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Composition and Some Biological Activities of the Essential Oils from an African Pasture Grass: *Elionurus elegans* Kunth.

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The volatile oils of the aerial parts and roots from a pasture plant, *Elionurus elegans*, were studied by GC-MS analyses. Both organs studied contained only terpenic constituents. The main components found in the extract essential oils of the aerial parts were campherenone (43.0%), caryophyllene oxide (4.9%), and bisabolone (4.9%), whereas those found in the root essential oils were campherenone (39.0%), *epi*-β-santalene (12.0%), and caryophyllene oxide (4.6%). Furthermore, the oils were tested for antibacterial and antifungal activities. The results obtained led to a nonsignificant inhibitory effect, although an increase of the lag stage was shown for the kinetics growth of *Candida albicans*, *Saccharomyces cerevisiae*, *Enterococcus hirae*, and *Staphylococcus aureus*. When α-tocopherol is used as a control, the antioxidant activities of the oils obtained from the aerial parts and roots were 30 and 46% IC_{50}, respectively.

KEYWORDS: ***Elionurus elegans*; Gramineae; essential oils; campherenone; GC-MS; antibacterial and antifungal activities; antioxidant**

INTRODUCTION

The genus *Elionurus* (Gramineae) comprises ∼26 species distributed throughout the high-temperature regions except for Europe (*1*). Also, 15 species of this taxonomic group are found in tropical and subtropical areas of Africa, America, and Australia (*2*).

Elionurus elegans is an aromatic grass widespread in open grasslands of western Africa. The plant is locally a pasture grass for both beef and sheep livestock and is also used as an antiseptic in traditional medicine. To our knowledge, the volatile oils of this plant have not been reported. On the other hand, *Elionurus muticus* was shown to be a palatable grass for beef cattle in Argentina (*3*), but the use of this plant in African folk medicine led to analyses of the essential oil contents. Geranial, neral, and geranyl acetate were shown as the main components in both roots and aerial parts of *E. muticus* (*4*).

As part of our contribution, the essential oils of *E. elegans* were studied in terms of their composition with emphasis on some of their biological properties.

MATERIALS AND METHODS

Plant Material. Samples of *E. elegans* were collected at the fructification stage, randomly, in September 2000 near the city of Ouagadougou (Burkina-Faso). Botanical identification was carried out by Prof. Guinko S., and a voucher specimen was deposited in the herbarium of the Laboratory of Dynamique et Ressources du Végétal, University of Aix-Marseille-I.

Isolation of Essential Oils. Plant materials were air-dried at room temperature conditions. Aerial parts (stems, leaves, and fruits) and roots were crushed separately prior to hydrodistillation with a Clevenger-type apparatus. Each distillation was carried out with 300 g of plant material for 2 h.

Chemical Analysis. The composition of the hydrodistilled extracts was performed through a Hewlett-Packard 5972 capillary GC-quadrupole MS system (EI, 70 eV). This gas chromatograph was equipped with a 25 m × 0.2 mm i.d. fused silica column coated with DB1, and He was used as carrier gas (1 mL min^{-1}). Oven temperature was set at 60 °C for 3 min and then programmed from 60 to 220 °C at 3 °C min^{-1}. The different compounds were identified by comparison with a published mass spectral database (*5*) and generated library of retention indices (*6*). Quantitative analysis of each oil component (expressed in percent) was carried out by peak area normalization measurements.

Bioassays. Crude essential oils of *E. elegans* were tested for bactericidal and fungicidal activities as previously described (*7*). The concentrations used were in the range of 0.78−100 μg mL^{-1}. Antibacterial and antifungal activities were determined in terms of minimum inhibitory concentration (MIC). As positive controls, penicillin G and nystatin were utilized.

Antifungal Assays. Two strains of fungi were used: *Candida albicans* (CIP 1180-70) and *Saccharomyces cerevisiae* (ATCC 28383). The test organisms were maintained for 24 h at 28 °C on Sabouraud medium.

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Antibacterial Assays. Both Gram-positive and Gram-negative bacteria were used: *Escherichia coli* (CIP:54127); *Staphylococcus aureus* (CIP:53154); and *Enterococcus hirae* (CIP:5855). These strains were grown for 24 h at 37 °C on Mueller Hinton medium.

Luminol-Dependent Chemiluminescence Analysis. The antioxidant capacity of the essential oils was tested using a luminometer (Lucy, Yelen Co., Ensues la Redonne, France). The reaction mixture (220 μL) contained 0.03 M 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), 50 μM luminol (luminescent Biostab reagent), and 20 μL of an appropriate dilution of α-tocopherol or essential oils. The latter compounds were previously dissolved in ethanol prior to a series of dilutions in distilled water.

