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Essential oil from two populations of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli (Chapéu de couro)

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ABSTRACT

Analysis by Gas Chromatography and Gas Chromatography/Mass Spectrometry of the essential oils obtained from leaves of *Echinodorus grandiflorus* ("Chapéu de couro") from two different populations (Big Leaves and Small Leaves), collected monthly between September 1998 and December 1999 revealed 17 components. Phytol was the major constituent for both populations. The main sesquiterpene representatives are $(E)-$caryophyllene, α-humulene and (E)-nerolidol.

Key words: *Echinodorus grandiflorus*, Alismataceae, essential oil, sesquiterpenes, diterpenes.

INTRODUCTION

Alismataceae is a primitive monocotyledonous family comprising 11 genera and 75 species occurring in tropical, subtropical and subtemperate regions in the Eastern as well as the Western Hemisphere (Crow 2003). These plants are aquatic or semi-aquatic herbs with milky sap. Of the two largest genera, *Echinodorus* is restricted to the neotropics, native in tropical America and it is formed by 26 species that occur from northern United States to Patagonia (Haynes and Nielsen 1994). In Brazil *E. grandiflorus* and *E. macrophyllus* are popularly known as "chapéu de couro". These plants have been used in the folk medicine as anti-inflammatory and diuretic. The chemical profile of *Echinodorus* species is represented basically by terpenoids (Manns and Hartmann 1993, Tanaka et al. 1997, Costa et al. 1999, Kobayashi et al. 2000a, b).

This paper aims to describe the leaf essential oils from two populations of *E. grandiflorus*.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Leaves of *E. grandiflorus* were collected monthly from September 1998 to December 1999, (except in February and July) in Tanguá, Rio de Janeiro State, Brazil. The plants have been cultivated in two different places in the area resulting in two morphologically distinct populations: one showing exuberant big leaves (BL), growing on the sandy

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bed of an irrigation ditch and the other, with scrubby small leaves (SL), occurring on a clayish bank formerly used as a decantation lake for fluorite mining residues. The mean of leaf dry weight along the fourteen months of collection was 4.0 g and 1.2 g respectively for BL and SL. Leaves of the specimens from the two populations were always harvested in the morning by 10.00 o'clock. The botanical materials were identified by the botanist Dr. Erika Santos Guimarães as *Echinodorus grandiflorus* (Chamisso and Schlechtendal) Micheli, and the vouchers are found at the Herbarium CESJ, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, under the register number 30.707.

Isolation of the Oils

About 3.00h after the collection the freshly picked leaves were hydrodistilled in a Clevenger-type apparatus (Gottlieb and Magalhães 1960) for 1.30h. The obtained oils were stored at –18°C to be analyzed.

Gas Chromatographic Analyses, GC

Capillary gas chromatography was performed using a Hewlett-Packard 6890 gas chromatograph; fused silica capillary column HP-5 (5% diphenyl and 95% dimethylpolysyloxane, 60m × 0, 25mm, 0,25μm film thickness); helium as carrier gas; and temperature programming from 70°C to 290°C (2°C/min); injector temperature 270°C and detector temperature 300°C.

Gas Chromatography / Mass Spectrometry, GC/MS

This analysis was carried out using a Hewlett-Packard 6890 gas chromatograph equipped with a fused silica capillary column (HP-5, 30m × 0.25mm, 0.25μm film thickness), helium as carrier gas with a flow rate 1.0ml/min; temperature programming from 70°C to 290°C (2°C/min), coupled to a Hewlett-Packard 5972 mass spectrometer. The MS operating parameters were: 70eV, ion source 250°C equipped with EI.

Identification of the Oil Components

The compound identifications were carried out by comparison of their retention indices (RI) with literature values; and the MS data with those from Wiley 275.1 mass spectral data base besides literature records (Adams 1995).

Retention Indices (RI)

These indices were calculated using a GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C22, performed at the same column and conditions as used in the GC analysis for the essential oils.

RESULTS AND DISCUSSION

The identified constituents of *E. grandiflorus* essential oils for the two analyzed populations (BL and SL) are shown in Tables I and II respectively, as well as with their percentage monthly recorded in GC analyses for the period September 1998 to December 1999, except in February and July. Both tables also show for each compound the mean percentage of oils, the mean retention time (RT) and the mean calculated retention indices (RI) as well as the literature RI recorded. Considering the relative area percentage, the total identified compounds was 58.9% for the BL and 60.3% for SL populations, being 39.9% / 32.0% due to mono- and sesquiterpenes and 19.0% / 28.3% to diterpenes for BL and SL populations respectively. Analyses of these tables show for the two populations phytol as the major oil constituent. The other significant sesquiterpene oil components are (E)-caryophyllene, (E)-nerolidol and α-humulene. Figures 1a and 1b shows the variability of the major sesquiterpene constituents from September 1998 to December 1999, for the two populations and (E)-caryophyllene showed to be the most representative product although for the BL population it has been closely followed by (E)-nerolidol.

