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Distribution of (-)-Hamanasic Acid A in South American Species of *Flourensia* and Phytotoxic Effects of Leaf Aqueous Extracts

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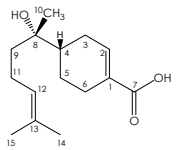
The presence of the phytotoxic sesquiterpene (-)-hamanasic acid A {(-)HAA; 7-carboxy-8-hydroxy-1(2), 12(13)-dien-bisabolene} isolated from *Flourensia campestris* (*FC*), was investigated in the South American species of the genus, together with the evaluation of the phytotoxic activity of their leaf aqueous extracts. (-)HAA was identified and isolated from *F. fiebrigii* (*FF*) and *F. oolepis* (*FO*), being chemically (GC-MS, NMR, $[\alpha]_D$) and biologically (bioassayed on lettuce) indistinguishable from that of *FC*, while no (-)HAA was found in *F. hirta* (*FH*), *F. riparia* (*FR*) and *F. niederleinii* (*FN*). Its leaf content in *FF* was similar to that found in *FC* (ca. 15 mg g^{-1} WT) and significantly higher than in *FO* (0.8 mg g^{-1} WT). The screening for the presence of (-)HAA in other species of *Flourensia* communities showed that its natural occurrence is restricted only to *Flourensia* species. No (-)HAA could be detected in any of the 37 -most representative- species of these communities (26 natives, 11 exotics), despite many of them belong to the same family and tribe as *Flourensia* spp. Leaf aqueous extracts of all *Flourensia* species exhibited strong inhibitory effects on lettuce germination and on root and shoot growth, regardless of the presence and content of (-)HAA. These results strongly suggest the existence of other powerful phytotoxic compounds in those *Flourensia* spp lacking (-)HAA. Our results clearly show that (-)HAA only pertains to some species of the genus *Flourensia*. Relative to previous exomorphologic groupings of the genus, our chemotaxonomic data would give support to the close link described between *FC* and *FF*, but not with *FR*. In addition, the fact that (-)HAA was also found in *FO*, which belongs to a second different line, also points out that species position in this lineage would deserve to be revisited. The restricted production of (-)HAA by *Flourensia* in their communities suggests its special link with the genus, and sustains its putative allelochemical role.

Keywords: *Flourensia*, (-)-Hamanasic acid A, Phytotoxic activity, Sesquiterpenes, Chemotaxonomy.

The genus *Flourensia* (Family Asteraceae; Subfamily Asteroideae; Tribe Heliantheae; Subtribe Enceliinae) comprises 25 species distributed throughout America, from which 18 are found in South America, and 12 in Argentina [1-3]. As part of our ongoing studies on the adaptive ecophysiological traits of native species of Argentina [4-6], we found that *F. campestris* Griseb. exhibited strong phytotoxic effects [7]. This species, endemic to the inland central region of the country, produces elevated amounts of a phytotoxic compound identified as (-)-hamanasic acid A (7-carboxy-8-hydroxy-1(2), 12(13)-dien-bisabolene {(-)HAA}), a new levorotatory form of the (+)-sesquiterpene previously described by Hashidoko *et al*. [8] in *Rosa rugosa* leaves (Rosaceae). At present, (-)HAA is only available by extraction as its chemical synthesis is still lacking. In bioassays using *Lactuca sativa* as a test system, (-)HAA drastically inhibited seed germination (by 90%), and completely arrested root and shoot growth at the higher concentrations tested (ca. 4 mM). This bisabolanoid is accumulated on plant surfaces in exceptionally high concentrations (1.6% of total leaf biomass), and is easily leached out by water from fresh and dry tissues suggesting a putative allelochemical role. Other *Flourensia* species have also been found to synthesize mixtures of phytotoxic compounds, where sesquiterpenes are always present, as was revealed in *F. cernua* [9], in *FC* -namely (-)HAA [7]-, and more recently in *F. oolepis* [10]. These findings pose a number of questions regarding the distribution and specificity of (-)HAA in South American species of the genus and the potential phytotoxic activity of their leaf aqueous extracts.