Chemiluminescence intensities of both blank (M_1) and assay (M_2) were monitored by integration over 1 min, and the percentage of inhibition (Inh%) was calculated using the following formula:

$$\text{Inh\%} = 100\,(1 - M_2/M_1)$$

The results are expressed as the concentration of the test sample that shows 50% inhibition of α-tocopherol chemiluminescence: IC_{50}.

RESULTS AND DISCUSSION

Chemical Characterization. The essential oils of the roots and aerial parts of *E. elegans* were obtained in 0.23 and 0.45% (w/w) yields of dry material, respectively. GC-MS analyses of these oils show only terpenic constituents, mainly sesquiterpenes accounting for >97% of the amount of the identified components (**Table 1** and **Figure 1**). The major constituent is campherenone, which represents more than one-third of the oil mixtures.

It should be noted that several components belong to the group of bisabolene or to bergamotane and santalane groups, which are bisabolene derivatives. To our knowledge, except for the camphor tree, campherenone seems to be present to a lesser content than in the other plants investigated (*8*, *9*). This is the first report on the occurrence of campherenone in the genus *Elionurus*. Thus, *E. elegans* may be a suitable chemotype in studies of the biosynthetic pathways of campherenone.

Although 39 compounds were clearly identified from the oils, it remains that further chemical analyses are needed to complete the identification of the oil components. Unidentified constituents from the aerial parts and roots account for about 18 and 28%, respectively.

Bioassays. The oils of both the aerial parts and roots of *E. elegans* showed mild activities against the test organisms except for *Escherichia coli* (**Table 2**). No complete inhibition was observed with the different concentrations used. However, the lag stage of the growth curve for *Enterococcus hirae* was drastically increased at the essential oil contents ranging from 50 to 100 μg mL^{-1}. Similar data were also obtained with *Candida albicans*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus* but from a higher content of the oil (100 μg mL^{-1}). Previous studies have pointed out that undiluted oils with oxygenated-rich components have significant effects on a large variety of bacteria (*10*). Although our volatile extracts showed partial inhibitions, further studies are required to characterize the major inhibitory components. Some constituents may act as synergists and/or antagonists with regard to the effect of the main inhibitors. The preliminary studies carried out have concluded to a noninhibitory effect of campherenone (**Table 3**).

Antioxidant Activity. In recent studies, chemiluminescence has been shown to be a sensitive and promising technique to evaluate the potential antioxidant activity of both biological and synthetic molecules (*11*−*13*). Therefore, it was tempting to investigate whether the essential oils of *E. elegans* may act as free radical scavengers.

Table 1. Composition of the Essential Oils from the Aerial Parts and Roots of *E. elegans*

peak	compound	RI[b]	RA[a] (%) aerial parts	RA[a] (%) roots
1	camphene	947	0.1	0.1
2	myrcene	984	0.4	
3	limonene	1019	1.6	
4	carvomenthene	1020		0.3
5	camphor	1245		0.4
6	piperitone	1255	0.1	0.2
7	geraniol	1256	0.2	
8	geranyl acetate	1392	0.4	
9	β-elemene	1396	0.1	
10	petasitene[c]	1400	0.2	
11	β-caryophyllene	1420	4.3	0.1
12	β-gurjunene	1433		0.5
13	*trans*-α-bergaptene	1436	1.0	0.3
14	α-humulene	1455	0.4	
15	(E)-β-farnesene	1458	1.9	
16	*epi*-β-santalene	1460	2.3	12
17	α-curcumene	1480	0.3	
18	(*E*,*Z*)-α-farnesene	1483	1.1	
19	β-bisabolene	1506	0.5	0.3
20	*cis*-γ-bisabolene	1512	0.6	0.2
21	β-sesquiphellandrene	1522	0.5	0.3
22	NI[d]	1527	0.3	0.2
23	*cis*-sesquisabinene hydrate	1540	0.3	
24	NI	1543	0.1	
25	elemol	1552	0.3	0.2
26	NI	1555	0.3	0.5
27	nerolidol	1562	0.2	0.1
28	NI	1567	1.3	0.1
29	NI	1572	0.2	
30	*trans*-sesquisabinene hydrate	1580	1.3	
31	caryophyllene oxide	1581	4.9	4.6
32	humulene oxide II	1608	0.5	0.4
33	bisabolene-2-ol	1618	0.5	0.4
34	β-eudesmol	1645	0.6	1.0
35	campherenone	1647	43.0	39.0
36	5,7-diepi-α-eudesmol	1663	1.6	0.9
37	NI	1664	3.0	2.5
38	(*Z*)-α-santalol	1667	1.2	0.2
39	α-eudesmol	1667	0.7	0.3
40	NI	1668	0.3	
41	NI	1680	4.9	8.5
42	NI	1681	0.4	
43	(*E*)-α-santalol	1687	0.6	0.8
44	*epi*-α-bisabolol	1689	2.7	2.6
45	α-bisabolol	1690	1.2	1.6
46	bisabolenol	1692	0.8	0.3
47	acorenone B	1696	0.2	
48	NI	1702	0.2	
49	(*E*,*E*)-farnesol	1725	0.8	
50	NI	1735		1.9
51	bisabolone	1750	4.9	3.9
52	NI	1753	0.2	
53	NI	1754	0.2	
54	NI	1760		8.6
55	NI	1770		0.3
56	NI	1773		0.3
57	NI	1810	5.9	4.8
	total identified		82.5	71.0

