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Phytochemical and Biological Profile of Essential Oils of *Elionurus muticus* (Spreng.) Growing in Northeastern Argentina

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Dedication to Nicolás Kolb Kozlobsky for inspiring us.

The purpose of this study was to investigate essential oils (EOs) from leaves of *Elionurus muticus* growing in Northeastern Argentina regarding their physicochemical profiles as well as their biological potential. Roots of a selected *E. muticus* population were investigated too. For this purpose, EOs of fresh materials were obtained by steam distillation and the chemical composition was characterized by gas chromatography GC/MS-FID. Antibacterial, antioxidant and eco-toxicity activities of the essential oils (EOs) were tested by *in vitro* assays. The EOs showed three *E. muticus* chemotypes: citral (neral + geranial), acorenone + bisabolone, acorenone + geranial. EO of roots of citral population contains mainly acorenone derivatives. EOs have high antibacterial effect against *Staphylococcus aureus*, being found minor antibacterial effect against Gram-negative bacteria. The half-maximal inhibitory concentration of EOs against DPPH$^{\bullet}$ were 7.1–30.0 mg/mL and the eco-toxicity was high with $LD_{50} < 39$ µg/mL. Based on the findings, given the high variability in their chemical composition and biological activity of *E. muticus* EO and the promising yields, it could be potentially chosen for industrial applications.

Introduction

The genus *Elionurus muticus* Willd (Family: Poaceae; tribe: Andropogoneae) is widespread in the tropical and subtropical regions of South America, Africa and Australia. *Elionurus muticus* (Spreng.) Kuntze, is a native grass in Argentina known as *'espartillo*, *aibé*, *pasto bravo*, *limoncillo* or *pasto limón'*. It is found in open grassland and high rainfall areas and grows wild or may be cultivated.[1]

E. muticus is a rich source of essential oil (EO) with a great variability in the composition. In some areas, different specimens of the same population shows different chemical profiles called chemotypes (CT).[2–6]

In a previous research, five CT from Misiones were reported: citral, geraniol, oxo-bisabolene, acorenone and nerolidol.[5] Especially for CT citral, a rich content of neral + geranial (> 65 %), was accompanied by high yield (> 1 %) and a great versatility for the commercial culture and exploitations.[4,5]

E. muticus constitutes a valuable resource renewable and with proper management, it can be used as a forage species to feed livestock.[1,7,8] Aerial part and its roots have been popularly used as a medicinal and aromatic plant since it has sudorific and fever-reducing properties.[9] Antioxidant and antibacterial activities of *Elionurus* sp. have been reported by some authors[3,8–11] and preliminary cytotoxicity assay on Vero cells and antifungal effect of *E. muticus* EOs on *Candida* sp. were performed in previous researches.[4]

As a part of our characterization of wild populations of *E. muticus* growing in Misiones province, the aim of this study was to determine the physicochemical and biological profile of essential oils of *E. muticus* from Misiones. Chemical composition, relative density and refractive index were assessed in leaves EOs and root EO of a selected population. For these same oils, antioxidant activity, eco-toxicity, antibacterial activity were determined by *in vitro* assays. The results were related to the chemical composition of the oils.

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Table 1. Essential oil yield, physicochemical properties and moisture content of the distilled plant materials.

EO	Moisture (%)[a]	Yield (%) (v/w d.w.)[b]	Yield (%) (v/w f.w.)[c]	Relative density	Refractive index
L1	63.7 ± 3.3	1.05 ± 0.34	0.40 ± 0.04	0.856 ± 0.014	1.495
L2	63.3 ± 5.1	0.80 ± 0.02	0.33 ± 0.03	0.850 ± 0.027	1.486
L3	64.4 ± 4.3	1.12 ± 0.04	0.38 ± 0.04	0.843 ± 0.018	1.477
R3	60.0 ± 5.4	0.60 ± 0.50	0.29 ± 0.03	1.045 ± 0.003	1.515
Lemongrass		1.93		0.872–0.979	1.438–1.489

[a] Moisture content of plant material expressed on a fresh weight basis. [b] Yield expressed in mL per 100 g of dry sample. [c] Yield expressed in mL per 100 g of fresh sample. Water density: 1.031 ± 0.024 g/mL (at 22 °C). Lemongrass oil (*Cymbopogon citratus*) in https://www.iso.org/obp/ui/#iso:std:iso:3217:ed-1:v1:en.

