Artigo

EVALUATION OF SEASONAL CHANGES IN CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Elyonurus muticus* (SPRENGEL) O. KUNTZE (GRAMINEAE)

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Aerial parts of *Elyonurus muticus* were collected in the four seasons of the year in the Brazilian Pantanal and subjected to extractrion with cold ethanol and to hydrodistillation. Sesquiterpenoids (E)-caryophyllene, bicyclogermacrene, spathulenol and caryophyllene oxide were the main components identified in the essential oils and their concentrations varied according to the plant collection period. The essential oils and the ethanolic crude extracts were active against *Bacillus cereus* MIP 96016, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 and were not active against *Escherichia coli* ATCC 25922. The antibacterial activities varied according to the plant collection period.

Keywords: *Elyonurus*; essential oil; antibacterial activity.

INTRODUCTION

The genus *Elyonurus* Humb. et. Bompl ex. Willd (Gramineae) is common in the tropical and subtropical regions of South America, Africa and Australia[1]. *E. muticus* is employed as medicinal and aromatic plant in Argentina, where it is known as "espartilho" or "aibe"[2], and also in Bolivia, where it is named "paja carona" or "Karunásh"[3] and Uruguay[4].

As part of integrated phytochemical – biological studies of plants from the Mato Grosso do Sul flora[5], we investigated *Elyonurus muticus* (Sprengel) O. Kuntze (Gramineae). In the Brazilian Pantanal, *E. muticus* is popularly known as "capim carona", a grass that reaches one meter high and grows on sandy poor soils not subjected to periodic flooding, in the region known as Nhecolandia, burning even green, due to the presence of essential oil[6]. *E. muticus* is not appropriate for cattle feeding as the bitter taste is transmitted to the milk, although the young plant is grazed[7,8]. It is periodically burned by the farmers, who consider this plant a problem for cattle breeding[6].

The aim of this study was to evaluate the chemical composition and the *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria, of the ethanolic crude extracts and essential oils of *E. muticus'* aerial parts collected in the region of Nhecolandia, Brazilian Pantanal, in the four seasons of the year.

EXPERIMENTAL

Plant material

Elyonurus muticus specimens were collected in the region known as Nhecolandia, in the Brazilian Pantanal (18° 59' S and 56° 39' W, Corumbá/MS, Brazil). The aerial parts of at least six plant samples each time were collected in April 20th, 2001 (autumn), August 31st, 2002 (winter), October 15th, 2001 (spring) and February 21st, 2002 (summer). The collected plant materials were kept under refrigeration, in the dark. Voucher specimens (*SMAC* 20121) were deposited in the Herbarium of Centro de Pesquisa Agropecuária do Pantanal (CPAP) – Embrapa, Corumbá, MS, Brazil and authenticated by MSc. S. M. A. Crispim (CPAP/Embrapa).

Extraction

The aerial parts of *E. muticus* were exhaustively extracted with cold EtOH, in the dark. From 1,124.00 g of the plant materials collected in the autumn were obtained 16.34 g of ethanolic crude extract (1.45% w/w); from the materials collected in the winter (750.00 g) were obtained 12.55 g of extract (1.67% w/w); from the materials collected in the spring (821.00 g), 7.42 g of ethanolic extract (0.90%); and from the aerial parts collected in the summer (625.00 g), 10.15 g of extract (1.62%).

The essential oils were obtained from fresh aerial parts by hydrodistillation in a Clevenger type apparatus for ten hours. The aqueous phase was extracted with diethyl ether, dried over anhydrous sodium sulphate and concentrated under low pressure and temperature to yield the essential oils. From 172.00 g of the plant materials collected in the autumn were obtained 0.40 g of essential oil (0.23% w/w); 171.00 g of the samples collected in the winter furnished 0.50 g of essential oil (0.29% w/w); 296.00 g of plant materials collected in the spring furnished 1.10 g of essential oil (0.37%); and from 263.00 g of the materials collected in the summer were obtained 0.65 g of essential oil (0.25% w/w).

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Analysis of the essential oils

GC analyse were conducted using an Hewlett-Packard 6890 apparatus fitted with an HP-5 fused silica capillary column (30 m, 0.25 mm, 0.25 μm). Sample volumes of 0.5–1.0 mL were injected, and pressure programming were used to maintain a constant flow (1 mL min^{-1}) of the helium carrier gas. The mass spectrometer Hewlett-Packard 5973 was used in the EI mode (ionization energy of 70 eV) and set to scan the mass range of 50–700 a.m.u. at the rate of 2.94 scans per sec. The interface temperature was maintained at 280 °C. The resulting data were processed by the Hewlett-Packard Chemstation Software package. Temperature programming for the analyse was from 50 to 290 °C at 4 °C/min. The injector temperature was 240 °C. The retention indices were obtained by co-injecting the oil samples with a $C_{11} – C_{24}$ linear hydrocarbon mixture and used to identify the compounds present, together with the comparison to the Wiley 275 mass spectra library and with the literature[9].