The relative percentages of diterpenes in the *E. grandiflorus* essential oils for the two populations (Figures 2a and 2b) besides the production of huge

TABLE I
Identified constituents of *E. grandiflorus* essential oils from BL-population with their percentage monthly recorded in GC-analysis for the period September 1998 to December 1999, together their mean percentage of oil (O), the mean retention time (RT) and the mean calculated retention indices (CRI) as well as the literature RI (LRI).

	Constituents	Sept	Oct	Nov	Dec	Jan	Mar	Apr	Mai	Jun	Aug	Sept	Oct	Nov	Dec	O	RT	CRI	LRI
1	linalool	0.0	0.4	0.6	1.6	1.2	0.9	0.0	0.5	1.4	1.4	1.0	1.2	0.0	0.2	0.7	15.5	1104	1098
2	dihydroedulan	3.3	0.9	1.6	2.1	3.7	3.1	0.0	1.7	1.3	1.1	0.9	0.8	0.6	0.9	1.5	26.7	1300	–
3	(*E*)-caryophyllene	20.2	20.3	14.8	13.5	10.5	10.9	0.4	0.3	21.1	18.6	10.3	14.2	0.0	2.0	11.5	34.3	1428	1418
4	*alpha* humulene	9.6	7.5	5.7	5.6	5.6	5.5	0.0	0.3	9.0	6.8	4.0	5.6	0.7	1.8	4.9	36.2	1460	1454
5	(*E*)-farnesene	0.0	0.7	0.7	1.1	1.1	0.8	0.3	1.9	2.2	0.7	0.9	0.7	0.0	2.4	1.0	37.8	1487	1458
6	*beta* selinene	0.0	0.4	0.3	0.4	0.6	0.0	0.5	0.4	0.8	0.4	0.3	0.4	0.0	0.7	0.4	38.6	1501	1485
7	*alpha* farnesene	0.0	0.8	1.0	0.5	1.6	1.5	0.9	0.5	0.2	0.4	0.6	1.1	8.1	8.2	1.8	39.8	1522	1508
8	*delta* cadinene	0.0	0.0	0.0	0.6	0.6	0.5	0.0	0.5	0.8	0.4	0.3	0.5	1.9	0.5	0.5	40.2	1529	1513
9	(*E*)-nerolidol	21.0	9.7	7.2	6.7	8.6	9.9	6.6	3.4	7.8	5.3	4.6	7.9	11.8	2.1	8.2	42.5	1570	1564
10	caryophyllene oxide	13.3	0.0	4.1	4.0	6.8	7.4	3.5	19.6	2.2	3.1	5.2	5.0	0.0	1.0	4.5	43.7	1591	1576
11	humulene epoxide	0.0	0.0	1.1	0.9	1.5	1.5	0.6	4.2	0.7	0.8	1.0	1.2	0.0	0.3	1.0	45.1	1617	1581
12	bisabolone	0.0	0.3	0.3	0.2	0.0	0.4	0.3	1.4	0.4	0.0	0.2	0.3	0.7	0.3	0.3	50.5	1717	1650
13	drimenol	1.5	3.9	3.8	2.6	3.9	4.9	1.3	2.0	4.4	2.4	2.4	5.3	4.2	1.6	3.1	53.2	1770	1759
14	neocembrene	0.0	0.3	0.8	0.4	0.6	0.7	0.3	0.3	0.5	0.3	0.3	0.8	0.7	0.3	0.5	60.8	1924	1959
15	echinoic acid	0.0	1.1	1.9	5.1	0.9	1.6	2.6	7.6	3.1	2.1	1.0	4.1	4.6	6.9	2.9	63.1	1972	**
16	cembranoid	0.0	1.1	1.6	5.3	0.8	1.3	10.2	2.4	1.8	1.3	1.2	0.3	0.7	4.8	2.2	69.6	2116	–
17	phytol	7.8	22.2	18.0	2.7	7.4	16.8	45.3	5.6	2.6	2.8	5.6	11.4	12.3	28.7	13.4	69.9	2123	***
18	*(1–13)	68.9	45.0	41.2	39.9	45.7	47.2	14.4	36.6	52.3	41.2	31.8	44.4	27.9	22.0	39.9	–	–	–
19	(14–17)	7.8	24.7	22.2	13.6	9.7	20.5	58.4	15.9	7.9	6.5	8.0	16.7	18.3	40.7	19.0	–	–	–
20	(1–17)	76.7	69.7	63.4	53.5	55.4	67.7	72.8	52.5	60.2	47.7	39.8	61.1	46.2	62.7	58.9	–	–	–

* 18 = identified mono- and sesquiterpenes (1–13); 19 = identified diterpenes (14–17); 20 = identified constituents (1–17). LRI = Adams 1995; ** = Tanaka et al. 1997; *** = (C.M.A. Tanaka, unpublished data).