Results and Discussion

Essential oil characterization

Yellow EOs extracted from leaves by steam distillation yielded 0.80-1.12 % (v/w on dry weight basis), while yielded 0.33–0.40 % (v/w on fresh weight basis) distilling 1000–2000 g of fresh material with a water content of 60.0–64.4 % (Table 1). Previous studies have shown a variation between 0.1 and 0.7 % in the essential oil yield (fresh weight basis) in wild populations of *E. muticus* from state of Rio Grande do Sul, Brazil and Zimbabwe.[12,13] Yellow EO from roots yielded 0.6 % (v/w on dry weight basis), and 0.29 % (v/w on fresh weight basis) distilling 1000–1500 g of fresh material with a water content of 60.0 % (Table 1).The yields obtained in the present study mainly from leaves, are promising for scale industrial applications.

Relative density is defined as the ratio of the densities of a given EO and water when both are at identical temperatures.[14] The relative density is influenced by the chemical composition, the mean molecular weight, also as the degree of unsaturation of the oil.[15] Relative density of EOs are presented in Table 1. Leaves L1–L3 shown low density (0.843–0.856) when compared with roots R3 (1.045). Relative density obtained from leaves EOs were in the same range that lemongrass oils of high quality (Table 1). Comparing the obtained results with the results provided for lemongrass (https://www.iso.org/standard/8421.html), relative density of EO of *E. muticus* leaves is lower (Table 1). On the other hand, roots provide EO with higher density than water.

The determination of the refractive index also represents a characteristic physical property of an oil, usually ranging from 1.450 to 1.590.[14] The refractive index (RI) is influenced by the chemical composition of the EO. The RI value is used in EOs and mixtures as a rapid measure of purity and quality.[16] Refractive index assessed in leaves EOs were in the same order that lemongrass oils (Table 1). R3 oil shows a high refractive index when compared with leaves EOs.

Essential oil composition

Table 2 presents the composition of the EOs of leaves and roots and the relative amounts (%) of identified volatile compounds. In general, the chemical profile of the populations demonstrates a high diversity of compounds. Main components found in leaf EOs were oxygenated monoterpenes and oxygenated sesquiterpenes (see Table 2). Roots R3 contain mainly oxygenated sesquiterpenes followed by minor percentages of monoterpene hydrocarbons.

The essential oil profile of L1 and L2 was similar, dominated by acorenone (55.5 % and 54.0 %), 6R,7R-bisabolone (23.1 %, L1) and geranial (10.7 %, L2), respectively (Figure 1). L3 oil presents higher amount of neral (22.9 %), geranial (33.3 %) and neryl acetate (24.2 %).

Species from genus *Elionurus* have shown marked variability in their essential oil chemical composition. In Brazil, the oil essential composition varies according to region. A study in the central region identified camphene (11.5 %), (*E*)-caryophyllene (17.9 %) and spathulenol (18.6 %) as major compounds,[17] while other reported bicyclogermacrene (33.3 %) and (*E*)-caryophyllene (14 %) at southwest of the country.[8] In southern Brazil, in the border region with Argentina, neral (31.5 %) and geranial (47.3 %) were found as the major compounds of *E. muticus* essential oil. However, borneol (27.7 %) was the major compound, followed by less significant compounds, such as bisabolone (17.2 %) and bornyl acetate (11.7 %) in wild population closest to Uruguay.[12] Similarly, neral (34.9 %) and geranial (44.5 %) are also major compounds in the *E. muticus* essential oil from Zimbabwe.[13] In Argentina, *E. muticus* is classified as five chemotypes according to the major compound that is present in the essential oil.[2,4,5] Our results are in accordance with the previously reported chemical profile for *E. muticus.* All of these named compounds are present in the