Prenylflavonoids and benzofuran derivatives [11,12,13a,b], together with similar surface deposited and volatile mono and sesquiterpenes [7,14-16] have been characterized in *Flourensia* species. Even though some of these molecules and structural types are repeatedly found among species, a clear terpene pattern implying chemosystematic correlations could not be detected. In this sense, the study of the sesquiterpene (-)HAA as an (-)-α-bisabolol derivative in *Flourensia* spp., and in coexisting species, may bring additional chemotaxonomical insights, as well as valuable information about its ecophysiological significance for the genus.

In the present work we addressed the following questions: a) is (-) HAA widely distributed among South American species of *Flourensia*? b) is (-)HAA production genus specific? c) are the phytotoxic effects of leaf aqueous extracts a common trait in *Flourensia* species? d) is phytotoxicity related to the amount of (-)HAA present?



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(-)HAA with numbering and absolute configuration.

The occurrence of (-)HAA was screened in *F. oolepis* (*FO*), which grows in the same biome as *FC,* but in different communities, and four other South American *Flourensia* species distantly distributed: *F. hirta* (*FH*), *F. riparia* (*FR*)*, F. niederleinii* (*FN*) and *F. fiebrigii* (*FF*), which together with *FC* comprises 50% of the total taxa described in Argentina [17]. The detection and quantification of (-)HAA were accomplished through a screening 2D-TLC technique and GC-FID, as described previously [7]. The identity of the compounds was confirmed through their isolation (LC), and further chemical (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMBC, HSQC and $[\alpha]_D$), and biological (bioassay on lettuce seeds) characterizations. Bioassays of leaf aqueous extracts from *Flourensia* species were performed on lettuce seeds [7]. The role of (-)HAA is discussed in relation to its value as a taxonomical character, as key to *Flourensia* phytotoxicity and the ecophysiological significance for the genus. In *Flourensia* species, the (-)HAA moiety was first revealed by 2D-TLC in *FO* and *FF*, and was absent in *FH*, *FN* and *FR*.

Taking into account the existence of the enantiomer (+)HAA already described in Rosaceae [8], and the possibility of other similar sesquiterpenes with similar chromatographic behavior, the identified compounds were isolated and chemically and biologically characterized, and results were compared with those from *FC* (-)HAA. *FF* was primarily selected owing to its higher concentration. The compound from *FF* was isolated in a similar yield to that from *FC* (*ca.* 3% vs. 4% DW basis, respectively). Obtained as a colorless syrup, the ^{1}H, ^{13}C, and ^{1}H-^{1}H COSY NMR spectroscopic data were the same as those previously described for *FC* (-)HAA (7-carboxy-8-hydroxy-1(2),12(13)-dien-bisabolene; [7]), and consistent with those for its enantiomer (+)-hamanasic acid A [8]. The rotational value, $[\alpha]_D$ - 64.9, was technically the same as that of the bioactive sesquiterpene found in *FC* ($[\alpha]_D$ - 63.0; [7]), in contrast to the $[\alpha]_D$ - 94.4 value that was documented previously as preliminary data [18]. The isolated (-)HAA from *FF* was also biologically characterized through bioassays on lettuce, giving ECg_{50}=4.8±1.0 mM, ECr_{50}=2.8±0.6 mM and ECs_{50}=3.6±0.9 mM. These results were not significantly different from those exerted by *FC* (-)HAA in the same experimental test system [7]. Overall results showed that *FF* (-)HAA was chemically and biologically indistinguishable from *FC* (-)HAA, discarding possible chromatographic misidentifications of optical isomers and of other related sesquiterpenes. Very similar results were obtained for *FO* (data not shown).

