American Journal of Analytical Chemistry, 2023, 14, 95-120
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Chemical Composition of Different Extracts of *Conyza bonariensis*: Insecticidal and Nematicidal Activities

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How to cite this paper: Adande, K., Eloh, K., Simalou, O., Bakaï, M.-F. and Caboni, P. (2023) Chemical Composition of Different Extracts of *Conyza bonariensis*: Insecticidal and Nematicidal Activities. *American Journal of Analytical Chemistry*, **14**, 95-120.
https://doi.org/10.4236/ajac.2023.142006

Received: December 31, 2022
Accepted: February 25, 2023
Published: February 28, 2023

Abstract

C. bonariensis (L.) Cronq. known as hairy fleabane was first described in Argentina but it is now widely spread through most warmer regions of Europe, Africa, Asia, the Caribbean and Central America. In this work, a chemical analysis by liquid and gas chromatography coupled with mass spectrometry of the whole plant, aerial part, flowers and roots extracts of *C. bonariensis* harvested in Togo (West Africa) was carried out. Two acetylenic compounds *Lachnophyllum* ester and limonene were identified as the main components of essential oils while *Lachnophyllum* and *Matricaria* lactones were dominant in chloroform extracts. Based on the plant chemical compositions, essential oils and chloroform extracts were tested on cowpea weevil *Callosobruchus maculatus* adults which are considered as one of the most cosmopolitan pests of stored beans, and on freshly hatched second-stage juveniles of root-knot nematode *Meloidogyne incognita*. Results showed that the whole plant essential oil demonstrated an $LC_{50/24h}$ value of 1.75 µL oil/L air on *C. maculatus* while at 3.91 µL oil/L air, it showed 100% mortality. Furthermore, the plant root chloroform extracts partitioned in diethyl ether-hexane mixture showed the strongest nematicidal activity with an $LC_{50/72h}$ value of 0.47 mg/mL. Our findings suggest that the widely diffused plant *C. bonariensis* and its acetylenic constituents could be considered as potent botanical insecticidal and nematicidal agents.

Keywords

Conyza bonariensis, Insecticidal Activity, Nematicidal Activity,

Callosobruchus maculatus, *Meloidogyne incognita*

1. Introduction

Conyza bonariensis (L.) Cronquist or *Erigeron bonariensis* is an invasive plant of the Asteraceae family, native to South America. It is often found in tropical and subtropical regions and is widespread in most warm regions of Europe, Africa, Asia, the Caribbean and Central America [1]. *C. bonariensis* is a plant known for its medicinal properties and is therefore widely used in traditional medicine for the treatment of rheumatism, dental pain and headaches; it is also attributed with anti-ulcerogenic and anticoagulant activities. *C. bonariensis* is rich in essential oils (EOs), with an oil content of 0.1% - 0.5% for the whole plant. Studies conducted on the chemical composition showed that its EO is rich in terpenes (limonene and (E)-β-farnesene) and acetylenic compounds (cis-*Lachnophyllum* ester and *Matricaria* ester) [2] [3]. However, to the best of our knowledge, no chemical study was reported on the plant acclimatized in the African Sub-Saharan Region.

Recently, we reported electron-deficient synthetic alkynes bioactive on root-knot nematode *Meloidogyne incognita* [4]. We found that the conjugation of electron-withdrawing carbonyl groups to an alkyne triple bond was extremely proficient in inducing nematode paralysis and death. Naturally occurring acetylenics are of particular interest since many of them display important biological activities. They are of great interest for medicine, pharmacology, medicinal chemistry, and pharmaceutical industries and could be valorised in pest management [5]. This evidence prompted us to investigate herein the nematicidal and insecticidal activities of a plant from Asteraceae family, known to be rich in acetylenic compounds.

Callosobruchus maculatus F. (Coleoptera: Bruchidae) is an insect pest that attacks cowpea seed stock, resulting in rapid crop deterioration. Losses caused by this insect can be as high as 36.4% after two months of storage, and as high as 100% within few months [6] [7]. Phytoparasitic nematodes are microscopic worms that cause plant diseases whose typical symptoms are stunted growth, wilting, leaf discoloration, and deformation of plant organs. This results in reduced crop quantity and quality [8]. Root-knot nematodes of the genus *Meloidogyne* are for instance involved in the decline of tomato production in Togo [9].

If synthetic pesticides are usually used to control insects and nematodes, concerns about their safety to environment and human health are more and more risen. Plant-based insecticide and nematicide would be affordable and less dangerous to the environment and food security. In the present study, chemical constituents of essential oils along with the crude oil of *C. bonariensis* were evaluated for their insecticidal and nematicidal activities on the adult stages of stored-bean pest *C. maculatus* and juvenile stages of *M. incognita*. We therefore

chose in this work to determine the chemical composition of essential oils (EO) and selectively extracted acetylenic components from different parts of *C. bonariensis* by GC-MS and LC-MS. On the other hand, this study evaluated the biological activities of the different extracts on cowpea weevils and parasitic root-knot nematodes.

2. Materials and Methods

2.1. Materials

Chemicals and instruments

Methanol, diethyl ether, deuterated chloroform and hexane used were of high-performance liquid chromatography grade. Reactions were monitored by TLC on 0.25 mm E. Merck silica gel plates (60F-254) visualized under UV light and by applying a phosphomolybdic acid solution in EtOH followed by heat. High-quality reagents were purchased at the highest quality that was commercially available and was used without further purification.

Plant material

Plant materials of *C. bonariensis* were collected in the middle of May 2021 at Dagni Koudzragan in Togo with geographical coordinates Lat: 7.13396 N 7˚8'2.27178" long: 0.67799 E 0˚40'40.75512". The biomass was left under air conditioning for different extractions.

2.2. Methods

Essential oil and solvent extractions

Harvested aerial parts and flowers of *C. bonariensis* were dried at a temperature of about 15˚C under air conditioning for 5 days. Furthermore, the obtained biomass was submitted to 4 h water steam-distillation using a Clevenger-type apparatus. Resulting essential oils were dried over anhydrous sodium sulphate before being transferred to Teflon-sealed cap dark glass vials and stored at 4 C until use.

For the solvent extraction; dried plant roots (200 g) were extracted with chloroform for 30 min by ultrasonication and left overnight at room temperature for maceration (3 × 600 mL). After 24 h, the extract was filtered and evaporated under reduced pressure to dryness at 40˚C to give a gummy product that was suspended in MeOH/H_2O (3:1). The mixture is subsequently partitioned with hexane (3 × 600 mL) to eliminate fats. The methanol/water phase was evaporated at reduced pressure (40˚C) to eliminate methanol before being partitioned with Et_2O (3 × 100 mL). The Et_2O and hexane extracts rich in acetylenic compounds were concentrated to dryness under reduced pressure.

Essential oil analysis by gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC/MS) analysis was performed on an Agilent 5973 equipment with a 60 m × 0.25 mm × 0.25 µm HP1-MS apolar column at an initial temperature of 60˚C isothermal for 10 min, then at a final temperature of 300˚C for 20 min; the gradient was 2˚C/min. The carrier gas (he-

lium) flow rate was 1 mL/min. The mass spectrometer detector was HED/EM (High Energy Dynode/Electron Multiplier 0 - 3000 V) with an energy of 70 eV; the other parameters remained the same. One microliter of essential oil diluted with hexane (10 thousand times) was injected. Unknown mass spectra of peaks obtained from chromatograms were compared to known spectra from literature in NIST Database. Available compounds were eluted for retention time confirmation. However, Unavailable compounds were confirmed by Kovats indices calculation using alkanes.

LC-MS analysis of Diethyl and hexane phases

The diethyl and hexane phases obtained from *C. bonariensis* plant were analyzed by reverse-phase liquid chromatography on an Agilent 1200 series LC system using a Kinetex EVO C18, 100 Å, 5 µm, 150 × 2.1 mm (Phenomenex, Castel Maggiore, Italy). The LC conditions were as follows: flow rate: 0.3 mL/min; solvent A: 0.1% formic acid in bi-distilled water; solvent B: methanol; and gradient was from 10% to 100% B over 10 min and kept 10 min. Eight microliters of filtered samples dissolved in methanol were then analyzed by Electrospray ionization in positive and negative modes using an Agilent 6520 Time of Flight (TOF) MS. Mass spectral data were acquired in the m/z range of 100 - 1500 with an acquisition rate of 1.35 spectra/s, averaging 10,000 transients. The source parameters were adjusted as follows: drying gas temperature 250˚C, drying gas flow rate 5 L/min, nebulizer pressure 45 psi. Based on the original acquisition files, we performed a pre-processing step with MetAlign software used for automated baseline correction and alignment of all extracted mass peaks across all samples. Results were stored as CSV file. ESI/QTOF MS data were then analyzed using the molecular feature extraction algorithm of the MassHunter Workstation software (version B 03.01 Qualitative Analysis, Agilent Technologies, Santa Clara, CA, USA). The molecular feature extraction algorithm took all ions into account exceeding 1000 counts with a charge state equal to one. Blank runs showed maximum 10 features with intensity threshold at 1000 counts. Isotope grouping was based on the common organic molecules model.

Nuclear magnetic resonance analysis of isolated compounds

Nuclear magnetic resonance (NMR) spectra of different isolated compounds were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). The chemical shifts (δ) are reported in part per million downfield from tetramethylsilane (TMS), which was used as internal standard, and the spectra were recorded in deuterated chloroform ($CDCl_3$).

Isolation of *Lachnophyllum* ester and *Lachnophyllum* lactone: Nuclear magnetic resonance (NMR) analysis

Isolation of the Lachnophyllum ester by recrystallisation

(Z)-*Lachnophyllum* ester was obtained by dissolving *C. bonariensis* EO in hexane. One milliliter of EO was dissolved in 10 mL of hexane and kept at −20˚C. After 24 h, (Z)-*Lachnophyllum* ester crystallizes and the supernatant solvent is removed. (Z)-*Lachnophyllum* ester crystals are purified with 10 mL of hexane 6 times to obtain almost pure crystals as demonstrated by GC-MS analysis. The

crystals obtained were used for chemical analysis and nematicide tests.

Isolation of Lachnophyllum lactone by preparative HPLC

LC-MS analysis of diethyl-ether fraction revealed the two compounds as major components: *Lachnophyllum* ester and *Lachnophyllum* lactone. Preparative HPLC was used to isolate the latter. Compounds were eluted on a C18 column with an isocratic mobile phase MTBE: Hex (1:4). A diode array detector with absorption wavelengths of 254, 310 and 360 nm coupled with a refractive index detector was used. The HPLC pump was stabilized at a flow rate of 2 mL/min. Different obtained fractions were submitted to thin layer chromatography before nuclear magnetic resonance (NMR) analysis.