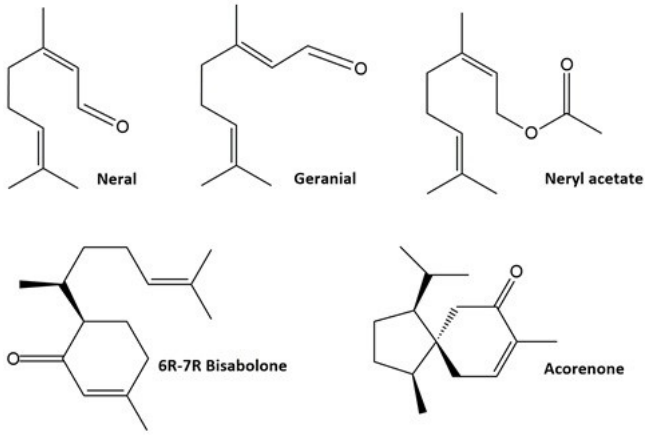

Figure 1. Main components in EOs of *E. muticus* growing in Misiones.

Table 2. Chemical composition of EOs obtained by steam distillation of leaves of L1, L2 and L3, and roots R3 of *E. muticus*.

No.	Components	RI[b]	RI[c]	L1 [%][a]	L2 [%][a]	L3 [%][a]	R3 [%][a]
1	Tricyclene	919	920	tr	–	–	–
2	α-Pinene	942	939	tr	0.18	–	0.14
3	Camphene	950–954	954	tr	0.16	0.09	0.20
4	β-Pinene	991	979	3.43	tr	1.72	–
5	6-Methyl-5-hepten-2-one	987	985	–	4.95	0.36	–
6	Limonene	1030	1029	–	0.49	–	–
7	(*Z*)-β-Ocimene	1039	1037	0.44	0.61	0.23	–
8	β-Ocimene	1049	1050	0.47	0.06	0.39	–
9	Perillene	1097	1100	tr	0.68	–	–
10	Linalool	1099	1096	tr	0.15	1.65	–
11	*trans*-α-Necrodol	1149	1148	–	–	0.10	–
12	(*E*) Isocitral	1181	1180	–	tr	1.02	–
13	Decanal	1205	1201	tr	tr	–	–
14	Neral	1238–1240	1238	0.16	7.15	22.87	1.89
15	Geraniol	1252–1253	1252	–	2.73	5.17	0.29
16	Geranial	1268	1267	0.24	10.71	33.32	3.17
17	Isobornyl acetate	1283	1285	–	0.10	tr	0.36
18	Neryl acetate	1365–1367	1361	–	–	24.19	–
19	Cyclosativene	1364	1371	tr	7.92	–	–
20	Linalool isobutanoate	1378	1375	tr	–	–	0.63
21	2-epi-α-Funebrene	1380	1382	0.26	tr	–	–
22	β-Elemene	1386	1390	tr	1.32	–	–
23	Sativene	1393	1391	–	–	0.10	–
24	Longifolene	1404	1407	–	–	–	–
25	Decyl acetate	1409	1408	–	–	tr	–
26	(*E*)-Caryophyllene	1414	1419	0.48	0.19	2.23	0.37
27	β-Dupreziaiene	1430	1422	0.12	0.05	–	–
28	β-Gurjunene	1426	1433	–	–	–	2.48
29	*trans*-α-Bergamotene	1430	1434	tr	–	–	–
30	(*Z*)-β-Farnesene	1440	1442	0.91	tr	–	–
31	Thujopsen-13	1446	–	0.10	tr	–	–
32	α-Himachalene	1447	1451	–	–	–	0.23
33	Amorfa-4,11-diene	1454	1451	0.13	–	–	–
34	α-Humulene	1452	1454	–	–	0.66	–
35	*cis*-Muurola-4(14),5-diene	1470	1466	–	–	–	4.00
36	Dauca-5,8-diene	1468–1471	1472	tr	0.07	–	–
37	γ-Muurolene	1471–1479	1479	tr	0.05	–	–
38	ar-Curcumene	1479	1480	0.48	1.28	–	–
39	γ-Curcumene	1475–1479	1482	0.12	0.29	–	–
40	Germacrene D	1486	1484	tr		–	–
41	Bicyclogermacrene	1491–1494	1500	4.78	0.11	0.92	–
42	α-Muurolene	1494–1502	1500	tr	tr	0.11	–
43	(*E*,*E*)-α-Farnesene	1501–1505	1505	tr	tr	–	–
44	β-Bisabolene	1504–1508	1505	0.22	0.24	–	–
45	(*Z*)-α-Bisabolene	1507	1507	0.70	0.52	–	–
46	γ-Amorphene	1514	1512	0.62	0.13	–	–
47	*trans*-Calamenene	1520	1522	–	–	–	0.48
48	β-Sesquiphellandrene	1521	1522	1.19	0.10	–	–
49	δ-Cadinene	1539	1523	–	–	–	1.93
50	(*E*)-γ-Bisabolene	1524	1531	tr	–	–	–
51	Germacrene B	1556–1570	1561	tr	0.50	–	–
52	(*E*)-Nerolidol	1559	1563	0.28	-	–	–
53	Geranyl butanoate	1572	1564	–	–	0.22	–
54	Spathulenol	1572–1577	1578	0.65	0.23	-	–
55	Caryophyllene oxide	1577–1579	1582	0.18	0.08	0.35	–
56	1,10-di-epi-Cubenol	1618	1619	1.71	0.14	–	–
57	γ-Acorenol	1629–1630	1630	1.37	0.13	–	–
58	γ-Eudesmol	1620	1632	0.36	–	–	–
59	α-Muurolol	1652–1655	1646	0.94	0.12	–	–
60	Cubenol	1658	1646	0.35	0.11	–	–
61	Acorenone	1688–1689	1692	55.52	54.04	2.16	–
62	6R,7R-Bisabolone	1741	1742	23.13	3.29	0.61	–
63	*cis*-Thujopsenal	1706	–	–	–	–	3.61
64	epi-α-Cadinol	1641	–	–	0.11	–	-
65	Acorenone derivative 1	1746	–	–	–	–	33.52
66	Acorenone derivative 2	1794–1800	–	–	–	0.11	44.88
	Total			99.34	98.81	98.54	98.83

