans*-caryophyllene and δ-amorphene were found to be the major constituents in the oil of the leaves; bicyclogermacrene, α-terpinolene and germacrene D were the major constituents in the oil of the flowers and β-pinene, α-terpinolene, β-fenchyl alcohol and α-pinene were the major constituents in the oil of the fruits. The *in vitro* antimicrobial activity of the three essential oils and of some of their major constituents against five *Gram* (±) bacteria and one fungus is reported.

Keywords: *Annona cherimola*, essential oil, Gas Chromatography-Mass Spectrometry, bicyclogermacrene, *trans*-caryophyllene, α-amorphene, α-copaene, α-terpinolene, germacrene D, linalool, β-fenchyl alcohol, β-pinene, α-pinene.

Resumen. La composición química de los aceites esenciales obtenidos por arrastre de vapor de las hojas, flores y frutos frescos de *Annona cherimola* fue analizada por Cromatografía de Gases-Espectrometría de Masas (GC/MS). Sesenta componentes fueron identificados en los aceites esenciales. Mientras que el biciclogermacreno, el *trans*-cariofileno y el δ-amorfeno se identificaron como los constituyentes mayoritarios en el aceite esencial de las hojas; el biciclogermacreno, el α-terpinoleno y el germacreno D fueron los constituyentes mayoritarios del aceite esencial de las flores, y el β-pineno, el α-terpinoleno, el alcohol β-fenchílico y el α-pineno fueron los principales componentes del aceite esencial de los frutos. La actividad antimicrobiana *in vitro* de los tres aceites esenciales y de algunos de sus contituyentes mayoritarios fue evaluada contra cinco bacterias *Gram* (+), (-) y un hongo.

Palabras clave: *Annona cherimola*, aceite esencial, Cromatografía de Gases-Espectrometría de Masas, biciclogermacreno, *trans*-cariofileno, α-amorfeno, α-copaeno, α-terpinoleno, germacreno D, linalool, alcohol β-fenchílico, β-pineno, α-pineno.

Introduction

The Annonaceae family includes 80 genera and about 850 species distributed in tropical and subtropical areas of America, Africa and Asia. Only four genera of this family are of economic importance, and the genus *Annona* is one of them. *Annona cherimola* is highly appreciated for its exquisite fruits and for its use in traditional medicine in the treatment of skin diseases [1], tumors and cancer [2], and is reported to have antimicrobial and insecticidal properties [3,4]. Although several reports on the chemical composition of *A. cherimola* have been published [1-9], to date there are no reports on the chemical analysis of the essential oil of this species. We present here the chemical composition and the antimicrobial activity of the essential oils from the leaves, flowers and fruits of *A. cherimola.*

Results and discussion

CG/MS analysis of the three oils led to the identification of sixty constituents, which are listed in Table 1 along with their quantitative data. The identification of each component was based on a comparison of its mass spectrum with those contained in the HP CHEMSTATION-Wiley275.L Library. A high proportion of the essential oils is constituted by four main compounds: more than 40 % of the essential oil from the leaves is composed by bicyclogermacrene (18.20 %), *trans*-caryophyllene (11.50 %), α-amorphene (7.57 %) and α-copaene (5.63 %); similarly, 34.02 % of the essential oil from the flowers corresponds to bicyclogermacrene (11.73 %), α-terpinolene (9.75 %), germacrene D (7.01 %) and linalool (5.53 %); finally, almost 45 % of the essential oil from the fruits corresponds to β-pinene (15.48 %), α-terpinolene (13.59

Table 1. Chemical composition of the essential oils of leaves, flowers and fruits from *A. cherimola*.