Insecticidal and nematicidal bioassays

Insecticidal activity

Insecticidal activity was evaluated on young generations of cowpea bruchids, *C. maculatus*. The rearing was carried out by introducing 50 adult couples on 200 g of healthy cowpea seeds sterilized at a temperature of −18˚C for 3 days in freezer. The insects were removed after 48 h and infected seeds were put in incubation from which first generation was emerged after 21 days. Newly emerged adults were used for insecticidal activities. To determine insecticidal activity of essential oil of *C. bonariensis*, 20 couples of newly emerged adults were selected and placed in one-liter jars with different concentration (1, 2, 3 and 5 µL) of essential oil which were placed on 5 cm diameter filter paper discs. For each dose, 4 trials were carried out in addition to 4 controls that had not received the product. After 24 hours, insects were removed and placed in Plexiglas petri dishes for another day to monitor their agony and mortality rate of adults in each treatment was determined by following the formula:

$$\text{Mortality rate (\%)} = (\text{Ntr} - \text{Nte})/\text{NT} \times 100$$

where Ntr is number of dead insects in the treatment, Nte is number of dead insects in control and NT is total number of insects tested [10].

Nematicidal activity

Population of *Meloidogyne incognita* originally obtained from tomato (*Solanum lycopersicum* L.) roots harvested in a greenhouse was used for rearing on tomato plants *cv. Belladonna*, a cultivar that is very susceptible to root-knot nematodes. All plants were maintained in a greenhouse at 25˚C - 28˚C, 60% relative humidity, and 16h photoperiod in plastic pots (18 cm diameter) containing a 10:1 (v/v) mixture of peat and perlite. After 40 days, the plants were uprooted, and the roots were washed free of soil and cut into 2 cm pieces. Eggs were extracted using the sodium hypochlorite procedure [11] and second instar larvae were obtained using the modified Baermann method at 28˚C. All J2 hatched within the first 3 days were discarded and subsequent generations were collected and used in the experiments.

Nematicidal activities of essential oils of different parts of *C. bonariensis*, diethyl ether extract and isolated *Lachnophyllum* ester were tested for loss of mobility of J2, and EC_{50} values were calculated. Stock solutions of tested samples

were prepared in methanol, while the final solutions were obtained by dilution with water containing the surfactant polysorbate 20 (Tween-20). Final concentrations of methanol and Tween-20 in each well never exceeded 1.0% and 0.3% v/v, respectively, because preliminary tests showed that the mobility of nematodes exposed to these concentrations was similar to the mobility of nematodes maintained in pure water [12]. For the assays, 96-well microplates were used. The test well constituted of 200 µL solution containing 25 - 30 J2s. After 24, 48 and 72 h, mortality (motility) of nematodes was checked under a reversed microscope (Zeiss, Germany). Every sample was replicated 6 times and the experiment was repeated at least twice at different times. The percentages of dead J2 were corrected by eliminating the natural death in the water Tween 20 0.3%/MeOH (2:98 v/v) control (less than 5% of total number of J2) according to the Schneider Orellis formula: [13]

$$\text{corrected } \% = \frac{\text{mortality } \% \text{ in treatment} - \text{mortality } \% \text{ in control}}{100 - \text{mortality } \% \text{ in control}} \times 100$$

and they were analyzed (ANOVA) after being combined over time. Since ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of death J2 treated with tested compounds were subjected to nonlinear regression analysis using the loglogistic equation proposed by Seefeldt *et al.* in 1995 [14]:

$$Y = C + \frac{D - C}{1 + e^{b \log x - \log EC_{50}}}$$

where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test compounds concentration required for 50% death/immotility of nematodes after elimination of the control (natural death/immotility). In the regression equation, the test compounds concentration (% w/v) was the independent variable (x) and the immotile J2 (percentage increase over water control) was the dependent variable (y). The mean value of the six replicates per compound concentration and immersion period was used to calculate the EC_{50} value.

3. Results and Discussion

3.1. Essential Oil Extractions

Extraction of fresh aerial part of *C. bonariensis* plant gave a yellow EO with a yield of 0.12% of fresh material. The flower gave 0.3% while a percentage yield of 0.01% was found for the root. When studying the same plant, Barbosa *et al.* [3] reported yields varying from 0.04% to 0.32% for the extraction of different parts of *C. bonariensis* harvested in Brazil. Maia *et al.* [2] reported percentage yields between 0.1% - 0.5% for 5 samples harvested in different geographic areas. The extraction yield found herein is within the general found range and still high for a medicinal endemic plant. This work, report for the first time, EO extraction from *C. bonariensis* acclimatized in West Africa.

3.2. Chemical Composition of the Extracted Essential Oils

GC/MS analysis of the aerial part and root of the EO identified 35 compounds (Table 1) representing more than 95% of the crude essential oils. Major components were methyl lachnophylum ester and lactone, limonene, trans β-farnesene, trans β-ocimene, β-caryophylene and germacrene D. As expected, acetylenic compounds were the major components of the EO. Monoterpene hydrocarbons made up to 21.69% and sesquiterpene hydrocarbons 18.75% for the aerial part while the root showed less.

Chemical composition of the aerial part of the EO of *C. bonariensis* acclimatized in Tunisia and Italy was studied by Hammami *et al.* [15]. For Tunisian chemotype, nearly 90% terpenes were found and major compounds were caryophyllene oxide (18.7%), spathulenol (18.6%) and α-curcumene. The major compounds of the plant harvested in Sardinia, Italy, were cis-*Lachnophyllum* ester (14.2%) and (E)-β-farnesene (12.0%). We found in this work the same components even though the previous work did not identify *Lachnophyllum* lactone. This may be explained by the less sensitivity of this compound to electronic ionization and absence of the lactone in previous NIST mass spectra libraries. Slight difference in chemical composition of the EO of the plant acclimatized in Togo was probably caused by harvesting location combined with the extraction method. In fact, Hammami *et al.* [15] demonstrated that neophytadiene (53.2%) was the major component when supercritical fluid extraction is used while caryophyllene oxide (18.7%) was the major compound when hydrodistillation extraction was used on a sample harvested in Tunisia.

The results obtained from a chemotype of different parts of *C. bonariensis* plant from Brazil [3] showed the presence of methyl *Matricaria* ester (76.4%), manool (25.3%), limonene (29.6%) and carvone (21.1%) as major compounds of the root, stem, flower and leaf, respectively. cis-*Lachnophyllum* ester which is the major compound of our work was not identified but its trans isomer was identified at 21.3% in the root. These findings highlight the need to evaluate chemical composition of EOs from different regions before use. Likewise, Maia *et al.* [2] identified in different chemotypes harvested in Amazon region of Brazil, limonene (58.4%), (E)-β-farnesene (30.9%), manool (25.3%) and carvone (21.1%) in different parts of *C. bonariensis* .

3.3. LC-MS/Q-TOF Analysis of Different Extracts of *C. bonariensis*

To check the overall acetylenic components of the West African acclimatized *C. bonariensis*, different selective extracts were made. With an initial chloroform extract, diethyl ether and hexane fractions were obtained by liquid-liquid extraction from aerial and root parts of the plant. After evaporation, extracts were submitted to liquid chromatography coupled with a quadrupole time of flight detector (LC-MS/QTOF). Four fractions were analyzed: aerial part diethyl fraction ($CBAPET_2O$) and hexane fraction (CBAPHex); and root diethyl fraction ($CBRET_2O$) and hexane fraction (CBRHex). Chromatograms and exact mass

Table 1. Volatile compounds identified in the essential oil of *C. Bonariensis*.

Compounds	Whole plant	Leaves	Flowers	Roots	IR*
β-pinene	0.99	0.44	0.13	-	939
Limonene	11.69	12.75	5.57	-	1031
β-ocymene	8.29	5.75	0.93	-	1041
3,5-Decadiyne 2,2dimethyl-	-	-	-	1.48	1217
1-Octadecyne	-	-	-	1.18	1238
γ-pyronene	-	2.58	3.11	-	1338
α-Copaene	0.02	0.14	0.83	-	1377
β-cubebene	-	1.30	-	0.02	1387
(-)*β*-elemene	0.23	-	-	0.16	1392
Longifolene-V4	-	-	0.36	-	1402
β-caryophyllene	4.78	16.19	21.08	2.94	1418
trans *α*-bergamotène	0.20	-	-	-	1438
Aromandendrene	-	-	-	0.08	1440
β-gurjunene	0.89	-	-	-	1442
(-)aristolene	-	-	0.73	-	1455
Humulene	-	1.95	1.64	1.75	1455
β-farnesene	6.16	15.53	12.90	25.74	1458
Alloaromadendrene	-	0.49	5.72	-	1460
Acoradiene	-	-	0.56		1471
β-cadinene	-	-	0.45		1472
(+)-*γ*-gurjunene	0.16	1.05	0.35		1473
Germacrene-D	4.12	6.77	5.06	5.04	1480
α-curcumene	-	0.74	-	-	1482
β-guaiene	-	1.21	1.08	-	1488
(+)-epi-bicyclosesquiphellandrene	-	2.83	3.22	-	1490
Nerolidiol	-	-	-	0.88	1494
Bicyclogermacrene		2.51	3.77	-	1494
(-)-zingiberene	0.16	0.27	-	-	1500
3-Phenyl-2-propyn-1-ol	-	-	-	1.19	1504
γ-cadinene	0.15	5.34	5.65	-	1509
α-farnesene	0.91	1.2	0.61	0.90	1509
Cada-1,4-diene	-	-	1.37	-	1524
δ-cadinene	-	-	1.79	-	1526

Continued

5,9,9-triméhyl-spiro[3.5]non-5-èn-1-one	-	1.8	2.30	-	
methyl cis-*Lachnophyllum* ester	57.97	9.76	-	50.62	1539
Nerolidol	-	-	0.78	0.88	1554
Spathulenol	-	0.94	-	0.15	1578
caryophyllene oxide	0.16	1.25	2.71	1.21	1583
Tetradecanal	-	0.24	-	-	1618
Patchoulane				0.10	
Oxacyclotetradeca-4,11-diyne	-	-	-	0.44	1639
T-Cadinol	-	-	-	0,44	1640
α-cadinol	0.99	0.35	-	0.55	1652
Cadalene	0.15	-	0.18	-	1674
Pentadecanal	-	-	-	0,64	1715
5,7-Dodecadiyn-1,12-diol	-	-	-	0.03	1724
9-octadecyne	-	0.46	-	-	1786
Hexadecanal	-	0.33	0.29		1831
Dibutyl phthalate	-	-	-	0,16	1965
9,12,15-Octadecatrienal	-	-	-	0,11	2045
9,12-Octadecadienal	-	-	-	0.28	2150
trans-Phytol	-	-	-	0.14	2114
10,12-Octadecadiynoic acid	-	-	-	0,23	2202
Total identified	97.035	91.34	80.99	96.91	

extractions of potential acetylenic components are shown in **Figures S1-S4**. *Lachnophyllum* methyl ester, *Lachnophyllum* lactone, *Lachnophyllum* ethyl ester, *Matricaria* lactone and *Matricaria* ethyl ester were identified in the different extracts (**Table 2**).