[a] Percentage peak area of EO components. [b] RI, experimental retention indices in reference to C_7–C_{24} *n*-alkanes. [c] Literature data.

populations that were studied in this work, but at a lower percentage.

There are still very few data available on the full identity of components of essential oil of root of *E. muticus*. In our article, we have identified acorenone derivatives as major compounds in R3 oil (Figure 2). We believe that compounds 65 and 66 are derivatives of acorenone because in their mass spectra they show good agreement in their profile with respect to the *m/z* and relative abundance of the main peaks. Further study will be necessary to confirm this assumption. Aristolone was identified as major component in oil of root from crops in Zimbabwe. The major components in its leaf oil were geranial (44.7 %) and neral (35.4 %).[13]

On the other hand, the volatile oils of the aerial parts and roots from *E. elegans* were studied previously. The main components found in both organs analyzed were camphorenone (43.0–39.0 %) and caryophyllene oxide (4.9–4.6 %), respectively.[18]

Identification and quantification method: GC/MS. All compounds were identified based on their RI and mass spectra with literature data (Adams, 2007; WILEY/NIST, 2008), and a spectra library built up from pure substances and components of known oils; tr: traces (< 0.05 %).

Antioxidant activity

DPPH• is a stable free radical, which provides a good indication of a sample's anti-radical potential.[19] The antioxidant activity for the essential oil against DPPH• radical was tested and is displayed in Table 3. Results are expressed as the IC_{50} value, defined as the concentration that results in 50 % inhibition of free radical in solution. A low IC_{50} value corresponds to a high antioxidant activity. A concentration-dependent potential was observed in all assayed samples. The root of *E. muticus* gave a percentage scavenging activity smaller than the aerial part plant, showing that the roots contribute little to whole plant antioxidant activity.

