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Essential oil composition of *Ambrosia cumanensis* (Asteraceae) from Costa Rica

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Abstract
Ambrosia is an Asteraceous genus of flowering plants, which has about 40-45 species, and their geographical distribution ranges from the USA, Mexico, and Central America to South America. The aim of this work was to study the chemical composition of the essential oil of *A. cumanensis* growing in Costa Rica. The essential oils were obtained through the steam distillation process in a Clevenger type apparatus. The chemical composition of the oils was analyzed by capillary GC-FID and GC-MS using the retention indices on a DB-5 type capillary column in addition to mass spectral fragmentation patterns. A total of 137 compounds were identified, accounting for about 90% of the total amount of the oils. *Ambrosia cumanensis* produced terpenoid rich oil, whose composition was dominated by bicyclogermacrae (14.7-23.4%), germacrene D (10.1-16.9%), α-pinene (7.8-12.8%), β-pinene (4.5-6.7%), chrysanthenone (6.2-8.7%), limonene (3.5-5.8%), and filifolone (2.9-4.0%).

Keywords: *Ambrosia cumanensis*, essential oil composition, bicyclogermacrene, germacrene D, α-pinene, β-pinene, chrysanthenone, limonene, filifolone, Costa Rica.

1. Introduction
Asteraceae is one of the largest families of flowering plants with a world-wide distribution and with more than 1600 genera and over 24000 species of herbs, shrubs and trees [1]. From the economic and ecological points of view, this plant family has an appreciable value. Several species are used as food crops such as endive (*Cichorium endivia* L.), artichoke (*Cynara scolymus* L.), and lettuce (*Lactuca sativa* L.) used chiefly as salad vegetables, and sunflower (*Helianthus annuus* L.) as a source of edible oil. Other well-known plants are important spices such as tarragon (*Artemisia dracunculus* L.), and Mexican tarragon (*Tagetes lucida* Cav.) utilized in Costa Rica, mainly fresh, as a tarragon substitute; and as herbal medicines, e.g. arnica (*Arnica montana* L.), purple coneflower [*Echinacea purpurea* (L.) Moench], and chamomile (*Matricaria chamomilla* L.) used extensively for several thousands of years. Also many Asteraceae are relevant in horticulture as ornamental plants and for making floral bouquets (*Chrysanthemum* spp., *Dahlia* spp., *Gaillardia* spp., *Gerbera* spp., *Tagetes* spp., *Zinnia* spp.); as a source of insecticides like pyrethrum [*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.], and some members are noxious weeds (*Senecio* spp., *Ambrosia* spp., ragweed).
Ambrosia is a medium-sized genus in the Asteraceae family (tribe Heliantheae) that includes about 40-45 species found predominantly in the Americas, mostly growing in North America. The plants are distributed generally in desert and semi-desert regions of the southwestern United States and northwestern Mexico, but a few species are found in Central America, mainly growing in disturbed areas, and in Andean South America. In the United States, some species of this genus are considered noxious because their pollen is often the cause of hay fever [2, 3].
In Costa Rica, *Ambrosia cumanensis* Kunth is commonly known as *altamisa* and *gotas amargas* (bitter drops). It is a perennial erect, ramified, aromatic herb usually less than 1m tall. The stems are white-hirsute with long, spreading hairs; the leaves are deeply pinnatifid and densely strigose, 3 to 10 cm long, with dentate segments. The inflorescences are long and thin, almost without branching, like spikes, with many spherical heads of greenish-yellow flowers [2, 4].
In traditional medicine, the leaf decoction of *A. cumanensis* is used in several countries of Middle America as emenagogue, as febrifuge, for stomach ache and as vermifuge [5, 6]. In Venezuela, a decoction of the aerial parts of the plant is used to treat epilepsy and as a diuretic to expel gravel and stones from the bladder and, in bathing, for relieve rheumatic pain [5]. This weed is used as medicinal by communities of Highland Maya of Chiapas (Mexico) for treatment

of vomiting and diarrhea [7]. The infusion of the plant is used in Costa Rica for liver complaints and drunk to calm the nerves [8].