If *Lachnophyllum* and *Matricaria* methyl esters are frequently reported in *Conyza* species [3] [16], *Lachnophyllum* and *Matricaria* ethyl esters identified herein are less reported. They were identified only in diethyl ether extracts. Ethyl esters were identified before in *C. albida* by Pacciaroni *et al.* [17]. Nevertheless, this is the first time the ethyl esters of *Lachnophyllum* and *Matricaria* are detected in *C. bonariensis*. More investigations are needed to isolate those compounds from *C. bonariensis* for structure elucidation. Furthermore, *Matricaria* and *Lachnophyllum* lactones were more abundant in solvent extract than essential oils. Frequently, lactones derivatives show more bioactivity than ester derivatives [18] [19]. Chloroform extraction with subsequent fractionations demonstrated in this work could raise *C. bonariensis* bioactivity.

Table 2. Distribution of acetylenic compounds in different extracts of *C. bonariensis*.

Compound	Number	Retention time	M + H adduct	Distribution of compounds in Extracts
Lachnophyllum methyl ester	**1**	12.87	177.0922	All extract
Lachnophyllum lactone	**2**	10.96	163.0766	All extracts but more in aerial parts
Lachnophyllum ethyl ester	**3**	8.36	191.1067	$CBRET_2O$ fraction
Matricaria lactone	**4**	10.76	161.0600	$CBRET_2O$ fraction
Matricaria ethyl ester	**5**	8.34	189.0910	$CBRET_2O$ fraction

3.4. Isolation of Two Known Acetylenic Compounds

cis-*Lachnophyllum* methyl ester isolation

The major component of the aerial part EO was isolated by recrystallisation in hexane. It was a white needle-like crystals that melt very rapidly at room temperature (around 28˚C). GC-MS, ^1H, ^{13}C, HMBC and HMQC NMR analyses confirmed the structure of the compound (**Figure 1**). At the best of our knowledge, this work is the first report of isolation of cis-*Lachnophyllum* methyl ester by recrystallisation.

Proton NMR spectrum showed the presence of a triplet at δ 2.36 representative of 2 H-8 protons coupled with the H-9 protons (J = 7 Hz) that appeared at δ 1.59. Coupling between H-8 and H-10 protons gave a multiplet at δ 1. More, a long-range coupling between H-8 protons and H-2 proton of the double bond is shown at δ 6.15 (J = 1 Hz). The other H-3 proton of the double bond at δ 6.19 gave a doublet with its neighbor. Their coupling constant J = 11 Hz confirmed the cis geometry of the C-2,3 double bond. Finally, the methyl of the ester appears as a singlet at δ 3.77.

On the decoupled ^{13}C NMR spectrum (**Figure S5** and **Figure S6**), we can easily observe C-10 methyl at δ 13.461, the two carbons C-9 and C-8 at 21.569 and 21.753 respectively. Acetylenic carbons C-7, 6, 5 and 4, were shown at δ 90.70; 86.570; 70.83; and 65.185, respectively. Furthermore, peaks of C-3 and 2 double bonds carbons were observed at δ 130.674 and 122.501, respectively. The peak δ 164.775 should be C-1 carbon. The last carbon of the acetylenic compound that is the methyl group of the ester is resolved at δ 51.593.

If from two previous spectra, the presence of multiple bonds is almost confirmed, there are still doubts regarding the long-range couplings. To elucidate these neighboring relationships, further studies were carried out in HMBC and HMQC. In HMBC spectrum, a spot at C-2 and H-8 intermediary was observed (**Figure S2**). This confirms the majority of multiplets obtained in ^1H and ^{13}C interpreted as arising from long distance couplings beyond seven bonds. HMQC spectra did not change this decision.

These magnetic resonance analyses confirmed the structure of the cis-*Lachnophyllum* methyl ester proposed in previous line. The trans isomer of this

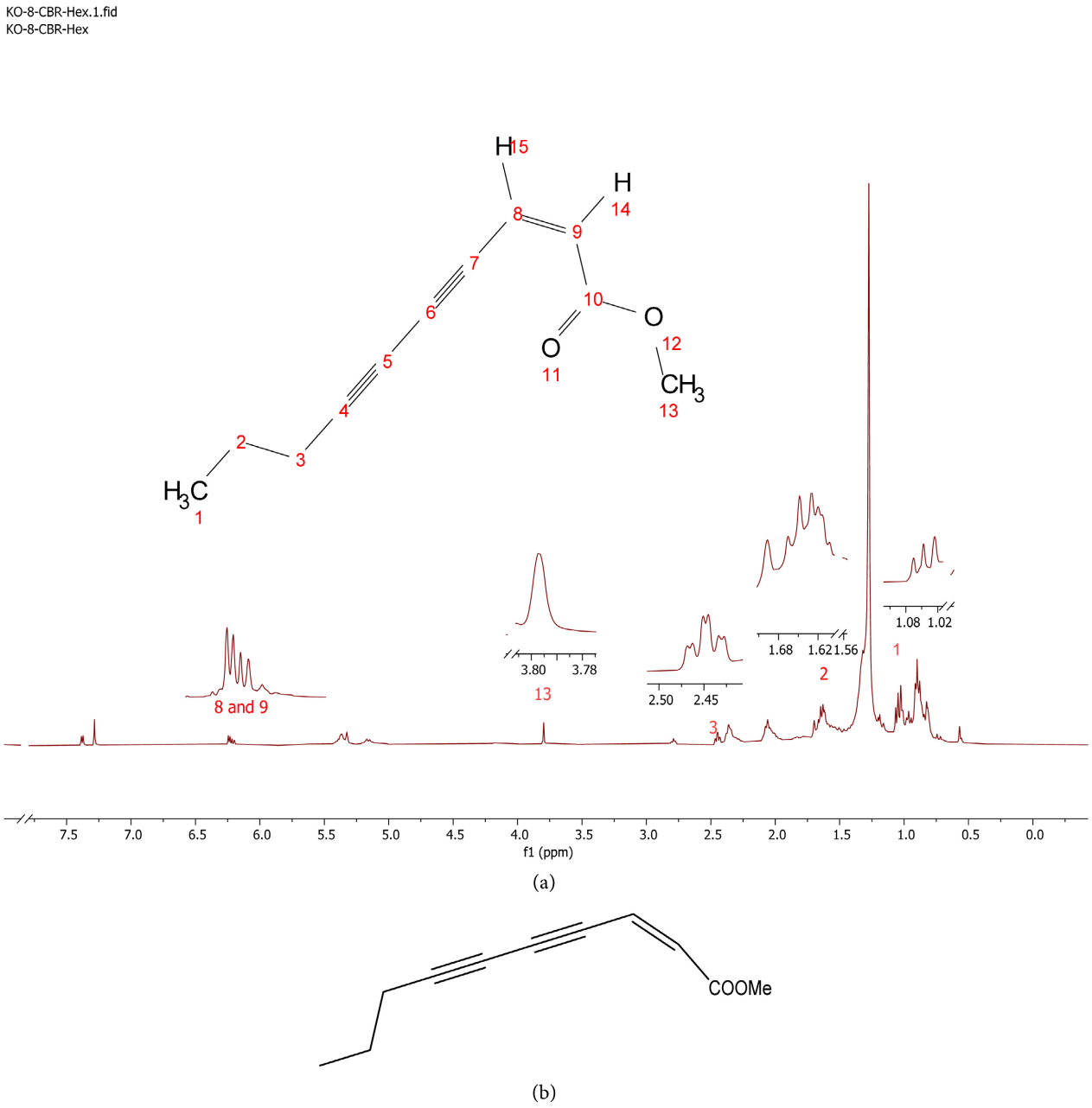


Figure 1. NMR spectra (a) and structure (b) of cis-*Lachnophyllum* methyl ester.

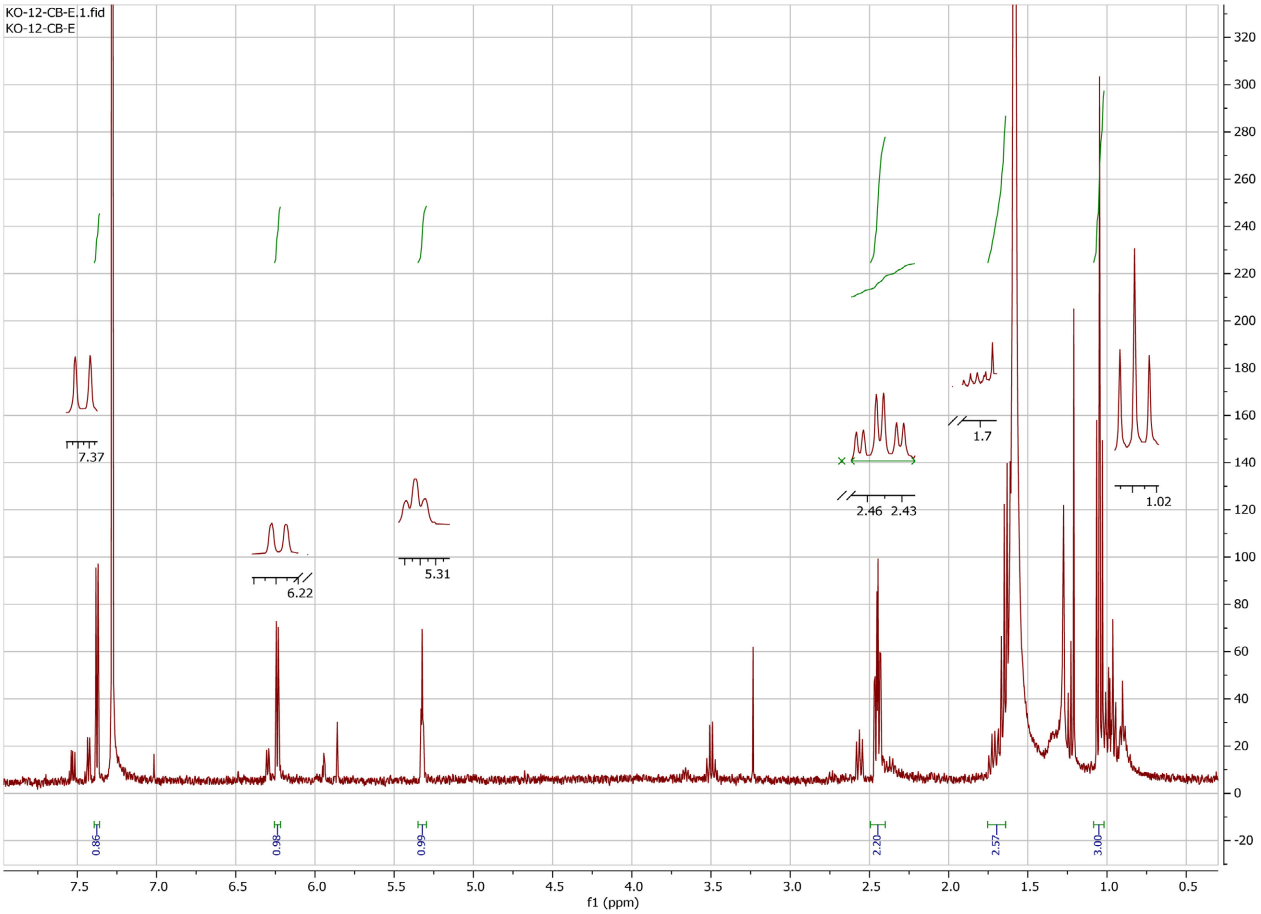
compound has already been isolated by Barbosa *et al.* [3] and analyzed by 1H NMR, they obtained almost the same peaks as in this work notably the carbons of double bond H-2 and 3 respectively at δ 6.83 and 6.32. The difference is in coupling constants of the two protons, *i.e.* 15.7 Hz against 11 Hz for our analyses. This difference confirms the identified double bond configurations. In the same paper, *Matricaria* methyl ester was isolated and the C-2 and C-3 carbons that were of cis geometry gave a coupling constant of J = 10.8, similar to our findings. Our experiment was isolated with a relatively easy method, *Lachnophyllum* methyl ester by recrystallization. The compound could serve as inter-

mediary for chemical synthesis of bioactive compounds.