This can be due to the fact that the molecules that act as free radicals scavengers are synthesized in the aerial parts. Regarding the antiradical activity against DPPH•, Xu et al. reported that there is a correlation between the citral content of an essential oil and its free radical scavenging capacity.[20] Our results are in agreement with Xu report: L3 (citral CT) showed greater antioxidant activity than L2 and L1 (rich in acorenone).

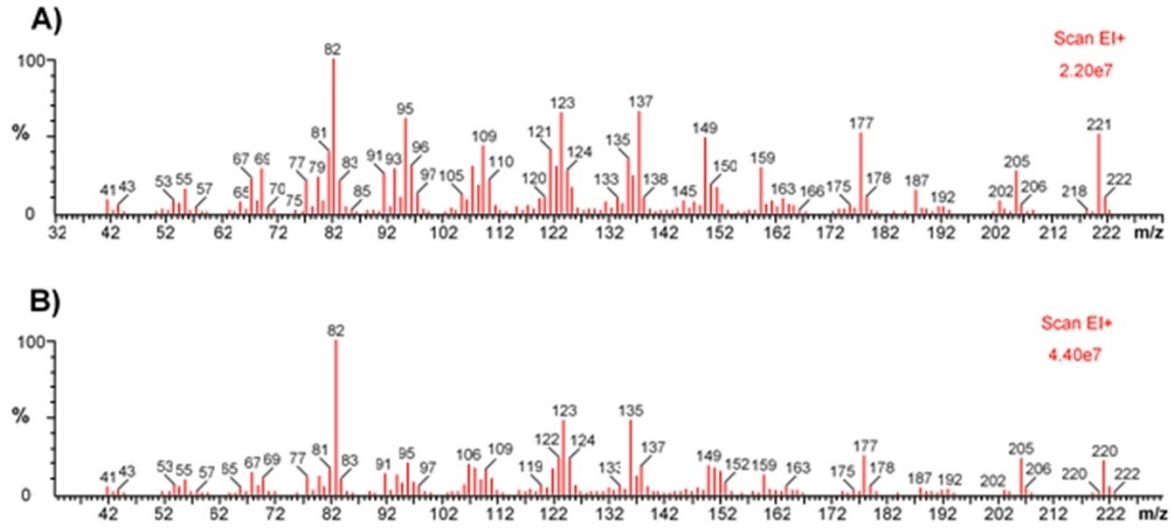

Figure 2. Mass spectra of A) Acorenone derivative 1, *m/z* (%): 82 (100); 137 (71.2); 123 (65.4); 95 (58.1); 221 (53.8); 177 (52.7); 149 (47.8); 205 (26.5); 222 (8.6) and B) Acorenone derivative 2, *m/z* (%): 82 (100); 123 (46.2); 135 (42.8); 177 (22.3); 205 (22.7); 220 (20.6).

Table 3. Antioxidant activity (IC_{50}) and general toxicity of EOs (LD_{50}) of *E. muticus*.

Samples	IC_{50}		LD_{50}	
	µL/mL	mg/mL	µL/mL	µg/mL
L1	$12.1 \pm 0.6^{[c]}$	10.3 ± 0.5	< 0.01	< 8.0
L2	$10.8 \pm 0.3^{[b]}$	9.1 ± 0.3	0.027 [0.012; 0.039]	23.0 [5.3; 36.8]
L3	$8.4 \pm 0.2^{[a]}$	7.1 ± 0.2	0.046 [0.037; 0.057]	39.0 [28.3; 36.8]
R3	$28.7 \pm 1.8^{[d]}$	30.0 ± 2.0	< 0.01	< 10.0

IC_{50}: scavenging activity against DPPH•. LD_{50}: median lethal dose against *A. salina*. [a–d] Means with different superscripts are significantly different (*p*-value < 0.05).