The phytochemical studies of *A. cumanensis* have been directed primarily to the extraction, isolation and structural elucidation of sesquiterpene lactones [9-19], some of them, with characteristic pseudoguaianolide and psilostachyin carbon skeletons [20]. In addition, the chemical research of few *Ambrosia* species afforded some polyines, one *p*-hydroxy acetophenone derivative, one lupeol derivative, germacrene D [13], coniferaldehyde and syringaldehyde [18]. The composition of the essential oil obtained from leaves of *A. cumanensis* has been the subject of one previous study in which Payne *et al.* [21] identified caryophyllene (20.4-36.2%), humulene (4.0 to 11.7%), terpinene (0.1 to 7.8%) and two farnesene compounds: farnesene (tentatively identified) (2.7 to 7.3%) and a 'farnesene isomer' (22.3 to 33.8%), as major constituents and twenty-four additionally compounds, most of them not identified. In addition, nineteen isolates obtained by steam distillation, from leaves of different species of rag weeds from USA and Mexico, were studied using packed stainless steel columns for chromatographic separations, and also it was found that they were composed by terpenoids [21].

In the literature, there are several studies on the chemical composition of essential oils of various species of *Ambrosia*, especially in the last twenty years [22-33]. In general, the investigated oils were predominantly terpenoid in nature and their major constituents are summarized in Table 1.

The present paper investigates the chemical composition of the essential oil of *A. cumanensis* growing in Costa Rica.

Table 1: Main compounds of diverse *Ambrosia* species essential oils.

Species	Location	Major constituents
A. artemisiifolia L.	Belgrade (former Yugoslavia)	Germacrene D, limonene, α-pinene, myrcene, (*E*)-β-ocimene, and borneol [26].
A. elatior L.	Japan	Limonene, α-curcumene, α-pinene, δ-cadinene, γ-cadinene [22].
A. elatior L.	Argentina (Corrientes)	β-Caryophyllene, spathulenol, isoborneol, chrysanthenone, and camphor [29].
A. hispida Pursh.	Cuba (Pinar del Río)	Borneol, spathulenol, Germacrene D, camphor, myrtenol, and bicyclogermacrene [27].
A. microcephala DC.	Brazil (Bujarú, PA)	β-Bisabolene, β-himachalene, bornyl acetate, camphor, and *ar*-curcumene [25].
A. peruviana Willd.	Venezuela (Guasdualito)	γ-Curcumene, *ar*-curcumene, bornyl acetate, camphor, *cis*-epoxy-ocimene, borneol, and junenol [32].
A. polystachya DC.	Brazil (Lavras, MG)	Germacrene D, (*E*)-β-ocimene, β-caryophyllene, limonene, globulol, β-pinene, and bicyclogermacrene [33].
A. scabra Hook. & Arn.	Argentina	Limonene, caryophyllene oxide, and β-caryophyllene [30].
A. tenuifolia Spreng.	Mauritius	α-Thujone and sabinene [24].
A. tenuifolia Spreng.	Argentina	Germacrene D and bicyclogermacrene [30].
A. trifida L.	Northeast China (Shenyang)	Bornyl acetate, borneol, caryophyllene oxide, α-pinene, and β-caryophyllene [28].

2. Materials and methods

2.1 Plant Materials

Flowering aerial parts of *A. cumanensis* (Asteraceae) were collected in June-September, 2014, in the locality of San Rafael, Montes de Oca, Province of San José (9º56'37.00''N, 84º01'01.80''O), at an altitude of 1335 m. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 77483).

2.2 Isolation of the essential oils

The oils were isolated by hydrodistillation at atmospheric pressure, for 3 h using a Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulfate, filtered and stored at 0-10 °C in the dark, until further analysis. The yield of the pale yellowish oil was 0.19 % (v/w) in fresh plant material and 0.22% (v/w) from dried sample.

2.3 Gas chromatography (GC-FID)

The oils of *Ambrosia cumanensis* were analyzed by GC-FID (gas chromatography with flame ionization detector) using a Shimadzu GC-2014 gas chromatograph. The data were obtained on a 5% phenyl-/95% dimethylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm), (MDN-5S, Supelco), with a Lab Solutions, Shimadzu GC Solution, Chromatography Data System, software version 2.3. Operating conditions were: carrier gas N_2, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min, 280 °C (2 min); sample injection port temperature 250 °C; detector temperature 280 °C; split 1:60.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The analyses by gas chromatography coupled to mass selective detector were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST computer databases. The data were obtained on a 5% phenyl-/95% dimethylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm), (MDN-5S). Operating conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min; sample injection port temperature 250 °C; detector temperature 260 °C; ionization voltage: 70 eV; ionization current 60 µA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

2.5 Compound identification

Identification of the components of the oils were performed using the retention indices which were calculated in relation to a homologous series of *n*-alkanes, on a 5% phenyl-/95% dimethylpolysiloxane type column [34], and by comparison of their mass spectra with those published in the literature [35] or those of our own database. To obtain the retention indices for each peak, 0.1 µL of *n*-alkane mixture (Sigma, C_8-C_{32} standard mixture) was injected under the same experimental conditions reported above. Integration of the total chromatogram (GC-FID), expressed as area percent, has been used to obtain quantitative compositional data.