Lachnophyllum lactone isolation

LC-MS analysis showed that *Lachnophyllum* lactone was readily present in the roots of the plant. To isolate more acetylenic compounds, we performed a preparative HPLC on the diethyl ether extract that gave different fractions. Fortunately, a fraction showed to be a pure *Lachnophyllum* lactone as confirmed by mass spectrometry analysis and ^{1}H, ^{13}C analyses (Figure 2).

^{1}H proton NMR analyses showed (400 MHz, $CDCl_3$) δ 1.03 (t, 3H, J 5.2 Hz, CH_3), 1.62 (sex, 2H, J 7.2 Hz, CH_2), 2.43 (td, 2H, J 7.2, 2.4 Hz, CH_2), 5.31 (t, 1H, J 2.4 Hz, CH), 6.22 (d, 1H, J 5.2 Hz, CH), 7.37 (d, 1H, J 5.2 Hz, CH); ^{13}C NMR



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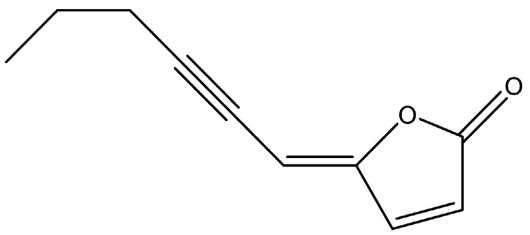

(b)

Figure 2. NMR spectra (a) and structure (b) of 4-Z Lactone *Lachnophyllum*.

(125 MHz, $CDCl_3$) δ 168.9, 156.0, 142.6, 120.1, 104.6, 95.1, 74.8, 22.1, 21.8, 13.5; MS/MS (EI, 70 eV) m/z (%) 162.0 $[M^+]$ (precursor ion), 147.0 (23) $[M - CH_3]^+$, 133.0 (25) $[M - C_2H_5]^+$, 119.1 (31) $[M - C_3H_7]^+$, 105.1 (14) $[M - C_2H_5 - CO]^+$, 91.1 (47) $[M - C_3H_7 - CO]^+$, 82.0 (100) $[C_4H_2O_2]^+$. This compound is generally found in plants of the Asteraceae family. It was isolated in extracts of the aerial part *Conyza canadensis* [20] [21]; also in the essential oil of root of *Erigeron acris* [22].

3.5. Insecticidal Activity of *C. bonariensis* Essential Oil

After the determination of chemical composition of different extracts of *C. bonariensis*, we chose to test the aerial part EO with the higher yield on cowpea weevils *C. maculatus*. When we applied a concentration of 1 µL/L, a mortality of 56% of adults was observed after 24 hours (**Figure 3**). When concentrations were raised, we observed 73% mortality at 2 µL/L, 83% at 3 µL/L and 100% at 4 µL/L. To the best of our knowledge, no work reported before the insecticidal activity of *C. bonariensis* plant. Otherwise, some biological activities of the plant have been reported. The insecticidal activity of the plant may be due to its acetylenic compounds and limonene present in in the essential oil. [17] [23] [24]. Recently, (4Z)-*Lachnophyllum* lactone was demonstrated to have phytoxicity activity on *Cuscuta* species, obligate parasitic plants by Fernández-Aparicio. [24]

Compared to other studies on insecticidal activity of EOS against cowpea bruchid, results reported by Ilboudo *et al.* [25] showed that the EOs of *Ocimum americanum*, *Hyptis suaveolens*, *Hyptis spicigera* and *Lippia multiflora* with thymol were toxic to *C. maculatus*. Reported LD_{50} values were 0.23 mL/L, 1.30 mL/L, 5.53 mL/L and 6.44 mL/L, respectively. It could be concluded that *C. bonariensis* EO had a similar toxicity to the weevils as *O. americanum* and *H. suaveolens*, but more toxic than *H. spicigera* and *L. multiflora*. According to the work of Nyamador *et al.* [26], EOs of *Cymbopogon nardus* and *Cymbopogon giganteus* acclimatized in Togo induced at the concentrations of 40 mL/L, 50%

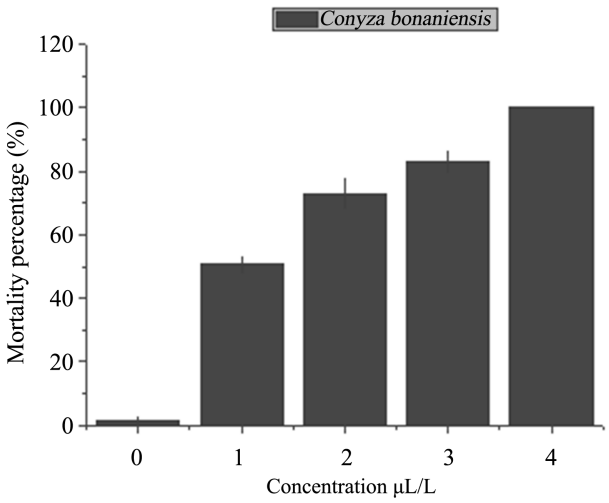

Figure 3. Nematicidal activity of essential oils of different parts of *C. bonariensis*.

and 90% mortality, respectively of *C. maculatus* adults after 24 hours. *C. bonariensis* could be classified among the best EOs against cowpea weevils. Noteworthy, *C. bonariensis* is an endemic not edible plant and could give cheap biopesticide.

3.6. Nematicidal Activity

EOs of different parts of *Conyza bonariensis* were tested on second instar (J2) juveniles of root-knot nematode of genus *M. incognita*. EC_{50} values are reported in Table 3.

EO of the whole plant was more effective than the EO of leaves and flowers. This difference would be due to variation in chemical composition of EOs of different parts. From the previous lines, we demonstrated that bioactive components were more abundant in root parts of the plant. The lack of nematicidal activity of the flowers and leave of the plant are explained by absence of those compounds. The effectiveness of the whole plant EO was probably due to the presence of acetylenic components that are the major compounds in the EO. Kimura *et al.* [27] reported the nematicidal activity of *Matricaria* and *Lachnophyllum* methyl esters. Those compounds present herein in the EO, could be responsible of bioactivity.

Nematicidal activity of diethyl ether extract of *C. bonariensis* root

To confirm our hypothesis of nematicidal activity of the detected acetylenic compounds by mass spectrometry, we tested the diethyl ether root extract (rich of acetylenic compounds) on *M. incognita*. The concentrations 250, 500 and 1000 mg/L were assessed. Results are reported in Figure 4.

Results showed that all tested concentrations induced nematode mortality. The $LC_{50/72h}$ value was estimated by probit analysis to 470 mg/mL. Chemical composition showed earlier in this work strong presence of two major acetylenic compounds including *Lachnophyllum* and *Matricaria* lactones. These two compounds that are biologically active and have several properties including nematicidal properties [27] explained the strong nematicidal activity of the plant.

Nematicidal activity of cis methyl ester *Lachnophyllum*

We also demonstrated the nematicidal activity of an isolated acetylenic compound. *Lachnophyllum* methyl ester isolated from the EO of the whole plant by recrystallisation was tested alone on J2 of *M. incognita* at concentrations of 75, 150, and 300 mg/L. Results are reported in Figure 5.

Results of Figure 5 revealed a high mortality rate of nematodes in contact with the different concentrations of the compound with an $EC_{50/72h}$ estimated to

Table 3. Nematicidal activity of essential oils of different parts of C. bonariensis.

Treatment	$EC_{50/72h}$ (Confidence intervalle) (mg/L)
Flowers essence	>5000
Leaves essence	4600 (4468 - 4756)
Whole plant essence	1817 (1798 - 1836)

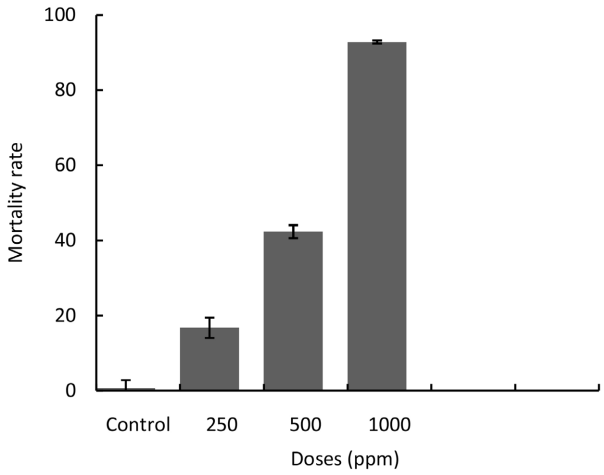

Figure 4. Nematicidal activity of diethyl ether extract of *C. bonariensis* root.