As regards to the genus, the bioactive sesquiterpene failed to serve as a chemotaxonomic marker, as it was only detected in three of the six *Flourensia* species investigated; 50 % of those described in our country. Dillon´s work [17] is the only comprehensive revision of the genus *Flourensia* and has not been revisited until now. He based the grouping of *Flourensia* species primarily on the comparison of exomorphic features and partial phytochemical data (Figure 2). In his phylogram, South American taxa are divided into three weakly differentiated lines; whilst our studied species are present in two of them. One of these lines is integrated by eight species geographically distributed from southern Bolivia to southern Argentina: *FR, FC, F. leptopoda, FN, FH, FF,* and *F. blakeana;* all species exhibit a trend toward fewer ray florets, with the majority possessing 5-8 ray florets. This lineage tends to occupy more arid environments at lower elevations. *FR* seems basal within the line. The other lineage, composed of *Flourensia suffrutescens, F. tortuosa, F. macroligulata, F. heterolepis* and *FO* is characterized by large capitula with broad phyllaries and 13-21 ray florets. The occurrence of prenylflavonoids was observed in at least four species: *F. heterolepis* [11], *FC, FR* [13a], and *FF* [13b], suggesting a chemotaxonomical relationship. *FC* was found to resemble *FR* in chemical constituents like benzofuranes and flavonoids. However, sesquiterpene lactones of the eudesmanolide type {(E,E)-farnesyl cation} identified in *FR* were not found in *FC* [13a]. The fact that (-)HAA, as a bisabolene derivative {(E,Z)-farnesyl cation}, is present in *FC*, but not in *FR* indicates the activation of different biosynthetic pathways of sesquiterpenes in both species.

Our data would give support to the close link between *FC* and *FF*, but this was not the case with other members of this line, namely *FR, FH* and *FN*. In addition, (-)HAA was also found in *FO,* which integrates a different lineage, posing the question as to whether *FO* should be included in this line. Future phytochemical studies comprising the screening of (-)HAA in the vast majority of species within the genus will certainly provide new insights for a revision of species composition within lineages.

Previous work demonstrated that in *FC*, the specific phytotoxic activity detected in the bioassays performed with the aqueous extracts was 3-fold higher than that observed with the isolated (-)HAA [7]. The difference in bioactivity observed could not be explained on the basis of (-)HAA concentration (alone). Taking into account that recovery of (-)HAA through EtOAc extraction from the aqueous phase was very efficient (more than 95%), the higher phytotoxicity of the aqueous extracts could be explained by the presence of other phytotoxins which have not been extracted with EtOAc (the phase used for bioassay-guided fractionation) and/or the action of natural detergents that would raise the solubility of (-)HAA, and thus its bioactivity. In order to ascertain the relationship between the presence of (-)HAA and the possible phytotoxic effects of the different *Flourensia* species, bioassays of leaf aqueous extracts from (-)HAA containing (*FO* and *FF*) and lacking (*FR*) *Flourensia* species were investigated, and compared with the corresponding content of (-)HAA detected in each species.

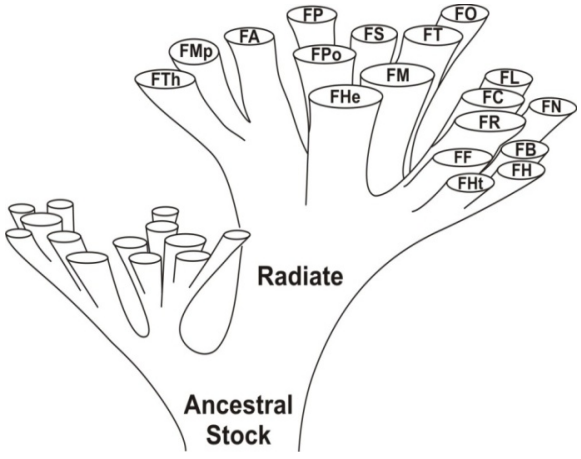

Figure 2: Dillon **[**17**]** phylogram of *Flourensia* species.

An enlarged version of the South American lines of *Flourensia* species relationships is shown. Key to abbreviations: FA= *F. angustifolia*; FB= *F. blakeana*; FC= *F. campestris*; FF= *F. fiebrigii*; FH= *F. hirta*; FHe= *F. heterolepis*; FHt= *F. hirtissima*; FL= *F. leptopoda*; FM= *F. macroligulata*; FMp= *F. macrophylla*; FN= *F. niederleinii*; FO= *F. oolepis*; FP= *F. peruviana*; FPo= *F. polycephala*; FR= *F. riparia*; FS= *F. suffrutescens*; FT= *F. tortuosa*; FTh= *F. thurifera.*

Table 1: (-)HAA leaf content and biological activity of aqueous extracts from *Flourensia* species.