3. Results and Discussion

From the hydrodistilled oils, a total of 137 compounds were identified by means of GC-FID and GC-MS techniques, accounting for 89.3-92.9% of the total composition of the essential oils. The compounds identified in the oils of *A. cumanensis* are presented in Table 2, where they are listed in order of elution on a MDN-5S column. Table 2 also includes the relative percentages of single components; their experimental retention indices (RI) with reference to a

homologous series of linear alkanes (C_8-C_{32}) and for comparison purposes, previously published values are also included (Lit. RI). *Ambrosia cumanensis* gave oils which were predominantly terpenoid in nature (mostly sesquiterpenoids) with several aliphatic and aromatic compounds as minor and trace constituents. In Table 3**,** the percentages of the various classes of constituents of the oils are indicated. The oils contained 55 monoterpenoids with α-pinene (7.8-12.8%), chrysanthenone (6.2-8.7%), β-pinene (4.5-6.7%), limonene (3.5-5.8%), and filifolone (2.9-4.0%) as major constituents. Other monoterpenoids were not particularly abundant, with the most prominent members being *iso*-chrysanthenone (1.3-1.7%), *trans*-verbenol (0.6-1.1%), (*E*)-β-ocimene (0.2-1.1%), sabinene (0.5-0.8%), and *cis*-chrysanthenol (0.4-0.6%). The majority of monoterpenoids that constitutes the oil presented the monocyclic *p*-menthane (4.5-7.1%) and the bicyclic pinane (19.8-28.2%) carbon skeletons. Besides the α- and β-pinenes, that are widespread compounds in *Ambrosia*, there were present chrysanthenone, *cis*-chrysanthenol, *trans*- (and *cis*-) verbenols, verbenone, myrtenol, myrtenal, *cis*-pinocamphone, and the rearranged compounds *iso*-chrysanthenone and filifolone that appear to be of restricted occurrence in this species. In addition to monoterpenoids previously mentioned, the composition of the oil of *A. cumanensis* was dominated by the sesquiterpenes bicyclogermacrene (14.7-23.4%) and germacrene D (10.1-16.9%). These compounds were accompanied by lesser amounts of β-cubebene (2.3-3.0%), β-caryophyllene (2.2-2.5%), β-bisabolene (0.5-2.1%), α-humulene (1.2-1.5%), δ-amorphene (0.8-1.1%), and muurola-4,10(14)-dien-1-β-ol (0.4-1.3%). The remaining 52 sesquiterpenoids were present only in small amounts or at the trace level. Our findings corroborate the presence of 11 compounds previously reported [21] (indicated by asterisk* in Table 2), whereas 126 are newly reported in the composition of the oil of *A. cumanensis*. The oil composition differs from the previously reported oil obtained from leaves of plants cultured from seeds in a greenhouse at the University of Illinois (Urbana, USA) originally collected from natural populations in San Luis Potosí and Veracruz (Mexico). They were characterized by the occurrence of caryophyllene (20.4-36.2%) and 'farnesene isomer' (22.3 to 33.8%) as main constituents.

Taking together our results and previously reported data from diverse *Ambrosia* species (see Table 1) it can be noted that the major components are terpenoids but the identity of the compounds varied greatly. Within the same species, there are qualitative and quantitative differences that could be caused by environmental factors or by the phenological phase of the plant, as it was suggested by the work of Pajević *et al*. [31] on *A. artemisiifolia*, wherein quantitative differences of biogenic volatile organic compounds in leaves, using headspace methodology, were observed by variation of the experimental condition of soil moisture. Furthermore, it is known that *Ambrosia* is a very diverse and complex genus of Asteraceae and, therefore, its constituent species have had problems in terms of taxonomic status because most of them present a high degree of morphological and chemical variability determined largely by natural environment [12, 42].

4. Conclusions

The aerial parts of *Ambrosia cumanensis* growing in Costa Rica produce a terpenoid-rich essential oil whose composition was dominated by bicyclogermacrene (14.7-23.4%), germacrene D (10.1-16.9%), α-pinene (7.8-12.8%), β-pinene (4.5-6.7%), chrysanthenone (6.2-8.7%), limonene (3.5-5.8%), and filifolone (2.9-4.0%). The major components appear to be different from those of samples studied a long time ago, originated from seeds collected in Mexico and cultured in Illinois, USA. Like other members of the Asteraceae family, the differences could be due to the occurrence of chemotypes.