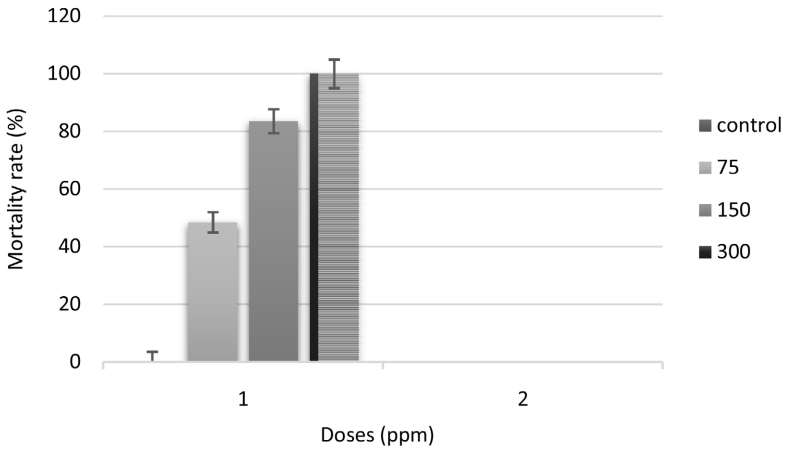

Figure 5. Nematicidal activity of cis-*Lachnophyllum* methyl ester.

78.61 mg/L. The efficacy of cis-*Lachnophyllum* methyl ester on nematodes was probably due to its chemical structure with conjugated triple and double bonds rising the compound bioactivity [28]. Previous studies reported several bioactivities of cis-*Lachnophyllum* methyl ester such as nematicidal [27], antifungal [29] [30] and antibacterial [30]. Our work is the first report of nematicidal activity of this molecule to root-knot nematodes of *Meloidogyne* spp. This compound could be vaporized to control plant parasitic nematode after further studies.

4. Conclusion

The work in this study was conducted on the essential oil of the whole plant and the chloroform extract of the root of *Conyza bonariensis*. The chemical composition of the essential oil of *C. bonariensis* was determined to be more than 98%, with the major compounds *Lachnophyllum* cis ester (57.97%) and limonene (11.69%). The chloroform extract was partitioned and fractionated to give a 4-Z Lactone *Lachnophyllum* compound and by recrystallisation the cis ester *Lachnophyllum* was isolated from the essential oil. The structure of both compounds

was determined by proton, carbon, HMQC and HMBC NMR. The neighbouring relationships confirmed the structures identified by other authors. On other hand, the essential oil of *C. bonariensis* has a very lightning insecticidal activity on insects as it induces 100% mortality at a dose of less than 4 µL/L during a 24 h application on *Callosobruchus maculatus* bruchids. The essential oil, partitioned extract and *Lachnophyllum* cis ester were very effective against J2 nematodes of the genus *M. incognita* with very high mortality rates of the different concentrations. Furthermore, *Lachnophyllum* cis ester and limonene also showed a synergistic effect against these nematodes. Thus, the use of plant extracts and biological molecules as biopesticides is interesting because they are natural and have little negative impact on health and the environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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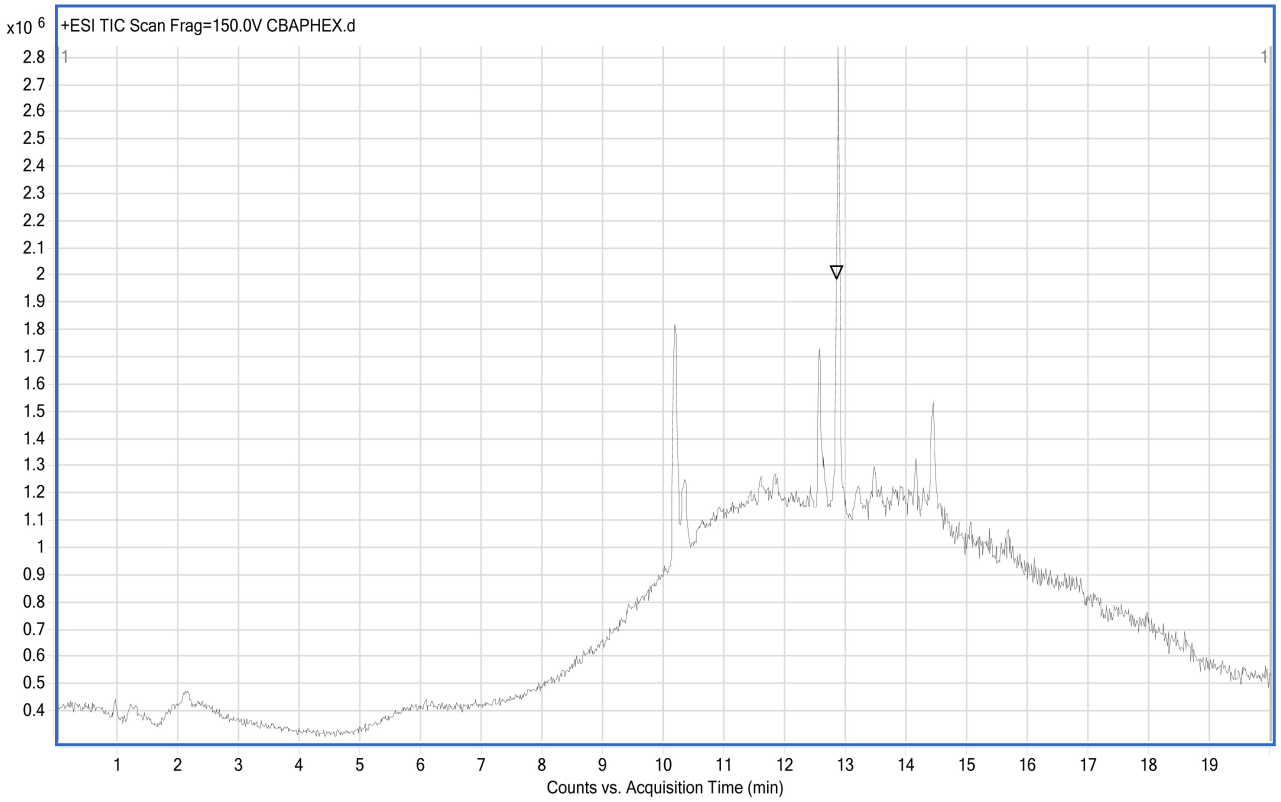
Supplements



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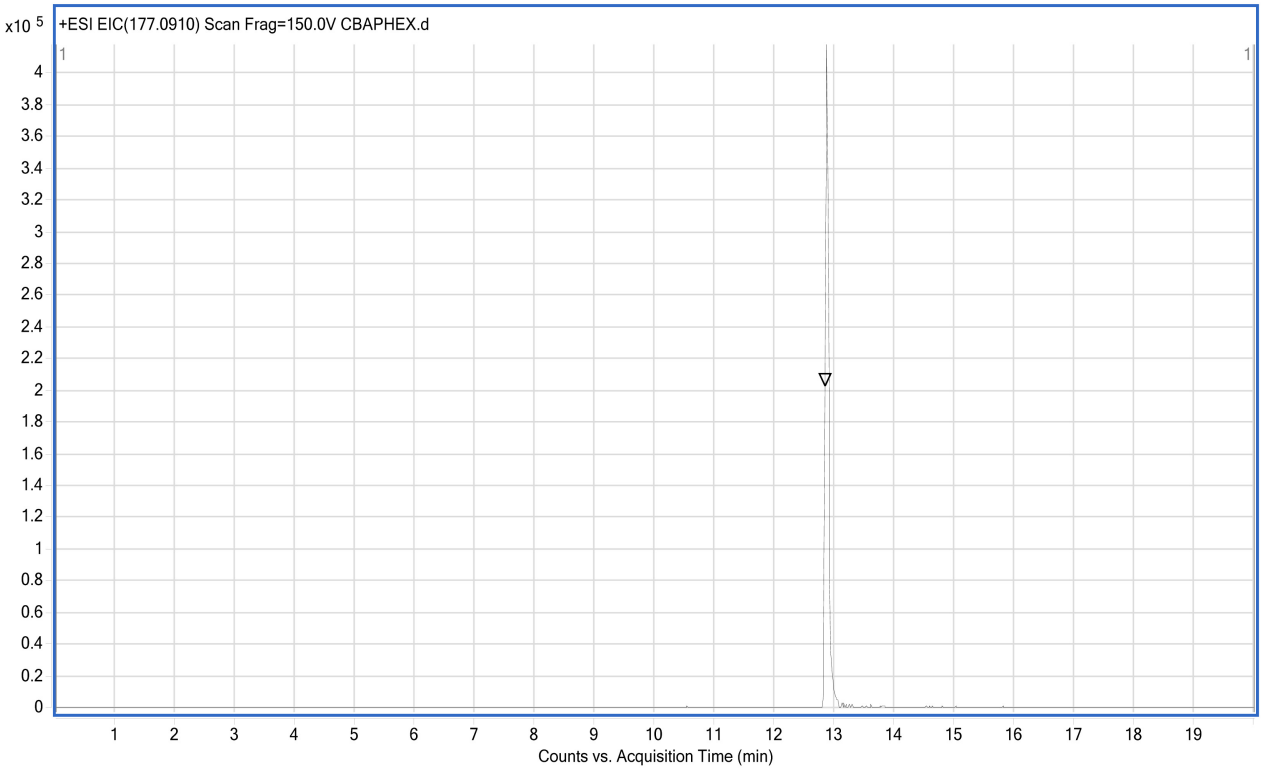