General toxicity assay

The EOs present high toxicity against *Artemia salina*. The results are showed in Table 3. The assay of eco-toxicity against *A. salina*, is used as a preliminary means to determine which plant extracts could be subjected to more elaborate bioassays in search of bioactive compounds with pharmacological activity.[21]

The criteria for classifying eco-toxicity against *A. salina* is the LD_{50} value; values of $LD_{50} > 1000$ μg/mL are considered non-toxic, those between 500 and 1000 μg/mL are slightly toxic, those between 100 and 500 μg/mL are moderately toxic and those < 100 μg/mL as strongly toxic.[22]

The test with *A. salina* revealed that the essential oils of all samples are highly eco-toxic. This result suggests that the toxic effect could be mainly due to the high content of acorenone and acorenone derivates found in the essential oil (L1, L2 and R3). However, the essential oil L3, with high content of citral and neryl acetate, showed the lowest toxicity. In agreement, several studies have shown the toxic activity of citral in various organisms and cell lines.[4,23,24] *A. salina* has purine metabolism similar to mammalian cells and has been demonstrated to have a good correlation with antitumor activity.[25] Even more, the lethality of *A. salina* may be used as an indicator of antitumor compound and insecticide activity.[22] Therefore, leaves and root may be chosen for the search of potential cytotoxic agents in future investigations.

Antibacterial activity

The EOs obtained from the aerial parts and root of *E. muticus* were tested against a set of strains. The result obtained are presented in Table 4.

Several authors have developed individual criteria to classify the tested natural products. According to Aligiannis et al. (2001), based on the MIC of plant extracts we can classify their inhibition as: strong inhibitors, with MIC up to 0.5 mg/mL; moderate inhibitor – MIC between 0.6 and 1.5 mg/mL; and weak inhibitor, with MIC greater than 1.5 mg/mL.[26] The MIC and MBC determined by microdilution tests reveal that the EO of *E. muticus* L3 was more active against *S. aureus*, and a minor effect were showed against Gram-negative strain being *E. coli* the most susceptible.

The antimicrobial capacity of tested EOs can be conditioned by their chemical composition. The L1 and L2 EOs rich in acorenone (Table 2) showed a weak inhibitory activity, in line with a report for oil from the aerial parts of *Niphogeton dissecta*.[27] While geranial and neral found in EO from L3, exhibit remarkable antimicrobial capacity against Gram-positive and Gram-negative bacteria.[28]

Previously, Cacciabue et al. (2005) using the disk diffusion method, presented a comparative study on antibacterial effect of espartillo essential oils showing that Gram positive strains were more sensible than Gram negative.[3] Besides, EOs of *E. muticus* rich in sesquiterpenoids ((*E*)-caryophyllene, bicyclogermacrene, spathulenol and caryophyllene oxide), were tested against *S. aureus* ATCC 25923 (MIC 0.5 mg/mL) and *E. coli* ATCC 25922 (MIC 2 mg/mL). Interestingly, the results reveal that the EO extracted from the aerial parts collected in the spring with percentage of caryophyllene oxide and spathulenol very much higher than in the oils obtained in the other seasons of the year, was four times more active against *S. aureus*.[8]

Conclusions

In this study, we report the physicochemical and biological profile of essential oils from leaves of three populations of *E. muticus* growing in Misiones. We further investigated the roots of a select CT of *E. muticus* from the same populations. We found great variability in the composition of leaf oils containing mainly neral, geranial, acorenone and bisabolone in defined proportions. While in the roots of citral CT two derivatives of acorenone were found as major components.

Leaves EOs showed promising results of antiradical activity against $DPPH^{\bullet}$. The essential oils of *E. muticus* present high toxicity against the nauplii of *A. salina*, being the EOs of leaves CT acorenone and root EO with derivatives of acorenone as main components the ones that present very high eco-toxicity. Therefore, leaves and root may be chosen for the search of potential cytotoxic agents in future investigations The results of antibacterial activity indicate that the EOs of leaves and roots of *E. muticus* "CT citral" have an interesting potential to be used in the treatment of bacterial infections.

Based on the results obtained and the yields of EOs, it is of interest to continue investigating the essential oils of leaves and roots of *E. muticus* from Misiones.

Table 4. Antibacterial activity of the essential oils isolated from *E. muticus*.