Flourensia species	Leaf (-)HAA content (mg g^{-1} DW)	Phytotoxicity of leaf aqueous extracts (% w/v) EC_{50}	ECr_{50}	ECs_{50}
FC	16.0 ± 0.7 **	3.1 ± 0.4 $^{a\,*}$	1.4 ± 0.1 $^{b\,***}$	2.2 ± 0.1 $^{a\,**}$
FO	0.80 ± 0.01 *	4.9 ± 0.1 $^{a\,**}$	4.0 ± 0.1 $^{b\,***}$	4.3 ± 0.2 $^{b\,***}$
FF	15.2 ± 0.7 **	4.6 ± 0.1 $^{a\,**}$	2.2 ± 0.4 $^{b\,**}$	1.9 ± 0.3 $^{b\,*\,**}$
FR	Not detected	2.5 ± 0.1 $^{a\,*}$	1.0 ± 0.02 $^{b\,*}$	1.3 ± 0.3 $^{b\,*}$

Data are expressed as mean ± se of triplicates {(-)HAA content}, and from dose-response curves (EC_{50}) of two different bioassays with three replicates for each concentration (aqueous extracts). Different letters (**a, b, c**) and symbols (*, **, ***) indicate significant differences between phytotoxic effects and species, respectively (p<0.05).

As far as we reviewed, the herbicidal bioactivity of leaf aqueous extracts of *FO*, *FF* and *FR* on lettuce had never been assessed before. The concentration of (-)HAA (mg g^{-1} DW) in the leaves of *FF* was similar to that in *FC*, and significantly higher than that found in *FO* (Table 1). As shown in Table 1, all the leaf aqueous extracts from the *Flourensia* species tested showed bioactivity at CE_{50} below 5% (g of leaves per 100 mL of water). These values fall within the range found in species with potential allelopathic activity [19, 20], as compared with other species whose leaf aqueous extracts would not exhibit bioactivity on lettuce even at concentrations as high as 10 or 20% [21, 22]. *FC* and *FR* showed similar inhibitory effects on germination, and were significantly higher compared with both *FO* and *FF* (p<0.05, Table 1). The phytotoxic effects of leaf extracts on germination among (-)HAA containing species (*FC*, *FO* and *FF*) was not consistent with their respective (-)HAA contents, confirming the presence of other phytotoxic compounds in the case of *FO*, as was already described [10]. Instead, the response of root and shoot growth were shown to be correlated with the (-)HAA concentration in these species; ECr_{50} and ECs_{50} were ca. 2x lower in *FC* and *FF* compared with *FO* (Table 1). Leaf extracts of *FR* also exerted a drastic inhibition of root and shoot growth, similar to that of *FC* and *FF*. Root growth was the most sensitive parameter affected by the leaf extracts of most of the tested species (*FC*, *FF* and *FR*), a result that is commonly reported in many studies dealing with allelochemicals [23, 24]. The unexpected strong phytotoxic effects of leaf aqueous extracts found in *FR*, a non (-)HAA containing species, clearly indicate the presence of powerful bioactive compounds which deserves further elucidation.

The natural occurrence of the bioactive compound {(-)HAA moiety} was restricted to *Flourensia* species in their communities, being absent in all the 37 *FC* and *FO* coexisting species tested (26 natives and 11 exotics, Table 2). From the 15 Asteraceae species studied, 6 belonged to Heliantheae, which correspond to the same tribe as *Flourensia* (Table 2). Despite similar C-7, C-8-dioxygenated bisabolanes having been isolated from other Asteraceae [24, 25], our results show that *Flourensia* was the only (-)HAA producing Asteraceae in these communities. The compound was not found in the three coexisting Rosaceae studied either, a family where its enantiomer (-)HAA had been firstly described by Hashidoko *et al*. [8]. Moreover, a screening performed in other domestic Rosaceae *(Spiraea cantoniensis* Lour., *Malus pumila* Mill., *Prunus persica* (L.) Stokes, and *Cydonia oblonga* Mill.) also failed to detect (-)HAA in any of these species (data not shown). It is puzzling how the two different HAA enantiomeric forms were found in phylogenetically distant plants such as those belonging to Asteraceae and Rosaceae. This aspect highlights the complexity of the sesquiterpene biochemical pathways characteristic of plants.