	Compound[a]	rt[b]	KI[c]	[d]GC Area % A[e]	B[e]	C[e]
1	Isoprene	2.41	421	0.35	0.08	—
2	α-Pinene	3.29	800	1.60	0.14	6.37
3	Camphene	3.41	804	—	—	0.45
4	β-Terpinene	3.67	813	—	0.08	—
5	β-Pinene	3.73	815	4.11	1.32	15.48
6	Mircene	3.85	819	0.23	2.75	—
7	β-Phellandrene	4.02	825	—	—	0.20
8	1,8-Cineole	4.30	837	0.32	0.30	4.02
9	*trans*-β-Ocimene	4.53	846	0.15	0.08	0.30
10	δ-3-Carene	4.67	853	—	—	0.19
11	*trans*-Linalool oxide	4.79	868	—	—	1.18
12	α-Terpinolene	5.13	876	—	9.75	13.59
13	Linalool	5.27	885	3.06	5.53	0.29
14	Borneol	6.00	939	—	—	2.02
15	α-Terpinene-4-ol	6.21	959	0.17	1.67	2.04
16	β-Fenchyl alcohol	6.31	970	—	1.70	8.81
17	α-Terpineol	6.66	982	—	0.15	0.83
18	Borneyl Acetate	7.60	989	—	—	0.21
19	α-Bisabolene	8.51	1085	1.69	1.74	1.38
20	α-Cubebene	8.67	1109	0.89	0.11	5.88
21	α-Copaene	9.04	1170	5.63	1.04	3.28
22	β-Cubebene	9.13	1189	—	—	1.09
23	β-Elemeno	9.19	1202	3.57	1.82	—
24	Unknown	9.35	1204	—	—	1.79
25	*cis*-Caryophyllene	9.40	1205	1.34	—	3.60
26	*trans*-Caryophyllene	9.60	1207	11.50	2.30	—
27	(-)Caryophyllene oxide	9.68	1209	—	0.16	0.81
28	β-gurjunene	9.70	1210	0.30	—	1.40
29	α-gurjunene	9.82	1213	1.37	—	—
30	(-)Isoledene	9.90	1215	0.60	—	0.33
31	α-Humulene	9.97	1217	3.05	0.24	1.48
32	Aromadendrene	10.05	1220	0.39	0.18	0.43
33	α-Amorphene	10.26	1226	2.20	—	—
34	Germacrene D	10.31	1228	3.75	7.01	1.44
35	Alloaromadendrene	10.40	1231	2.36	—	—
36	Bicyclogermacrene	10.46	1233	18.20	11.73	1.49
37	10-Hydroxy-α-gurjunene	10.55	1236	4.47	1.01	0.69
38	δ-Amorphene	10.61	1239	7.57	1.08	—
39	γ-Cadinene	10.69	1242	2.50	0.14	—
40	Unknown	10.77	1244	0.60	—	—
41	δ-Cadinene	10.81	1247	4.63	1.98	3.01
42	Cadina-1,4-diene	10.93	1251	1.27	0.12	—
43	9-Aromadendrene	10.99	1256	0.70	—	—
44	β-Elemol	11.11	1259	—	0.40	—
45	Nerolidol	11.19	1264	—	—	0.81
46	1(10),4,11-Germacratrien-9-ol	11.28	1269	2.99	4.57	1.01
47	γ-Cadinol	11.50	1281	0.24	0.86	2.00
48	Lauric acid	11.66	1287	—	0.58	0.27
49	T-cadinol	12.25	1407	0.42	3.40	2.03
50	α-Cadinol	12.39	1410	0.70	3.30	1.48
51	Muurolol	12.58	1415	—	2.39	2.48
52	Unknown	12.76	1430	—	—	1.21
53	Azuleno	13.27	1434	—	1.51	—
54	Unknown	13.79	1452	0.39	2.90	—
55	Dehydroaromadendrene	14.63	1493	0.23	—	—
56	9,10-Dehydroisolongifolene	15.14	1607	1.43	—	—
57	Cycloisolongifolene	15.20	1610	1.39	—	—
58	Unknown	16.34	1674	0.87	—	—
59	Unknown	16.92	1732	1.40	—	—
60	Palmitic acid	18.30	1801	0.27	12.97	—
61	(8 β,13β)-Kaur-16-ene	18.57	1811	0.49	—	0.44
62	Oleic acid	20.84	1886	0.57	6.64	—
63	Kauran-16-ol	21.56	1905	—	—	0.99
64	Stearic acid	21.62	1908	—	6.25	—
65	Kaur-16-en-19-ol	21.84	1913	—	—	0.64
66	Kaur-16-en-18-oic acid	24.52	2170	—	—	1.90
	Total of compounds identified (%)			96.70	97.08	96.25

[a] Composition listed in order of elution from a HP-1 column. [b]Retention times (rt) in minutes. [c]Kovats Indices (KI) on HP-1 capillary column
[d]Gas Chromatography. [e]A, B and C represent the essential oil of leaves, flowers and fruits of *Annona cherimola*, respectively
- no detected

Table 2. Proportion (%) of mono- and sesquiterpenes in the essential oils from *A. cherimola*.