Antimicrobial assay

The antibacterial activities of the essential oils and the crude ethanolic extracts were investigated by employing a microdilution method[10]. The assay was carried out with four bacterial species: *Escherichia coli* ATCC 25922 (American Type Culture Collection), *Bacillus cereus* MIP 96016, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Mueller-Hinton agar and broth (Difco Laboratories, Detroit, USA) were used for bacterial growth. The inoculum was an overnight culture of each bacterial species in Mueller-Hinton broth diluted in the same media to a final concentration of approximately 10^8 CFU mL^{-1}. Essential oils and ethanolic crude extracts were dissolved in dimethyl sulfoxide (DMSO) (10% of the final volume) and diluted with Mueller-Hinton broth to a concentration of 2 mg mL^{-1}. Further 1:2 serial dilutions were performed by addition of Mueller-Hinton broth to reach a final concentration in the range of 2 to 0.0156 mg mL^{-1}. 100 μL of each dilution were distributed in 96-well plates, as well as a sterility control (growth control contained Mueller-Hinton broth plus DMSO, without antimicrobial substance). Each test and growth control well was inoculated with 5 μL of a bacterial suspension (10^8 CFU mL^{-1} or 10^5 CFU $well^{-1}$). All experiments were performed in triplicate and the microdilution trays were incubated at 36 °C for 18 h. Bacterial growth was firstly detected by optical density (ELISA reader, CLX800-BioTek Instruments) and afterwards by addition of 20 μL of an alcoholic solution (0.5 mg mL^{-1}) of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) (Sigma). The trays were again incubated at 36 °C for 3 h, and in those wells where bacterial growth occurred, INT changed from yellow to purple. Any remaining yellow color indicated absence of growth. Before the addition of INT, a subculture was made from each well without apparent growth to determine MBC. MIC and MBC values were defined as the lowest concentration of each plant material, which completely inhibited growth or yielded no viable microorganisms, respectively. The results were expressed in milligrams per millilitre. Penicillin and tetracycline were used to assess the MIC of the reference strains.

RESULTS AND DISCUSSION

The crude ethanolic extracts obtained from the aerial parts of *E. muticus* were active against the Gram-positive bacteria *B. cereus* MIP 96016, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 and were not active against the Gram-negative *E. coli* ATCC 25922. The Minimal Inhibitory Concentration (MIC) values determined by microdilution tests are presented in Table 1. The results show that the ethanolic crude extract obtained from the plant materials collected in the autumn was the most active against *P. aeruginosa*, and the extract obtained in the spring was the activest against *B. cereus* and *S. aureus*. The yield (w/w) of the ethanolic crude extracts obtained from the aerial parts of *E. muticus* varied according to the plant collection period: 1.45% in the autumn, 1.67% in the winter; 0,90% in the spring; and 1.62% in the summer. The presented data indicate that the ethanolic extract obtained from plants collected in the spring was the most active against the Gram-positive bacteria and was present in the least concentration in the raw plant material.

Table 1. Minimal Inhibitory Concentrations against *B. cereus* MIP 96016, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 of the crude ethanolic extracts obtained from the aerial parts of *E. muticus*

Plant collection period	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923	*B. cereus* MIP 96016
Winter	>2.0*	2.0	2.0	2.0
Spring	>2.0	2.0	0.5	0.5
Summer	>2.0	2.0	2.0	1.0
Autumn	>2.0	1.0	1.0	1.0

*Expressed in mg mL^{-1}

The essential oils obtained from the aerial parts of *E. muticus* were tested against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. The MIC determined by microdilution tests are presented in Table 2 and the results reveal that the essential oil of *E. muticus* extracted from the aerial parts collected in the spring was four times more active against *S. aureus* than the oils obtained in the other seasons of the year, from the same part of the plant. Every tested oils were active against *E. coli*. The weight percentage of essential oil present in the aerial parts of *E. muticus* varied greatly, according to the season of plant collection. The highest essential oil yield (w/w) in relation to the plant material weight was obtained in the spring (0.37%), being 60.87% higher than the oil yield obtained in the autumn (0.23%), 48.00% higher than in the summer (0.25%) and 27.59% higher than in the winter (0.29%). The results indicate that in the spring the plant materials contain a higher concentration of essential oil and that this oil is more active against *S. aureus* than the essential oil present in the same plant, in the other seasons of the year.