Strains	L1		L2		L3		R3		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	2.1	4.2	0.5	1.1	0.1	0.3	0.3	0.6	0.01	0.05
E. coli	4.2	8.4	4.3	8.5	1.1	1.1	1.2	2.3	0.02	0.06
E. faecalis	4.2	8.4	4.3	8.5	2.1	2.1	1.2	2.3	0.02	0.12
P. aeruginosa	4.2	8.4	4.3	8.5	2.1	8.4	2.3	9.2	0.02	0.06
B. contaminans	4.2	8.4	4.3	8.5	2.1	8.4	2.3	4.6	0.02	0.03

MIC and MBC expressed in mg/mL.

Experimental Section

Standards and reagents

Gallic acid (GA) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) were purchased from Sigma–Aldrich (Steinheim, Germany), sodium chloride (NaCl), was purchased from Sigma–Aldrich (St. Louis, MO, USA). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) media were purchased from Liofilchem (Teramo, Italy). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Other chemical and reagents used were of analytical grade.

Plant material and collection data

Leaves of *E. muticus* (L1, L2, L3) in the vegetative stage were collected in October 2019 from three homogenous populations in departments Capital and Candelaria (Misiones, Argentina).

The sampling of the *E. muticus* populations were carried out in a randomized manner, ensuring the representativeness of the sampled populations. *E. muticus* roots (R3) from a selected population (L3) were collected too, washed and conditioned for the essential oil recovery. For L1 and L2, an eco-sustainable sampling was guaranteed. The roots of L1 and L2 could not be obtained.

The moisture content was determined by drying the samples (2–3 g) at 102 ± 2 °C to constant weight. The specimens plants were recognized and authenticated by Dr. Felipa Sánchez González (Taxonomist), Biology Department, National University of Misiones. Voucher specimens (HN036, HN037, HN038, HN039) from each plant were deposited in the Herbarium of the National University of Misiones. The faculty research committee approved the permission for plant collection (Project 16Q1119-PI).

Essential oil extraction

EOs were extracted from fresh samples of 1500–2000 g by steam distillation for 2.5 h using a lab scale extractor, condenser and Clevenger trap. Each distillation was performed in triplicate. The collected oils were dried (Na_2SO_4) and stored at 4 °C until treated and analyzed. The essential oil content was determined volumetrically on a fresh weight basis (f.w.) and a dry weight basis (d.w.).[29]

Physicochemical characterization of essential oils

Refractive index of essential oils was determined by using a laboratory refractometer.[30] Relative density (22 °C) was determined by using the pycnometer method.[31] Density measurements were repeated three times. Water density was used as reference for the assays.

Chemical profile of essential oils

EO composition was analyzed by GC/MS-FID. GC/MS analysis was carried out on a PerkinElmer Clarus 600 MS apparatus equipped with a DB-5 MS Ultra Inert (DB-5MS UI) column (30 m × 0.25 mm × 0.25 μm) and a GCMS software TurboMass version 5.4.2. The injector port was heated to 250 °C with helium as carrier gas at constant flow rate of 1.0 mL/min. The oven temperature was set at 40 °C during 5.33 min, increasing by 3 °C/min to 246 °C (total time: 74 min). All mass spectra were acquired in electron impact mode. Ionization was turned off for the first 3 min to avoid solvent overloading. The mass ranged from 50 to 450 *m/z*. The injection volume was 2 μL from a solution of 20 μL/mL (EO/ketone) in a splitless injection mode and the analysis was performed in the full-scan mode.

The components of the EO were identified by comparison of their Retention Indices (RI) and mass spectra with those from the literature data, the NIST or Wiley mass spectral library resident in the system, and those of a spectra library built up from pure substances and components of known oils.[32] The RI was determined relative to a series of n-alkanes (C7–C24).

Quantitative analysis of the EO was carried out using a GC-FID Konik 3000G equipped with a flame ionization detector and a DB-5MS UI capillary column (30 m × 0.25 mm with 0.25 μm). The oven temperature was set at 40 °C during 5.33 min, increasing by 3 °C/min to 246 °C. The injector and detector temperatures were 250 and 310 °C, respectively. Helium C-60 was used as a carrier gas at a constant flow rate of 1.5 mL/min, and 1 μL samples were injected using a split ratio of 1 : 20. The percentage composition of the products was calculated by normalization of the GC peak areas without response factors.