The possibility that (-)HAA may play a role as an alellopathic agent involved in plant-plant interactions within the communities

Table 2: *FC* and *FO* community coexisting species.

Species	Family
Natives	
Lithraea molleoides (Vell.) Engl.	Anacardiaceae
Acanthostyles buniifolium (Hook and Arn.) King and Rob	Asteraceae
Achyrocline satureioides (Lam.) DC.	Asteraceae
Ambrosia tenuifolia Spreng.	Asteraceae, H
Baccharis articulata (Lam.) Pers.	Asteraceae
Bidens pilosa L.	Asteraceae
Eupatorium viscidum Hook.et Arn.	Asteraceae
Heterothalamus alienus (Spreng.) Kuntze	Asteraceae
Parthenium hysterophorus L.	Asteraceae, H
Viguiera tucumanensis (Hook. Et Arn.) Griseb.	Asteraceae, H
Vernonanthura nudiflora (Less.) H.Rob. f. *nudiflora*	Asteraceae
Thelesperma megapotamicum (Spreng.) Kuntze	Asteraceae
Xanthium spinosum L. var. *spinosum*	Asteraceae, H
Zinnia peruviana (L.) L.	Asteraceae, H
Croton lachnostachyus Baill	Euphorbiaceae
Acacia caven (Mol.) Molina	Fabaceae
Apurimacia dolichocarpa (Gris.) Burkart	Fabaceae
Collaea argentina Griseb.	Fabaceae
Sophora linearifolia Griseb.	Fabaceae
Colletia spinosissima J. F. Gmel.	Rhamnaceae
Condalia microphylla Cav.	Rhamnaceae
Kageneckia lanceolata Ruiz and Pav.	Rosaceae
Cestrum parqui L´ Hér.	Solanaceae
Celtis ehrenbergiana (Klotzch) Liebm.	Ulmaceae
Aloysia gratissima (Gillies and Hook. Ex Hook.) Tronc.	Verbenaceae
Larrea divaricata Cav.	Zygophyllaceae
Exotics	
Carduus acanthoides L.	Asteraceae
Xanthium cavanillesii Schouw.	Asteraceae, H
Gleditsia triacanthos L.	Leguminosae
Robinia pseudoacacia L.	Leguminosae
Melia azedarach L.	Meliaceae
Ligustrum lucidum W.T. Aiton	Oleaceae
Ligustrum sinense Lour.	Oleaceae
Pinus taeda L.	Pinaceae
Cotoneaster franchetii Bois	Rosaceae
Pyracantha atalantioides (Hance) Stapf	Rosaceae
Ulmus pumila L.	Ulmaceae

List of 37 coexisting species studied from local *Flourensia* communities H, Heliantheae tribe.

investigated is partially supported by previous results highlighting the high amount of (-)HAA accumulated on leaves surface and the feasibility of being extracted with water [7], and reinforced by the present results showing that the compound is solely produced by *Flourensia* species. However, in order to confirm this hypothesis further investigations should be carried out in order to test species´ response to (-)HAA at the community level.

As concluding remarks, present work showed that (-)HAA originally isolated from *FC* was also identified in *FF* and *FO*, and was absent in other South American species of the genus, like *FR*, *FH* and *FN*. The isolated compounds were chemically and biologically characterized as (-)HAA, discarding the possibility of its isomer (+)HAA or other related sesquiterpenes with similar chromatographic behavior. In local communities, the sesquiterpene was not widely distributed, but restricted to the species of the genus *Flourensia*. According to previous genus exomorphologic groupings, our chemotaxonomic data support the close link between *FC* and *FF*, but not with *FR*. The phytotoxic effects of leaf aqueous extracts observed seem to be a common trait in this genus, and were not restricted to (-)HAA containing *Flourensia* species. This was the case of *FR* where the presence of other phytotoxic compounds is under investigation. Taken together with previous findings, the restricted production of (-)HAA by *Flourensia* in their communities confirms a specific link with the genus, and sustains its putative allelochemical role in the communities studied.