Table 2: Chemical and percentage composition of *Ambrosia cumanensis* essential oil from Costa Rica.

Compounda	RIb	Lit. RIc	Class	Sample 1 (fresh) %	Sample 2 (fresh)%	Sample 3 (dried) %	Identification method
(*Z*)-Hex-3-enal	795	797	A	-	-	t	1,2
(*E*)-Hex-2-enal	846	846	A	t	t	0.2	1,2
(*Z*)-Hex-3-en-1-ol	851	850	A	0.4	-	-	1,2
(*Z*)-Hex-2-en-1-ol	858	859	A	t	t	-	1,2
Hexan-1-ol	863	863	A	0.1	0.1	t	1,2
Heptan-2-one	886	889	A	-	t	-	1,2
(2*E*,4*E*)-Hexa-2,4-dienal	888	907	A	-	0.1	-	1,2
Heptanal	900	901	A	-	t	t	1,2,3
(*Z*)-Hex-3-enyl formate	914	913	A	-	t	-	1,2
Tricyclene	921	921	M	t	t	t	1,2
α-Thujene	925	924	M	0.1	0.1	t	1,2
α-Pinene*	934	932	M	7.8	11.4	12.8	1,2,3
α-Fenchene	944	945	M	0.1	t	t	1,2
Camphene*	950	946	M	-	0.1	t	1,2
Thuja-2,4(10)-diene	953	953	M	-	t	t	1,2
Sabinene*	973	969	M	0.6	0.8	0.5	1,2
β-Pinene*	975	974	M	4.5	5.3	6.7	1,2,3
Octan-3-one	980	979	A	-	t	-	1,2
6-Methyl-5-hepten-2-one	980	981	A	-	-	t	1,2
Myrcene*	987	988	M	0.3	0.1	0.2	1,2
2-Pentylfuran	988	984	Misc	-	-	0.4	1,2
Mesytilene	998	994	B	-	0.1	t	1,2
Octanal	998	998	A	t	-	0.1	1,2
α-Terpinene*	1018	1014	M	t	t	0.1	1,2
1,2,4-Trimethylbenzene (=*pseudo*-cumene)	1020	1021	B	-	-	t	1,2
p-Cymene	1022	1020	M	-	0.1	t	1,2