[a] Relative area. [b] Retention index as determined on a DB1 column. [c] Tentative identification. [d] Not identified.

Figure 2 shows some substantial antioxidant activities, although the inhibition rate was less than that of the control, 30 and 46% IC_{50} for the oils of the aerial parts and roots, respectively. The problem raised was thus to correlate the obtained activities with the chemical composition of the oils. A competitive chemiluminescence assay has concluded to a negligible antioxidant effect of camphor against free radicals (*14*). This finding was confirmed through two different models

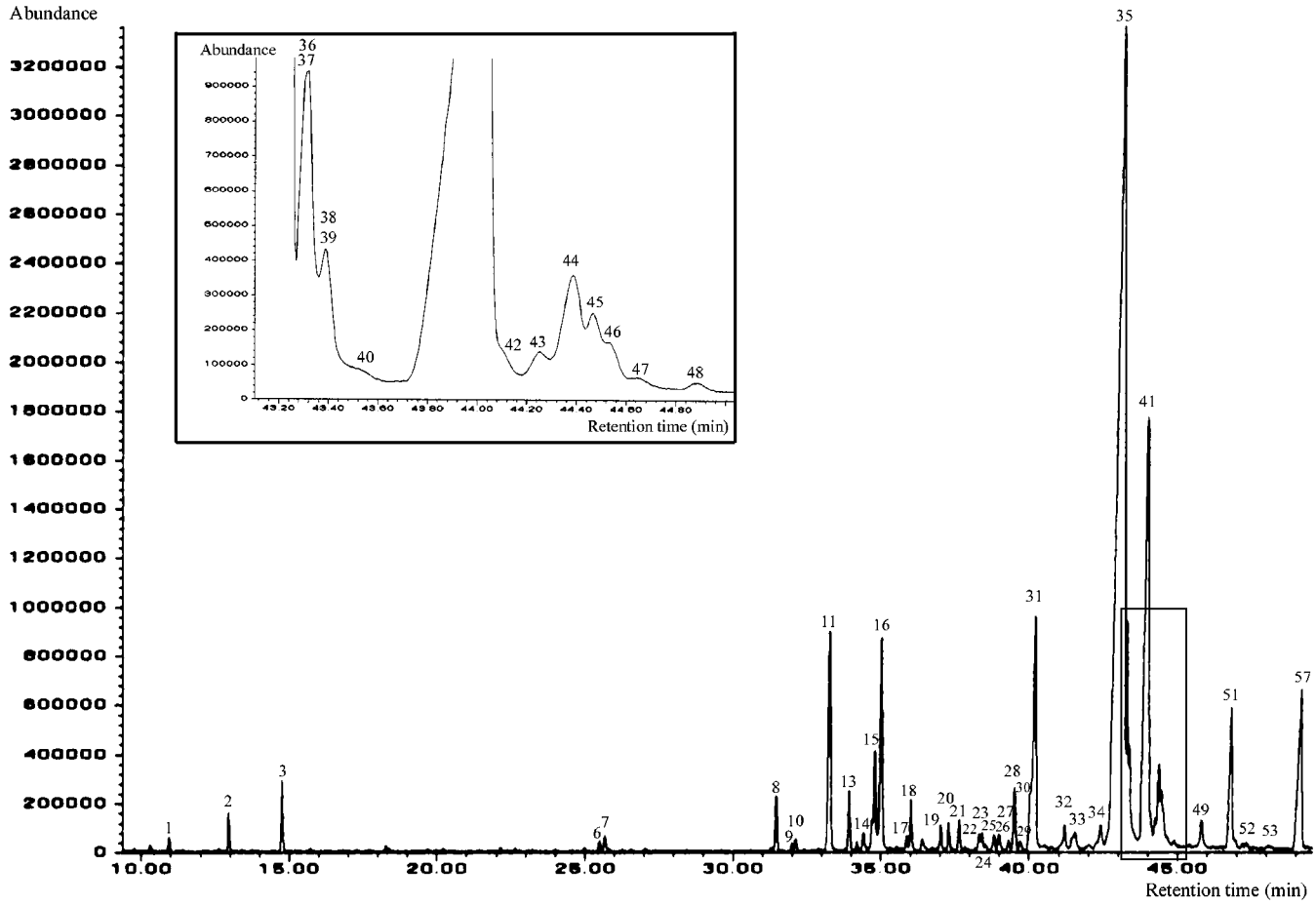

Figure 1. Typical gas chromatogram of the essential oil from the aerial parts of *E. elegans*.

Table 2. Antibacterial and Antifungal Activities of the Essential Oils from the Aerial Parts and Roots of *E. elegans*

		MIC (μg mL^{-1})			
source	organism	oils of aerial parts	oils of roots	penicillin G	nystatin
CIP:54127	*Es. coli*			50	nd[a]
CIP:53154	*St. aureus*		100	<0.4	nd
CIP:5855	*En. hirae*	50		6.25	nd
ATCC 28383	*Sa. cerevisiae*	100		nd	6.25
CIP 1180-70	*C. albicans*	100		nd	6.25

[a] nd, not determined.

Table 3. Antibacterial and Antifungal Activities of a Partially Purified Fraction of Campherenone from the Essential Oils of *E. elegans*

		MIC (μg mL^{-1})		
source	organism	partially purified campherenone	penicillin G	nystatin
CIP:54127	*Es. coli*		50	nd[a]
CIP:53154	*St. aureus*		<0.4	nd