Sample	Monoterpenes				Sesquiterpenes			
	hydrocarbons	alcohols	oxides	total	hydrocarbons	alcohols	oxides	total
Leaves	6.09	3.23	0.32	9.64	76.56	8.82	0.00	85.38
Flowers	14.12	9.05	0.3	23.47	31.00	15.93	0.16	47.09
Fruits	36.58	13.99	5.41	55.98	24.81	10.50	0.81	36.12

Table 3. Principal skeleta (%) of mono- and sesquiterpenes in the essential oils from *A. cherimola*.

Sample	Monoterpenes				Sesquiterpenes			
	acyclic monoterpenes	pinene	*p*-menthane	total	caryophyllene	germacrene	aromadendrene	total
Leaves	3.21	5.71	0.72	100.00	12.84	21.95	17.00	60.65
Flowers	5.61	1.46	14.70	92.70	2.46	18.74	2.09	49.45
Fruits	1.77	21.85	20.68	79.10	0.81	2.93	2.09	16.14

%), β-fenchyl alcohol (8.81 %) and α-pinene (6.37 %). The identified components represent between 96-97% of the total composition of the oils.

The monoterpenes and sesquiterpenes are the main type of compounds in the three essential oils (Table 2). The essential oil of the leaves have a high proportion of sesquiterpenes (85.38 %) and showed a weak antimicrobial activity against the assayed microorganisms (Tabla 4). In the essential oil of the flowers the proportion of sesquiterpenes was lowest (47.09 %), increasing the proportion of monoterpenes (23.47 %), and for this essential oil the MIC values observed were minor in all the assayed microorganisms, with the exception of *E. faecalis*. In the essential oil of the fruits the monoterpenes are the major constituents (55.98 %) being this essential oil the most active against *S. aureous* and *P. mirabilis*. These results indicate that the antimicrobial activity of these essential oils could be associated to the presence and amount of the monoterpenic compounds. The mono- and sesquiterpenes isolated could be classified as hydrocarbons, alcohols and oxides, being the hydrocarbons the major components of the three essential oils (Table 2).

In the three essential oils more than 79 % of the monoterpenes belongs to acyclic monoterpenes, and monoterpenes with pinene and *p*-menthane skeleton. In the essential oils from the leaves and flowers 60 % and 49 %, respectively, of the sesquiterpenes have the skeleton of caryophyllene, germacrene and aromadendrene, while in the essential oil from fruits the proportion of this sesquiterpenes is very lowest (16 %) (Table 3).

The three essential oils of *A. cherimola* showed a significant activity against *Gram*-positive, *Gram*-negative bacteria and one fungus (Table 4). Although no previous reports on the antimicrobial activity of the major constituents of the essential oil from the leaves were found in the literature, *trans*-caryophyllene showed moderate activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Shigella sonei*. The essential oil from the flowers was the most active against all the microorganisms tested, and the second most active against *S. aureus*, this activity could be associated with its high concentration of linalool, that was very active against all the microorganisms included in the Table 2, and which is known to possess antimicrobial and antifungal activity [10,11]. Previous reports have been carried out of the antimicrobial activity for α-pinene [10,12,13], who in our hands showed moderate activity, however, a very important activity was observed for β-fenchyl alcohol, both presents in high proportion in the essential oil of the fruits, which shows the best effect against the bacteria *S. aureus*, *E. faecalis* and *Proteous mirabilis*. Although at least one of the major constituents of each essential oil showed antimicrobial activity against the tested microorganisms, the MIC values obtained in each case are biggest that those of their corresponding essential oils, this suggest that the antimicrobial activity could be due to a synergistic effect between the constituents of each essential oil.

Experimental section

Plant material: The leaves (442 g), flowers (309 g) and green fruits with an average size of 2.5 cm (206 g) were collected from 10 individuals of a wild population of *A. cherimola*. The plant material was collected during the flowering and fruiting stage in April-May of 2002. A specimen (voucher No. 18854) was deposited at the Herbarium of the Universidad Autónoma del Estado de Morelos (HUMO), Cuernavaca, Morelos, México.