Antioxidant activities assay

The disappearance of DPPH• was monitored spectrophotometrically at 515 nm, according to Celaya et al. (2021) with modifications. Convenient dilutions of EOs in methanol (132–2 μL/mL) were tested against DPPH•. Each EO was performed in triplicate.[33] Mean values were compared using two way ANOVA and Tukey test to determine differences with statistical significance.

General toxicity assay

Eco-toxicity of EOs was determined against *Artemia salina* nauplii according to Celaya et al. (2022) with modifications.[34] Eggs from *A. salina* were hatched at 24–26 °C in seawater (pH 8.0) in contact with a light source (70 watt). After 48 h three tubes with groups of 10 *Artemia* nauplii were prepared for each dose. Test solutions at appropriate EO amounts (final concentration 0.1, 0.01 and 0.001 μL/mL) were prepared in DMSO and distilled deionized water, and transferred into 5 mL tubes. The maximum DMSO concentration did not exceed 0.1 % (v/v). The control group consisted of seawater, DMSO and nauplii. All tubes were maintained under illumination. The lethal concentration fifty LC_{50} (95 % confidence interval), was determined from the 24 h counts using the probit analysis method.[22] Each EO was tested in triplicate.

Antibacterial activity determination

The study included one Gram-positive bacteria: *Staphylococcus aureus* (ATCC 20231), and four Gram-negative bacteria: *Escherichia coli* (ATCC 30083), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (DMS 50071) and *Burkholderia contaminans* (LGM 23361). These species were selected due to their great importance in foods and health.[35–36] Cultures were obtained from the Plant Biochemistry Lab, Institute Leloir Foundation, IIBBA-CONICET (Argentina) and Center for Research and Development in Industrial Fermentations, Faculty of Exact Sciences, National University of La Plata (Argentina).

Minimum inhibitory concentration (MIC) was determined by employing broth microdilution methods based on the Clinical and Laboratory Standards Institute (CLSI) guidelines, reference document M100, with minor modifications.[33,38] Briefly, the suspensions of bacteria cultures were prepared in ampoules containing NaCl

0.85% suspension medium. After adjusting the turbidity to 0.5 McFarland, suspensions were diluted in Mueller Hinton broth medium (MHB, Britania SA, Argentina) until the final bacterial density of 1.5×10^6 CFU/mL. The MIC of extracts was determined by two-fold serial dilution method, in 96-well plates. Briefly, 100 μL of the bacterial suspension was added in each well, which contained 100 μL of EO dilutions in MHB medium. The maximum DMSO concentration did not exceed 2% (v/v). The initial concentration of EO was 20 μL/mL. The plates were incubated at 37 °C in a humidified atmosphere containing 500 ppm CO_2, without agitation for 18–24 h. The MIC was determined as the lowest concentration of dried extracts inhibiting the visual growth of the test culture on the microplate. Sterility and positive controls in MHB medium alone and with 2% of DMSO (v/v) were included. Positive control wells contained microorganisms without EO. The experiments were performed in duplicate and repeated independently three times, yielding essentially the same results.

MBC (minimum bactericidal concentration) of extracts was also accessed. The MBC was determined after 18–24 h of incubation for both Gram positive and Gram negative bacteria, by removing 20 μL of the contents from all wells showing no visible growth to MH agar medium plates. The plates were incubated at 37 °C. The MLC was defined as the lowest concentration showing 100% growth inhibition.

Author Contributions

LSC and PFM conceived and designed the study. ALV and PFM did data collection and entry. LSC and CIV performed physicochemical properties analysis. CIH and CIV performed the chemical composition analysis. LSC performed the biological activity analysis. PFM was responsible for the microbiology analysis. ALV, LSC and PFM carried out the data analysis and interpretation. LSC, PFM and ALV wrote the manuscript. CIV and CIH revised the manuscript critically. All authors approved the final version for submission.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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