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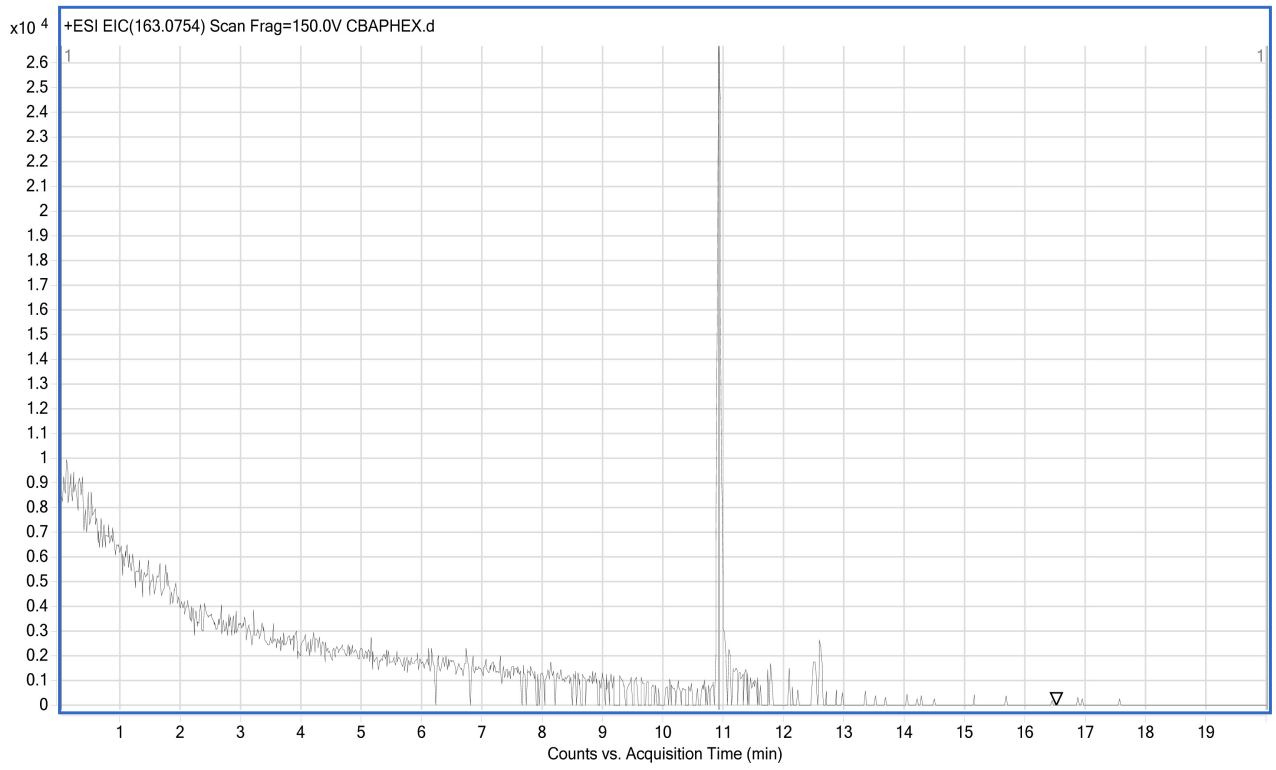
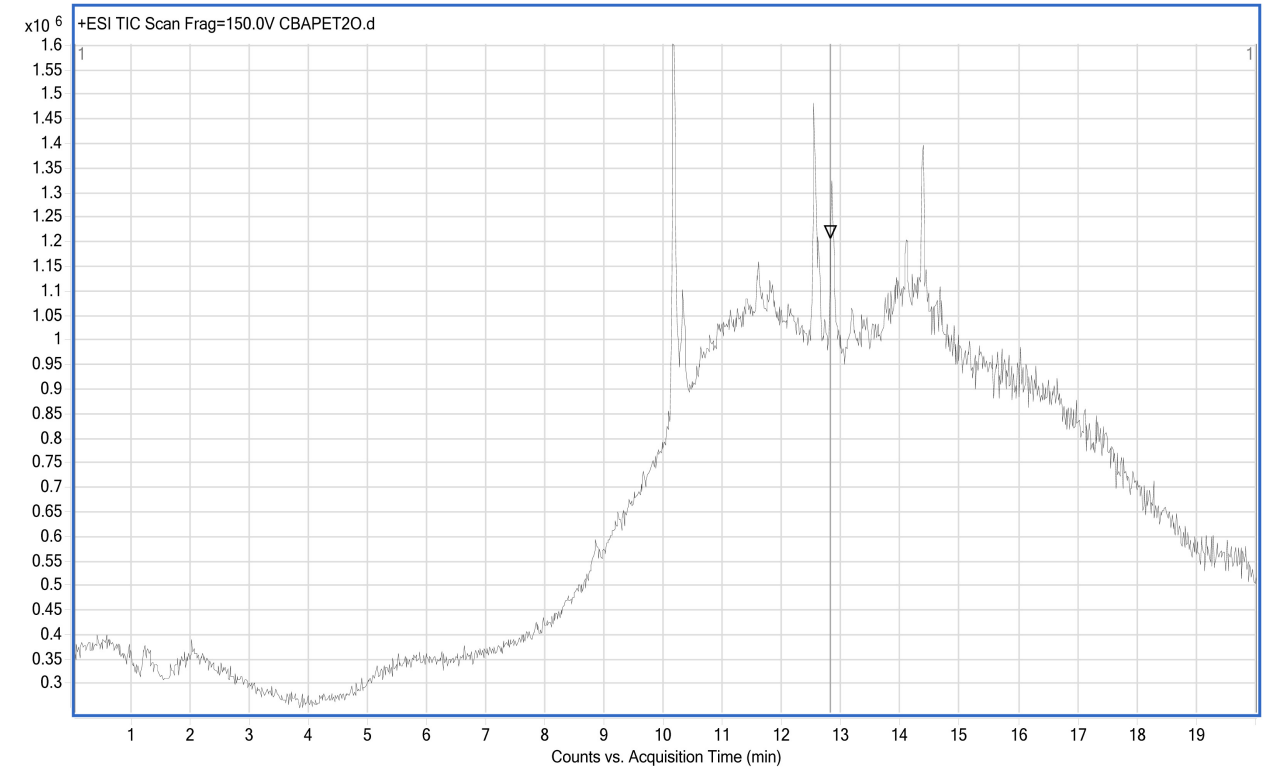


(c)

Figure S1. LC-MS Chromatograms of *C. bonariensis* aerial part hexane fraction (a) Total ion chromatogram; (b) *Lachnophyllum* methyl ester ion extract chromatogram; (c) *Lachnophyllum* lactone ion extract chromatogram.



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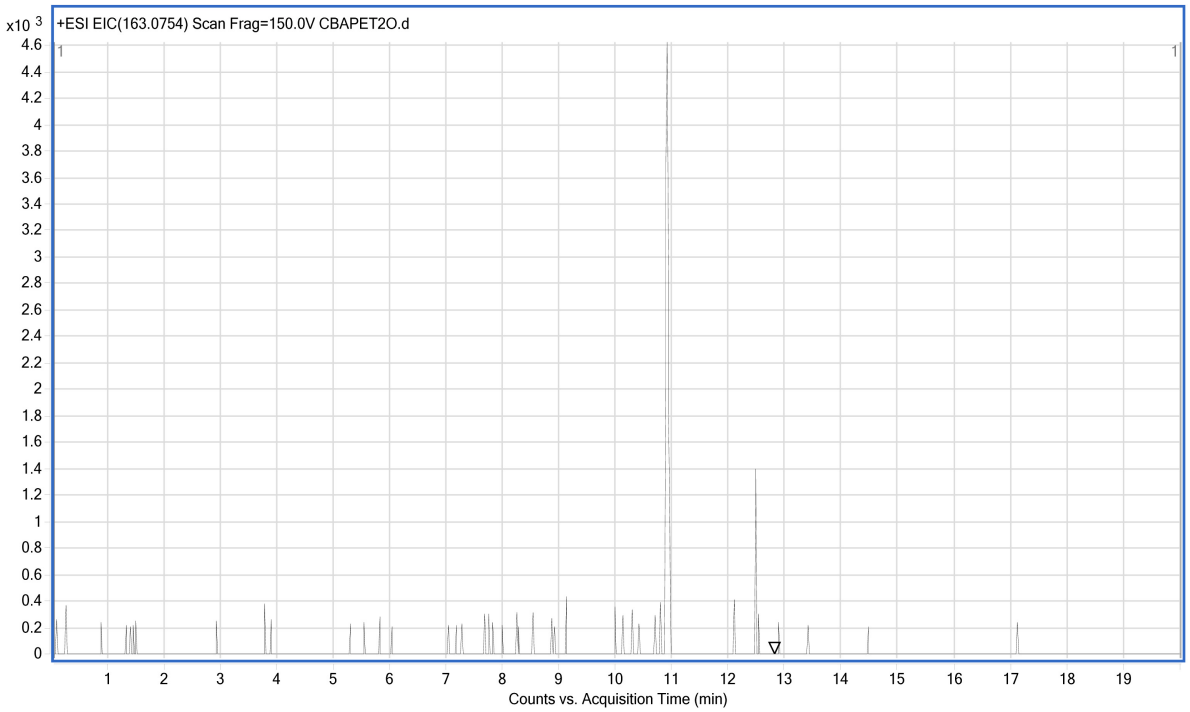

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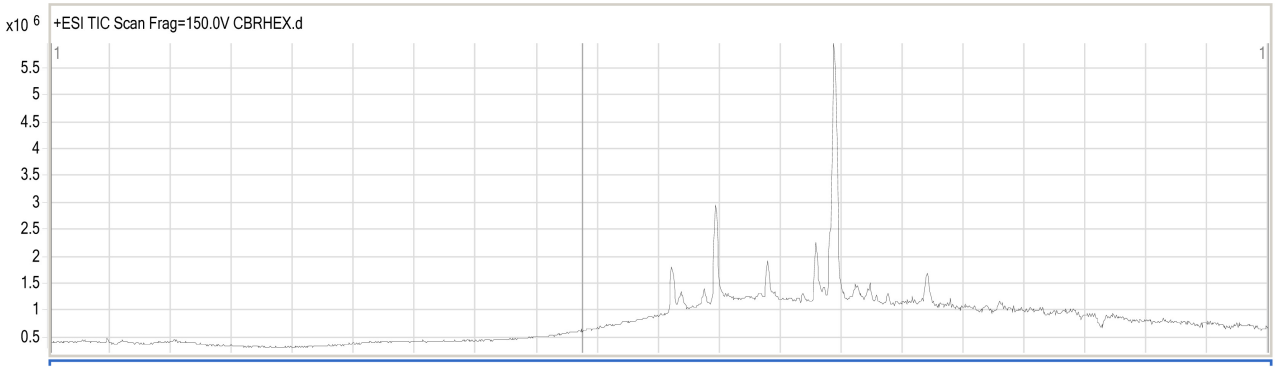
Figure S2. LC-MS Chromatograms of *C. bonariensis* aerial part diethyl fraction (a) Total ion chromatogram; (b) *Lachnophyllum* lactone ion extract chromatogram.