Experimental

Plant material: Air dried leaves of adult plants from natural areas were used. Endemics *Flourensia campestris* Griseb. and *F. oolepis* S.F. Blake were harvested in Punilla Valley (700-800 MASL),

Córdoba province, Central Argentina. Another four *Flourensia* species were collected within the geographic range of distribution of each species. *F. hirta* S.F. Blake (endemic), was collected in La Rioja province (1640 MASL), where it grows as a companion species in different bush-dominated communities (ca. 400 km northwest from Punilla Valley). *F. niederleinii* S.F. Blake (endemic) was also collected in La Rioja, on the slopes of Sierras de Velazco (1543 MASL), from almost pure stands. *F. riparia* Griseb (endemic) and *F. fiebrigii* S.F. Blake were both collected in the province of Salta (ca. 900 km north from Punilla Valley, 1000 MASL and 3000 MASL; respectively).

In order to evaluate the specificity of (-)HAA in the genus, the compound was screened in local *Flourensia* communities. The most conspicuous native species found growing together with *FC* and *FO* in their natural shrub dominated environments [26, 27], as well as exotic species that invade the same sites were collected (Table 2). All the species were determined by Drs J.J. Cantero and G.M. Tourn.

Identification and quantification of (-)hamanasic acid A by 2D-TLC: Briefly [7], powdered air dried leaves (50 mg) were extracted twice using 1.5 mL and 0.5 mL EtOAc, vortexed for 2 min and centrifuged (10 min, 300 *g*). EtOAc extracts were pooled and 3 µL was loaded on chromatograms (Silica gel 60 (Merck), aluminum sheets, F_{254}). Once developed in two dimensions using corresponding solvents, (-)HAA was elucidated at a final R_f=0.35 as a single blue spot through UV_{254} light, which stained strongly with I_2 vapors. Semi-quantitative results were obtained using different concentrations of pure (-)HAA as standard, which were in agreement with the accurate concentration of (-)HAA obtained by the GC-FID standard curve in plant extracts [7].

GC-FID: EtOAc extracts were re-suspended in toluene plus *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (950 + 50 µL, respectively) prior to analysis. The (-)HAA concentration was assessed by GC-FID (GC-hydrogen flame ionization detector, Agilent GC 6890) using a calibration standard curve (0.005–1.0 mg mL^{-1}), as already described [7]. The column was ZB-5HT (15 m x 0.32 mm i.d.). The injector temperature was set to 200°C with an injection volume of 4 µL, split ratio of 10 and N_2 flow of 2 mL min^{-1}. The oven temperature program began at 60°C with a ramp rate of 3°C min^{-1}. The final temperature was 290°C (200-245 ºC, 40ºC min^{-1}; 245-255ºC, 1ºC min^{-1}; 255-290ºC, 50ºC min^{-1}), which was held for 5 min making a total run time of 18 min per sample.

(-)HAA isolation: In order to increase the yield of (-)HAA originally isolated from *FC* through bio-guided fractionation [7], a more efficient methodology was devised, and then applied successfully to other plant materials where (-)HAA had been previously detected by 2D-TLC.

Powdered air dried *FC* leaves (25 g) were shaken vigorously with 200 mL EtOAc for 2 min and filtered. The obtained extract was partitioned with a polar solvent (200 mL H_20 + 10 mL MeOH + 4 mL of H_4NOH), mixed vigorously for 2 min and let stand for 24 h at 4ºC. The lower polar phase (*ca*. 250 mL) was partitioned with 255 mL of a solvent described by Hashidoko *et al*. [8] (125 mL *n*-hexane + 125 mL EtOAc + 5 mL formic acid), mixed vigorously for 2 min and let stand for 20 min at room temperature. The upper organic phase was evaporated *in vacuo*. The resulting extract (1.3 g) was fractionated by silica gel CC (1.5 cm i.d., 47 cm; 50 g silica gel 60 (Merck), 70-230 mesh) eluted at a flow rate of 8 mL min^{-1} using the described Hashidoko´s solvent. After eluting with 60 mL of solvent the collected 1 min fractions were subjected to TLC using $CHCl_3$–EtOAc–MeOH (5:1:0.25; Solvent 1), and pure (-)HAA as standard, detected under UV_{254} and with I_2 vapors. Selected fractions (Fr. 3-6) were pooled, dried at 30ºC with N_2 flow (*ca*. 200 mg) and further fractionated by an equivalent CC using Solvent 1. After eluting with 150 mL of solvent, the collected and dried 2 min fractions (Fr. 2-18; revealed by TLC) afforded *ca.* 100 mg of pure (-)HAA (*ca.* 4 %, DW basis). The described methodology improved 200 times the extraction yield in *FC* as compared with that originally performed [7], and, once applied to other species, yielded sufficient amounts of the compound for further chemical and biological characterizations.