Chemical analysis: The leaves, flowers and fruits of *A. cherimola* were finely cuted and subjected to steam distillation (1.5 h) using a modified Clevenger-type apparatus, to yield 0.63 %, 0.39 %, and 0.83 % of a yellow oil, respectively. The physical properties for each sample were: leaves ($[\alpha]_D^{25}$ + 16.1 ($CHCl_3$, c = 1.1), d^{25} 0.83), flowers ($[\alpha]_D^{25}$ + 6.4 ($CHCl_3$, c = 0.97), d^{25} 0.87) and fruits ($[\alpha]_D^{25}$ + 8.3 ($CHCl_3$, c = 0.92), d^{25} 0.84). The oils were subjected to GC / MS analysis in a Hewlett Packard 6890 GC / 5972 MSD chromatograph equipped with a HP-1 capillary column (length 30 m, id 0.25 mm, ft 0.25 μm). The carrier gas was helium and the linear gas velocity was 36 cm/s. The injector temperature was 250

Table 4. Antimicrobial activity of the essential oils of leaves, flowers and fruits from *A. cherimola*.

Sample	MIC (mg / mL)					
	Staphylococcus aureus	*Enterococcus faecalis*	*Escherichia coli*	*Shigella sonei*	*Proteous mirabilis*	*Candida albicans*
Leaves	0.25	0.5	10	5	5	5
Flowers	0.125	0.5	2	2.5	2	0.5
Fruits	0.06	0.5	2	2.5	1	2
trans-caryophyllene	8.0	16	16	16	> 16	> 16
Terpinolene	> 16	> 16	> 16	> 16	> 16	> 16
linalool	2	4	2	2	2	4
β-Pinene	> 16	> 16	> 16	> 16	> 16	> 16
α-Pinene	16	16	4	16	16	8
β-fenchyl alcohol	4	2	2	2	2	8
Gentamicin	0.004	0.004	0.008	0.008	0.008	—
Nystatin	—	—	—	—	—	0.004

ºC and the column temperature, initially at 60 ºC, was gradually increased at a rate of 10 ºC/min up to 160 ºC and then gradually increased at a rate of 5º C/min up to 220 ºC and kept at 220 ºC for 5 min. For detection, a flame ionization detector at 280 ºC, IE (Scan 30-550 uma) was used.

Standards of pure metabolites. *trans*-caryophyllene (Aldrich, C-9653), terpinolene (Fluka, 86485), linalool (Aldrich, L260-2), β-pinene (Fluka, 80608), α-pinene (Aldrich, 26,807-0) and β-fenchyl alcohol (Aldrich, 19,644-4) were obtained from commercial sources.

Antimicrobial Activity: The bacteria *Staphylococcus aureus* (ATCC 25213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453) and *Shigella sonei* (ATCC 11060) were maintained on Trypticase soya agar, while *Candida albicans* (ATCC 10231) was maintained on Sabouraud 4 % dextrose agar. The inoculum for each organism was 10^4 colony forming units (CFU)/ mL. The minimum inhibitory concentrations (MICs) were measured as described previously for essential oils [12]. Initial emulsions of the oils (20 mg/mL) and the standards of pure metabolites (16 mg/mL) were prepared in sterile distilled water with 10 % DMSO. Serial dilutions of the stock solutions in both media (100 μL of Muller Hinton broth or Sabouraud broth) were prepared in a microtiter plate and 2 μL of microbial suspension was added to each well. For each strain, the growth conditions and the sterility of the medium were proved and the plates were incubated 24 h at 37 °C for the bacteria, and 48 h at 28 °C for the yeast. Standard antibiotics (gentamicin and nystatin) were used as positive controls, and MICs were determined as the lowest concentrations preventing visible growth. To indicate the bacterial growth, *p*-Iodonitrotetrazolium violet (SIGMA I-8377) was added to the microplate wells, as described by Eloff [14].

Copies of the original GC and GC-MS chromatographs and spectra can be obtained from the author of correspondence.

Acknowledgements

We thank Enrique Salazar Leyva for technical assistance.

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