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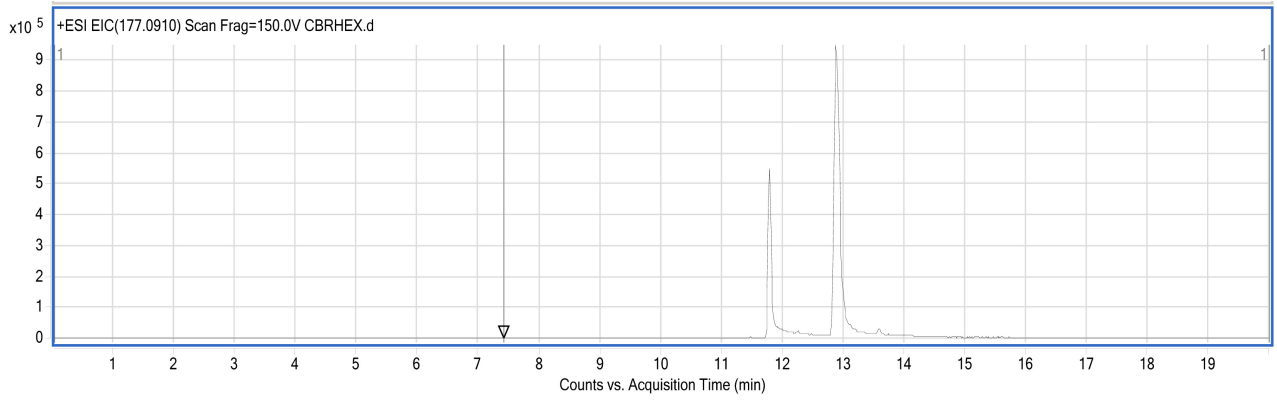

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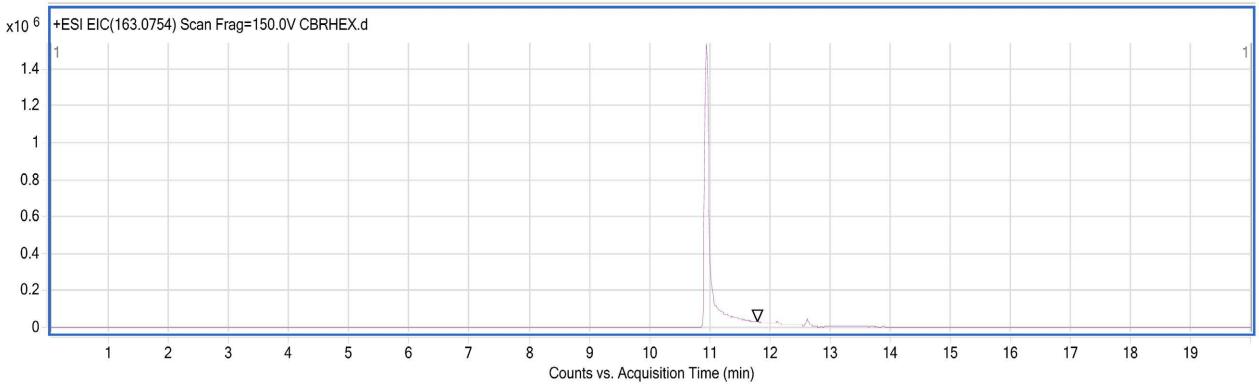

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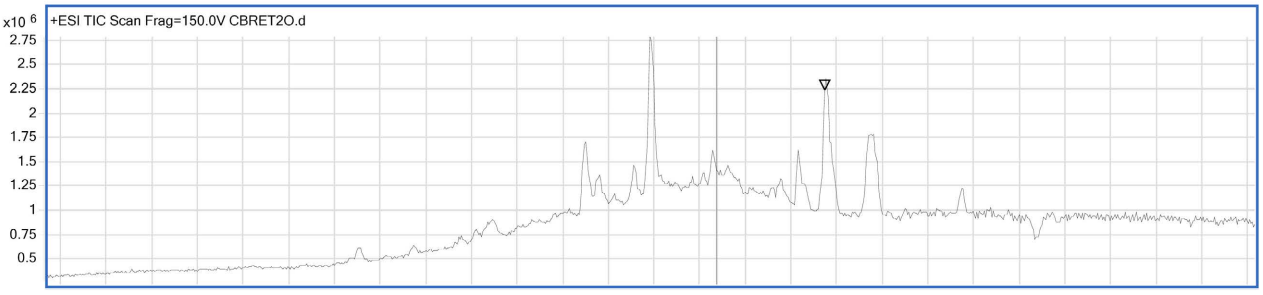
Figure S3. LC-MS Chromatograms of *C. bonariensis* root hexane fraction (a) Total ion chromatogram; (b) *Lachnophyllum* methyl ester ion extract chromatogram; (c) *Lachnophyllum* lactone ion extract chromatogram.



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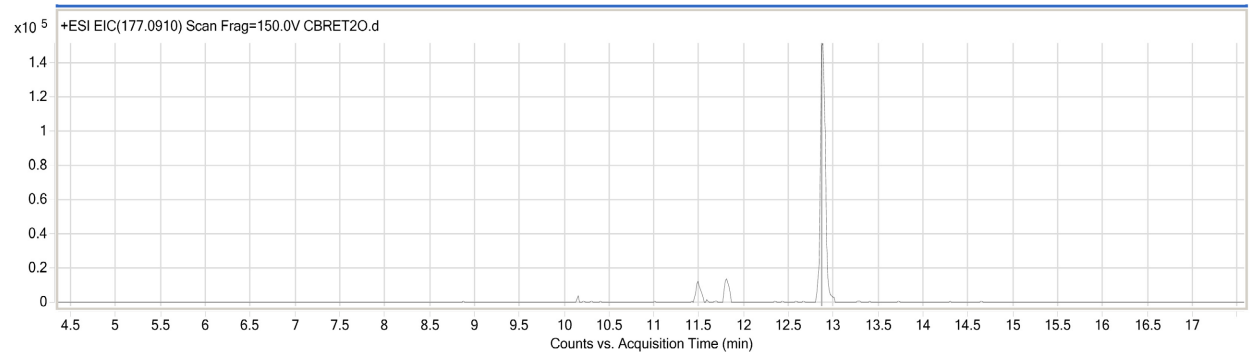

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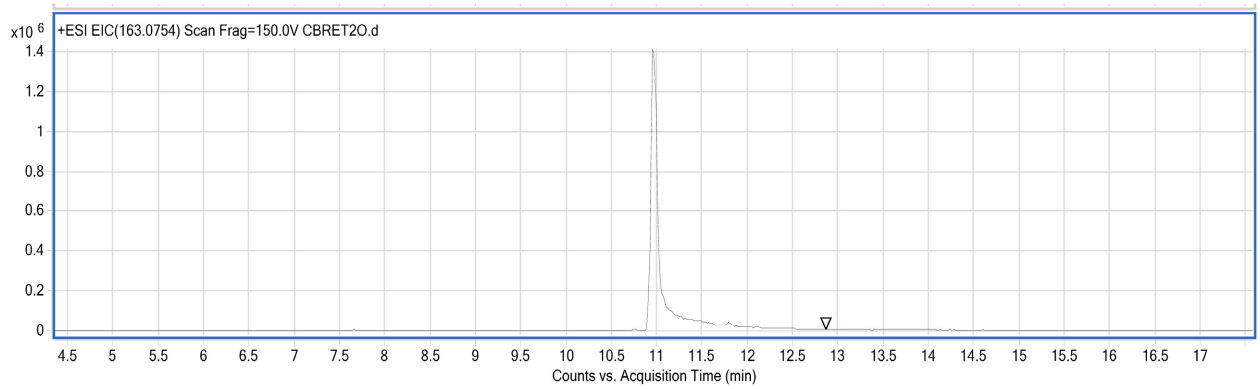

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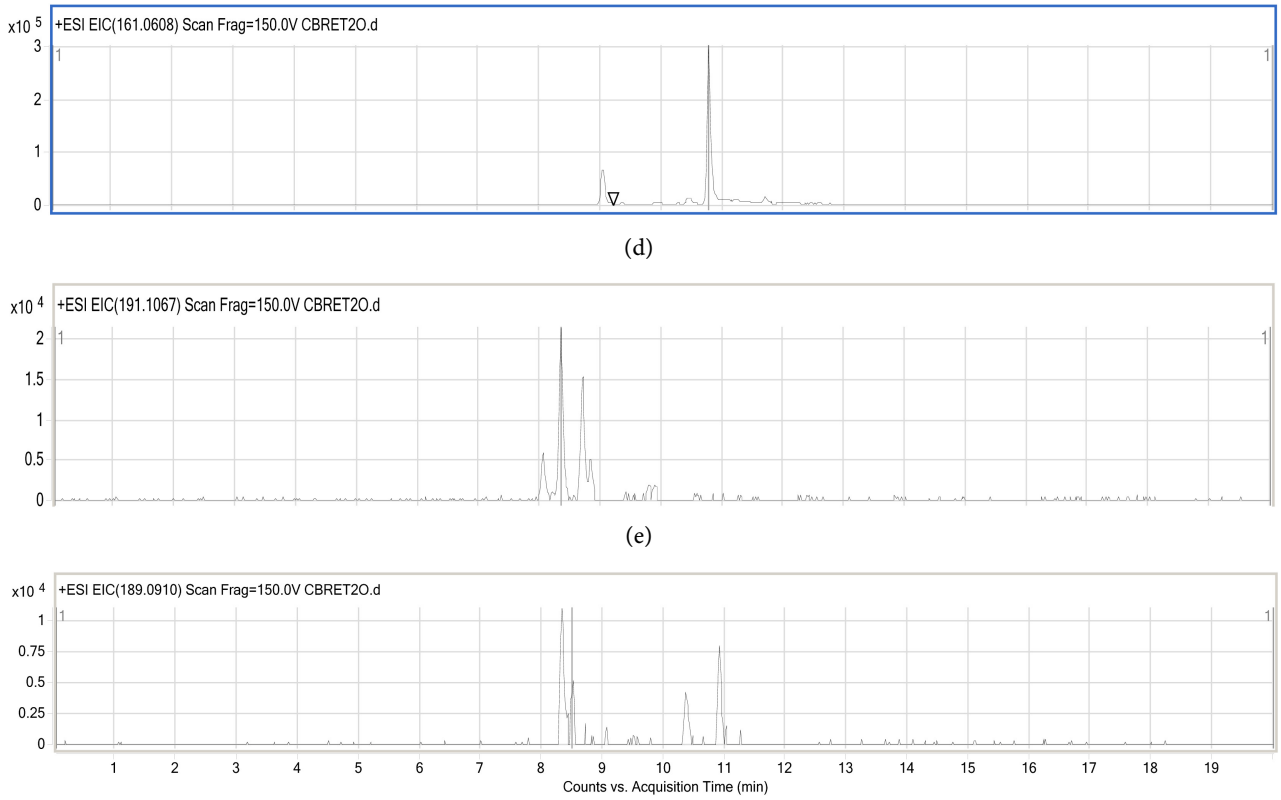
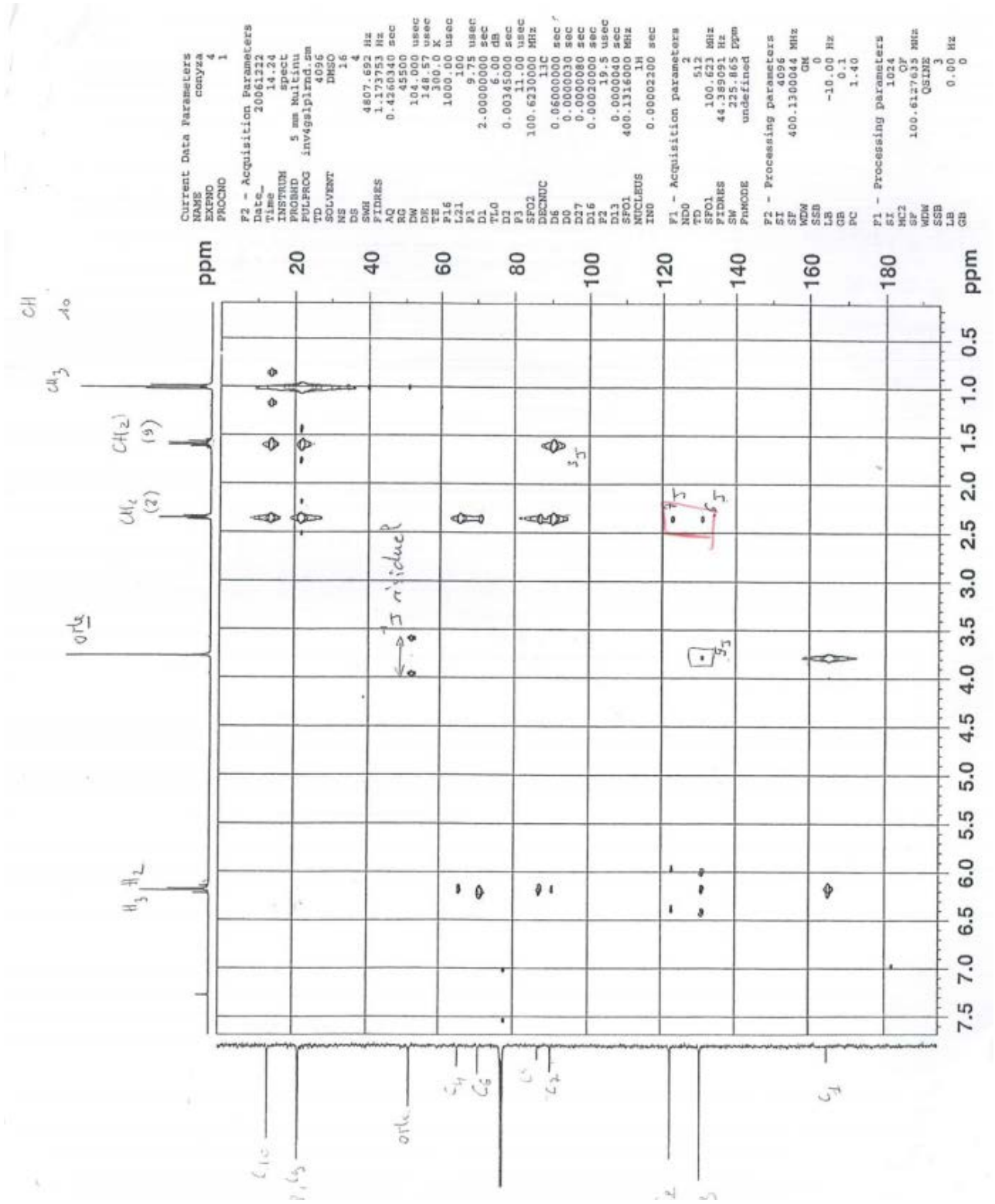