Table 2. Minimal Inhibitory Concentrations against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 of the essential oils present in the aerial parts of *E. muticus*

Plant collection period	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
Winter	2.0*	2.0
Spring	2.0	0.5
Summer	2.0	2.0
Autumn	2.0	2.0

*Expressed in mg mL^{-1}

The compositions of *E. muticus* leaves' essential oils are presented in Table 3. The sesquiterpenoids (E)-caryophyllene, bicyclogermacrene, spathulenol and caryophyllene oxide were the main components identified, and their percentage in the essential oils changed according to the period of plant collection. (E)-

Table 3. Percentage composition of the essential oils present in the aerial parts of *E. muticus*

Components	Percentage				RI calculated	RI literature[9]
	Winter*	*Spring*	Summer	Autumn		
Myrcene	1.7	0.3	7.1	0.5	991	991
Limonene	2.2	3.4	3.5	4.6	1027	1031
β-Elemene	2.0	2.0	2.4	2.3	1391	1391
α-Gurjunene	0.3	0.2	0.2	0.4	1409	1409
(E)-Caryophyllene	19.8	19.0	18.1	14.1	1417	1418
Epi-β-Santalene	0.7	0.8	0.5	0.5	1447	1449
α-Humulene	3.5	3.0	3.3	3.0	1451	1454
α-Patchoulene	2.1	1.5	1.7	2.3	1458	1456
9-Epi-(E)-Caryophyllene	0.7	1.2	0.7	0.9	1467	1467
Ar-Curcumene	1.3	1.6	1.0	0.8	1483	1483
Bicyclogermacrene	10.5	5.7	19.5	33.3	1495	1494
Trans-β-Guaiene	0.4	0.4	0.1	0.5	1503	1500
D-Cadinene	0.3	0.1	0.3	0.4	1523	1524
Ledol	0.9	0.6	0.7	1.0	1564	1565
Spathulenol	7.4	15.0	6.6	5.6	1576	1576
Caryophyllene oxide	4.0	13.4	2.4	5.1	1580	1581
Viridiflorol	2.6	1.7	2.1	3.1	1589	1590
Acorenone	0.6	0.3	0.3	0.3	1683	1685
Not identified	39.0	29.8	29.5	21.3		

*Plant collection period

Caryophyllene was the main component in the oils collected in the winter and spring, while bicyclogermacrene was the main component of the samples obtained in the summer and autumn. In the essential oil extracted from the leaves collected in the spring, the percentage of caryophyllene oxide and spathulenol were very much higher than in the oils obtained in the other seasons of the year. Considering that caryophyllene oxide[11] and spathulenol[12] have been reported to present notable antibacterial activity against *S. aureus*, the presence of this substances in high yield in the oil obtained from the plants collected in the spring may be related to the antibacterial activity presented by that oil.

In Argentina, *E. muticus* is classified into five chemical types, according to the major components present in the essential oils: citral, geraniol, acorenone, iso-acorone and 1,8-cineole. The two first types are the most usefull for industrial purposes[1,13]. Neral and geranial were, also, the main componentes of *E. muticus*' essential oils obtained from plants collected in Uruguay[4] and in Zimbabwe[14], but were absent in the oils evaluated in the present study, obtained from the same plant. A previous study[8] reported that the essential oil present in the leaves of *E. muticus* also collected in the region of Nhecolandia – Brazilian Pantanal, in April-1996, contained camphene (11,5%), (E)-caryophyllene (17,9%) and spathulenol (18,6%) as the major components, which differ from the results described in this report.

The region of Nhecolandia, where the plant materials were collected, is subjected to very diverse climate conditions, at different seasons of the year. In the winter, the rain is scarce, the mean temperature is around 20 °C and the minimal values reach near 0 °C. In the summer, the mean temperature is around 32 °C and the maximum values reach above 40 °C[15]. The results presented in this study indicate that biotic and abiotic factors present in the environment possibly interfere with the yield, the chemical composition and the antibacterial properties of the materials obtained from the aerial parts of *E. muticus*. The presented data suggest that this plant might be used as a source of materials for cosmetic or medicinal purposes but, considering the described seasonal variations, the plant materials should be continuously evaluated, before their use.

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