CIP:5855	*En. hirae*		6.25	nd
ATCC 28383	*Sa. cerevisiae*		nd	6.25
CIP 1180-70	*C. albicans*		nd	6.25

[a] nd, not determined.

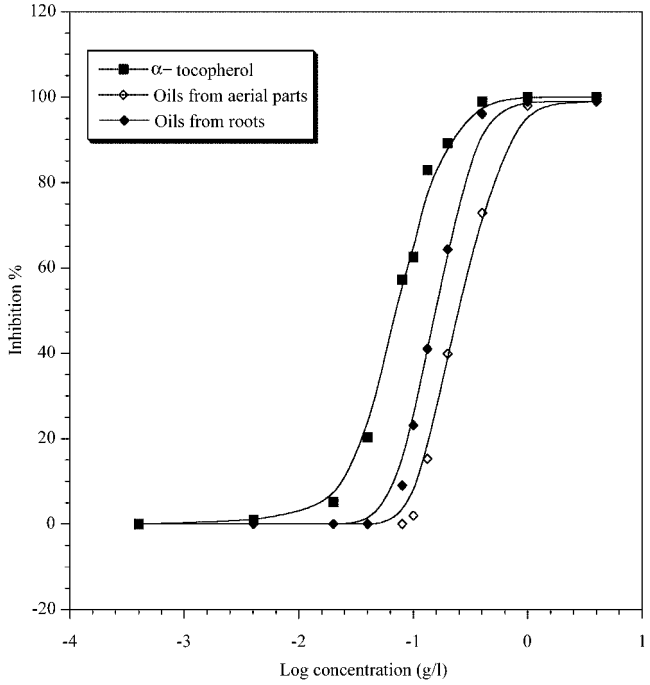

Figure 2. Antioxidant activities of the essential oils from the aerial parts and roots of *E. elegans* plus α-tocopherol in chemiluminescence assay (mean values of three independent extractions).

that evaluated the contents of the primary and secondary constituents occurring in lipid peroxidation (*15*). Also, similar results were obtained with camphene. Thus, it seems plausible that a nonsignificant activity may be accredited to campherenone. On the other hand, although limonene, geraniol, α-humulene, and α-bisabolol were found in lesser contents, they have been shown to be chain-breaking antioxidants (*15*). Given the above observations, the synergistic and/or antagonistic effects

of these constituents may explain the antioxidant effectiveness of the oils studied.

The work reported deals with some aspects of the phytochemistry and biological activities of an important pasture grass in western Africa. The plant is also used in local traditional medicine. Thus, the combined effects of the nutritional elements and the isolated essential oils would have an impact on both human and animal health. From this standpoint, phytochemical studies of *E. elegans* with regard to livestock dietary requirements are in progress in our laboratory.

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