Figure S4. LC-MS Chromatograms of *C. bonariensis* root diethyl fraction (a) Total ion chromatogram; (b) *Lachnophyllum* methyl ester ion extract chromatogram; (c) *Lachnophyllum* lactone ion extract chromatogram (d) *Matricaria* lactone ion extract chromatogram; (e) *Lachnophyllum* ethyl ester ion extract chromatogram; (f) *Matricaria* ethyl ester ion extract chromatogram.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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

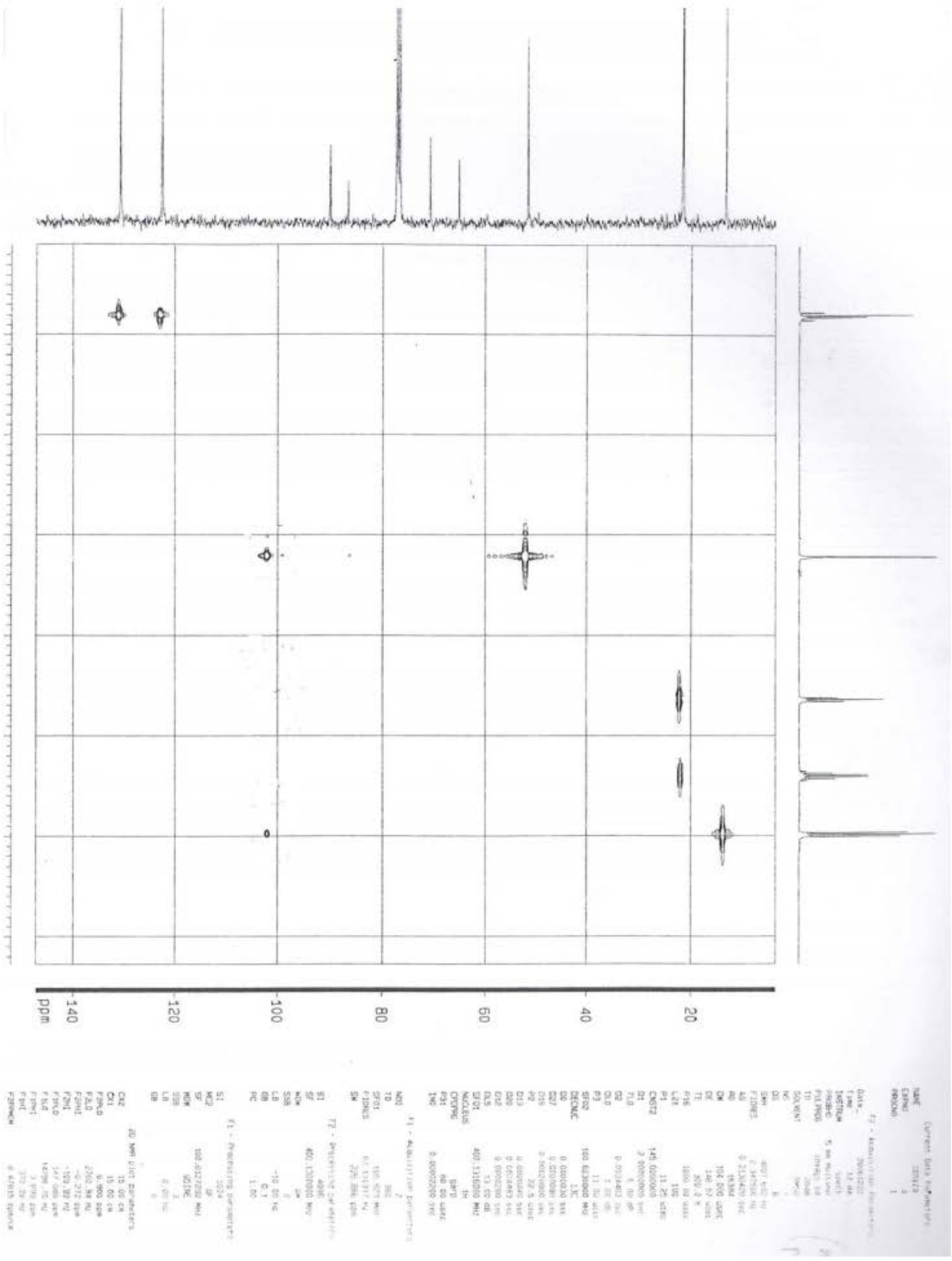

Figure S5. HMQC and HMBC of *Lachnophyllum* methyl ester.

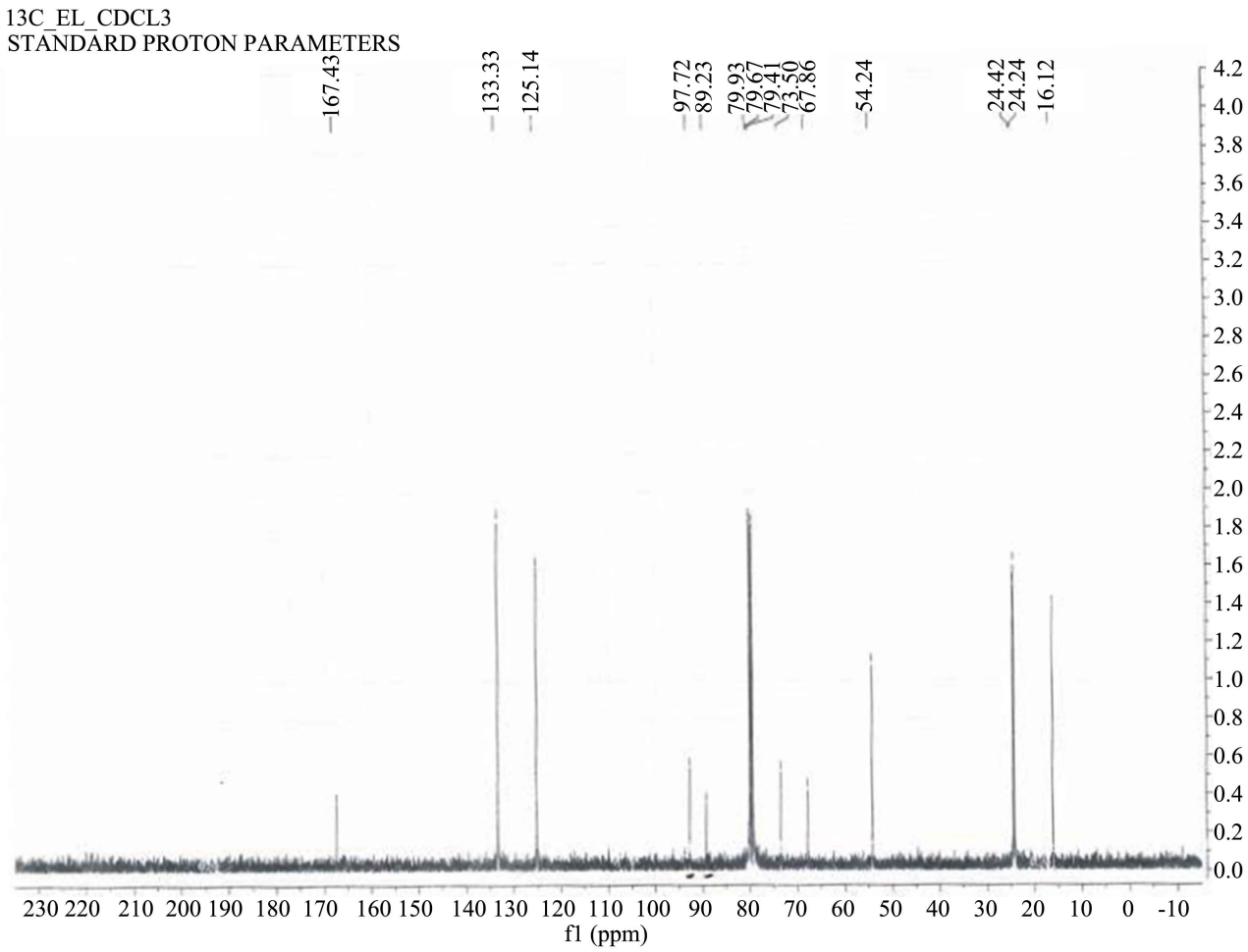

Figure S6. ^{13}C NMR of *Lachnophyllum* methyl ester.