Limonene*	1024	1024	M	5.4	5.8	3.5	1,2,3
β-Phellandrene*	1026	1025	M	-	-	t	1,2
(*Z*)-β-Ocimene	1030	1032	M	t	t	t	1,2
(*E*)-β-Ocimene*	1043	1044	M	1.1	0.4	0.2	1,2
γ-Terpinene	1054	1054	M	0.1	t	0.1	1,2
cis-Sabinene hydrate	1070	1065	M	0.1	t	0.1	1,2
p-Mentha-2,4(8)-diene	1084	1085	M	0.1	-	-	1,2
Terpinolene	1083	1086	M	-	0.2	0.1	1,2
p-Cymenene	1088	1089	M	t	-	t	1,2
Linalool	1100	1095	M	t	-	t	1,2,3
Filifolone	1100	1103 [36]	M	2.9	4.0	3.0	1,2
iso-Chrysanthenone	1103	1106 [37]	M	1.3	1.4	1.7	1,2
Nonanal	1104	1100	A	-	t	-	1,2
Perillene	1109	1102	Misc	0.1	0.1	t	1,2
trans-Thujone	1117	1112	M	-	0.2	-	1,2
Chrysanthenone	1122	1124	M	6.2	8.7	6.7	1,2
α-Campholenal	1122	1122	M	t	t	t	1,2
cis-Limonene oxide	1134	1132	M	0.1	0.1	t	1,2
cis-Verbenol	1136	1137	M	0.1	-	0.1	1,2
trans-Verbenol	1139	1140	M	0.6	0.6	1.1	1,2
(*E*)-Myroxide	1139	1140	M	-	t	-	1,2
p-Menth-3-en-8-ol	1142	1145	M	-	0.2	-	1,2
cis-Chrysanthenol	1161	1160	M	0.4	0.6	0.6	1,2
Borneol	1165	1165	M	0.1	0.1	-	1,2
p-Mentha-1,5-dien-8-ol	1169	1166	M	t	-	0.1	1,2
cis-Pinocamphone	1173	1172	M	t	t	t	1,2
Terpinen-4-ol	1178	1174	M	0.1	0.2	0.3	1,2,3
Cryptone	1183	1183	M	-	t	-	1,2
α-Terpineol	1191	1198 [38]	M	t	0.2	t	1,2
Myrtenol	1195	1194	M	0.1	0.3	0.2	1,2
Myrtenal	1196	1196	M	-	0.1	-	1,2
cis-Piperitol	1196	1195	M	t	-	t	1,2
Verbenone	1207	1204	M	0.1	0.1	t	1,2
trans-Piperitol	1210	1207	M	t	-	t	1,2
trans-Carveol	1215	1215	M	-	-	0.2	1,2
cis-Carveol	1224	1226	M	-	t	-	1,2
Methyl α-campholenate	1240	1232 [39]	M	0.2	0.3	0.3	1,2
Carvone	1242	1239	M	-	0.1	t	1,2,3
Piperitone	1249	1249	M	-	-	0.1	1,2
cis-Myrtanol	1252	1250	M	-	t	-	1,2
cis-Chrysanthenyl acetate	1255	1261	M	-	t	-	1,2
Perilla aldehyde	1273	1269	M	-	-	t	1,2
Isopiperitenone	1270	1272 [40]	M	0.1	0.2	0.1	1,2
Thymol	1290	1290	M	0.1	-	-	1,2
Silphiperfol-5-ene	1324	1326	S	0.1	-	-	1,2
δ-Elemene	1331	1335	S	0.6	0.5	0.4	1,2
α-Terpinyl acetate	1344	1346	M	t	t	-	1,2
Piperitenone	1340	1340	M	-	-	t	1,2
Silphinene	1345	1345	S	0.3	0.7	0.4	1,2
α-Cubebene	1345	1345	S	t	-	t	1,2
Eugenol	1354	1356	PP	t	t	0.1	1,2
Cyclosativene	1366	1369	S	0.1	0.1	0.1	1,2
α-Ylangene	1372	1373	S	t	t	-	1,2
α-Copaene	1374	1374	S	0.5	0.5	0.5	1,2
Daucene	1376	1380	S	t	t	0.1	1,2
Modheph-2-ene	1377	1382	S	0.8	1.0	-	1,2
α-Isocomene	1387	1387	S	-	-	t	1,2
β-Patchoulene	1377	1379	S	-	-	0.7	1,2
β-Bourbonene	1385	1387	S	t	0.1	t	1,2
β-Cubebene	1387	1387	S	2.6	3.0	2.3	1,2
β-Elemene	1389	1389	S	-	-	t	1,2
α-Gurjunene	1406	1409	S	0.7	0.6	0.6	1,2
β-Isocomene	1409	1407	S	0.5	0.5	0.5	1,2
α-Cedrene	1411	1410	S	t	t	t	1,2
β-Caryophyllene*	1418	1417	S	2.4	2.5	2.2	1,2,3
β-Cedrene	1423	1419	S	t	0.1	0.1	1,2
β-Copaene	1429	1430	S	0.1	0.1	0.5	1,2
α-*trans*-Bergamotene	1431	1432	S	0.6	0.5	0.5	1,2
Aromadendrene	1437	1439	S	t	0.1	0.2	1,2