(-)HAA chemical characterization: The putative (-)HAA identified and isolated from other species was chemically characterized from ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMBC and HSQC spectra in $CDCl_3$, by GC–MS and polarimetry, and compared with previously reported data, as already described [7]. Solvents were used from commercial sources without purification. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT, COSY, HMBC and HSQC spectra were recorded at room temperature with Bruker AC 400 spectrometers. The spectra were recorded in $CDCl_3$ and the solvent signals (7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C-NMR) were used as reference [28]. Coupling constants, *J*, are reported in Hertz (Hz). Optical rotation was measured on a Jasco P-1010 polarimeter for solutions in acetone (ca. 0.5%) at 25°C. GC-MS identification was obtained on GC-17A Shimadzu and QP-5000 MS Shimadzu machines. The GC column was HP 5% phenylmethylsilicone (Alltech) (30 m x 0.32 mm i.d.). The linear temperature program was from 150 to 300ºC, at the rate of 10ºC min^{-1}, and the carrier gas was He (1 mL min^{-1}). UV spectra were recorded on a Metrolab 2500.

The compound isolated from *FF* was obtained as a colorless syrup; $[\alpha]_D^{25}$ - 64.9° (*c* 0.70 , acetone). Results from ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMBC and HSQC spectra in $CDCl_3$, and GC–MS analysis, were the same as those already reported for (-) 7-carboxy-8-hydroxy-1(2),12(13)-dien-bisabolene [7].

(-)HAA biological characterization: Pure and dried (-)HAA isolated from other species was dissolved in EtOAc and bioassayed at 0.5, 1.0 and 1.5 mg mL^{-1} (1.0, 2.0 and to 3.0 mM, respectively). Bioactivity was evaluated on seeds of lettuce (*Lactuca sativa*, Grand Rapids) [7]. Briefly, 50 seeds of lettuce were placed in a 9.5 cm Petri dish lined with one sheet of filter paper previously moistened with each test solution (3 mL), and allowed to germinate in a growth chamber in darkness at 22 ± 1ºC. Solvents were evaporated at 40ºC before adding H_2O (3 mL) to both samples and controls. Two different bioassays with 3 replicates for each concentration were performed. Alternatively, bioassays using 25 seeds of lettuce placed in a 5.0 cm Petri dish lined with one sheet of filter paper previously moistened with each test solution (1.5 mL) were used. For comparison purposes, and as experimental controls, bioassays using pure *FC* (-)HAA were also performed.

Seed germination and the lengths of roots and shoots were assessed at 3 days, as previously described [7]. Controls showed (mean ± s.e.) 93.7 ± 0.5 % of germination and 2.1 ± 0.1 cm of root and 1.1 ± 0.1 cm of shoot growth. Effective concentrations capable of inhibiting 50% germination, root growth or shoot growth were calculated as ECg_{50}, ECr_{50} and ECs_{50}, respectively. The results were analyzed by ANOVA and Tukey´s test ($p < 0.05$).

Biological activity of aqueous extracts from* Flourensia *species: Air dried leaves were extracted in distilled H_2O (6 g of plant material in 100 mL) for 24 h at 22ºC. Aqueous extracts were

bioassayed at 6%, w/v, as well as serial dilutions with H_2O at 3.0 and 1.5%, as described above.

For comparison purposes, and as experimental controls, bioassays using aqueous extracts from *FC* leaves were also performed.

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