(*Z*)-β-Farnesene	1439	1440	S	0.2	0.1	0.1	1,2
α-Humulene*	1453	1452	S	1.4	1.2	1.5	1,2,3
(*E*)-β-Farnesene	1454	1454	S	t	t	t	1,2
cis-Cadina-1(6),4-diene	1460	1461	S	t	-	t	1,2
α-Acoradiene	1464	1464	S	t	t	0.2	1,2
9-*epi*-(*E*)-Caryophyllene	1465	1464	S	-	0.2	-	1,2
γ-Gurjunene	1472	1475	S	0.9	0.8	0.9	1,2,3
Germacrene D	1483	1484	S	16.9	10.1	14.8	1,2
trans-Muurola-4(14),5-diene	1490	1493	S	-	-	t	1,2
Bicyclogermacrene	1498	1500	S	23.4	14.7	18.1	1,2
(*E*,*E*)-α-Farnesene	1501	1505	S	t	0.1	0.5	1,2
β-Bisabolene	1506	1505	S	0.5	0.5	2.1	1,2
δ-Amorphene	1514	1514	S	1.1	0.8	-	1,2
δ-Cadinene	1517	1522	S	-	-	1.0	1,2
β-Sesquiphellandrene	1522	1521	S	0.5	0.2	0.6	1,2
Dauca-4(11),8-diene	1531	1530	S	0.1	t	t	1,2
trans-Cadina-1,4-diene	1532	1533	S	-	-	t	1,2
α-Cadinene	1537	1537	S	0.1	0.1	0.2	1,2
(*E*)-Nerolidol	1562	1561	S	0.1	0.6	-	1,2
Palustrol	1569	1567	S	0.1	0.2	1.0	1,2
Spathulenol	1576	1577	S	-	3.4	1.0	1,2
Caryophyllene oxide	1583	1582	S	-	0.5	-	1,2
Globulol	1591	1590	S	0.3	-	-	1,2
Salvial-4(14)-en-1-one	1591	1594	S	t	0.3	t	1,2
Viridiflorol	1592	1592	S	t	t	0.4	1,2
Carotol	1600	1599	S	0.9	0.8	-	1,2
Rosifoliol	1607	1600	S	t	-	-	1,2
1,10-di-*epi*-Cubenol	1621	1618	S	-	0.2	-	1,2
Junenol	1622	1618	S	0.7	0.7	-	1,2
1-*epi*-Cubenol	1627	1627	S	-	-	t	1,2
Muurola-4,10(14)-dien-1-β-ol	1631	1630	S	1.3	0.4	-	1,2
epi-α-Cadinol (T-cadinol)	1638	1638	S	0.2	-	0.5	1,2
epi-α-Muurolol (T-muurolol)	1643	1640	S	t	-	t	1,2
Cubenol	1643	1645	S	-	0.1	-	1,2
Desmethoxy encecalin	1649	1646	B	-	-	t	1,2
α-Cadinol	1655	1652	S	0.3	0.1	0.5	1,2
Selin-11-en-4-α-ol	1657	1658	S	0.1	0.1	0.5	1,2
Germacra-4(15),5,10(14)-triene-1-α-ol	1684	1685	S	-	-	0.3	1,2
Pentadecanal	1715	1715	A	0.1	0.3	-	1,2
Mint Sulfide	1739	1740	S	t	0.3	-	1,2
2-Heptadecanone	1902	1902 [41]	A	t	-	-	1,2
(*E*)-Phytol	1942	1942	D	0.1	0.1	-	1,2
Total identified				91.8	89.3	92.9	
Number of compounds				94	101	100	

[a]Compounds listed in order of elution from 5% phenyl-/95% dimethylpolysiloxane type column.
[b]RI = Retention index relative to C_8-C_{32} *n*-alkanes on the 5% phenyl-/95% dimethylpolysiloxane type column. [c]Lit. RI= J&W DB-5 [35]. [d]Method: 1 = Retention index on 5% phenyl-/95% dimethylpolysiloxane type column; 2 = MS spectra; 3 = Standard. t: Traces (<0.05%). En dash (-): not detected. * Compounds previously identified [21].

Table 3: The chemical class distribution in the essential oil of *Ambrosia cumanensis* from Costa Rica

	Leaves fresh 1		**Leaves fresh 2**		**Leaves dried**	
	%	NC	%	NC	%	NC
Aliphatics (A)	0.6	7	0.5	10	0.3	6
Alcohols	0.5	3	0.1	2	t	1
Aldehydes	0.1	3	0.4	5	0.3	4
Ketones	t	1	t	2	t	1
Esters	-	-	-	1	-	-
Terpenoids	91.1	86	88.6	88	92.1	88
Monoterpenoids (M)	32.6	37	41.7	42	38.8	42
Monoterpene hydrocarbons	20.1	15	24.3	16	24.2	18
Oxygenated monoterpenes	12.5	23	17.4	26	14.6	25
Sesquiterpenoids (S)	58.4	47	46.8	45	53.3	45
Sesquiterpene hydrocarbons	54.4	33	39.1	31	49.1	35
Oxygenated sesquiterpenes	4.0	13	7.4	13	4.2	10
Sulfur sesquiterpenes	t	1	0.3	1	-	-
Diterpenoids (D)	0.1	1	0.1	1	-	-

Aromatics	t	1	0.1	2	0.1	4
Benzenoids (B)	-	-	0.1	1	t	3
Phenylpropanoids (PP)	t	1	t	1	0.1	1
Miscellaneus (Misc)	0.1	1	0.1	1	0.4	2
Total	91.8	94	89.3	101	92.9	100

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