TABLE II
Identified constituents of *E. grandiflorus* essential oils from SL-population with their percentage monthly recorded in GC-analysis for the period September 1998 to December 1999, together their mean percentage of oil (O), the mean retention time (RT) and the mean calculated retention indices (CRI) as well as the literature RI (LRI).

	Constituents	Sept	Oct	Nov	Dec	Jan	Mar	Apr	Mai	Jun	Aug	Sept	Oct	Nov	Dec	O	RT	CRI	LRI
1	linalool	0.0	0.8	1.3	1.1	0.3	0.9	0.5	0.3	0.5	0.3	0.5	0.7	0.7	0.0	0.6	15.5	1104	1098
2	dihydroedulan	1.2	1.1	2.6	2.5	1.2	2.8	1.2	1.2	0.8	0.7	0.6	1.2	1.3	2.0	1.4	26.7	1300	-
3	(*E*)-caryophyllene	7.4	18.8	20.1	8.9	5.0	9.6	3.2	1.7	9.7	6.4	10.0	4.9	15.4	0.0	8.6	34.3	1428	1418
4	*alpha* humulene	3.2	7.6	7.9	3.6	2.7	4.1	1.7	0.9	4.5	3.3	4.0	2.4	7.6	0.3	3.8	36.2	1460	1454
5	(*E*)-farnesene	0.0	1.4	1.3	3.1	2.1	4.2	1.2	2.0	3.5	2.1	1.0	1.4	3.8	3.2	2.2	37.9	1489	1458
6	*beta* selinene	1.9	0.8	0.7	1.6	1.0	2.2	0.6	0.5	1.6	1.1	0.5	0.6	1.7	0.6	1.1	38.6	1501	1485
7	*alpha* farnesene	0.0	1.3	0.8	0.7	0.3	1.3	0.4	0.2	0.2	0.6	0.2	0.4	5.0	8.4	1.4	39.8	1522	1508
8	*delta* cadinene	0.0	0.7	0.7	0.3	0.3	0.3	0.3	0.2	0.4	0.2	0.5	0.1	1.4	0.9	0.5	40.2	1529	1513
9	(*E*)-nerolidol	13.5	8.4	6.7	4.6	4.8	6.6	5.3	2.2	3.1	3.5	3.1	2.7	4.1	1.0	5.0	42.5	1570	1564
10	caryophyllene oxide	7.2	7.3	6.0	5.1	2.2	8.0	2.7	2.3	2.2	2.1	2.9	1.6	0.0	0.3	3.6	43.6	1589	1576
11	humulene epoxide	0.0	1.7	1.2	1.0	0.6	1.4	0.6	0.7	0.6	0.6	0.6	0.3	0.0	0.0	0.7	45.0	1615	1581
12	bisabolone	0.0	0.0	0.0	0.2	0.3	0.4	0.3	0.8	0.4	0.6	0.0	0.0	0.3	0.4	0.3	50.5	1717	1650
13	drimenol	8.0	5.1	3.7	2.4	3.0	1.5	1.9	1.6	2.5	4.4	1.9	1.6	4.0	0.6	3.0	53.2	1770	1759
14	neocembrene	0.0	0.4	0.5	0.3	0.3	0.5	0.4	0.3	0.5	0.2	0.4	0.2	0.3	0.0	0.3	60.7	1922	1959
15	echinoic acid	0.0	3.0	2.8	1.7	2.2	1.8	2.6	3.6	3.1	1.6	2.7	2.2	0.3	2.2	2.1	63.0	1970	**
16	cembranoid	3.9	3.7	4.6	3.4	4.3	3.2	3.8	1.8	2.6	3.1	5.4	4.1	1.5	1.7	3.4	69.8	2120	-
17	phytol	12.0	6.6	2.2	32.2	45.5	14.4	40.0	25.5	16.9	23.9	17.1	47.5	11.2	20.0	22.5	70.2	2130	***
18	*(1-13)	42.4	55.0	52.9	35.1	23.7	43.2	19.8	14.3	30.0	25.9	25.7	17.7	45.3	17.7	32.0	–	–	–
19	(14-17)	15.9	13.7	10.1	37.6	52.3	19.9	46.8	31.1	23.1	28.7	25.6	54.0	13.1	23.9	28.3	–	–	–
20	(1-17)	58.3	68.7	63.0	72.7	76.0	63.1	66.6	45.4	53.1	54.6	51.3	71.7	58.4	41.6	60.3	–	–	–

* 18 = identified mono- and sesquiterpenes (1–13); 19 = identified diterpenes (14–17); 20 = identified constituents (1–17). LRI = Adams 1995; ** = Tanaka et al. 1997; *** = (C.M.A. Tanaka, unpublished data).

amount of phytol, the presence of echinoic acid as the second major component must be mentioned since it has followed the phytol variability during the fourteen months in study. The diterpenes have been biosynthesized in greater amount by the BL-population except phytol that is produced 1.6 times more by the SL-population. Comparison of variability of the two major components in the *E. grandiflorus* oils, phytol and (*E*)-caryophyllene, through the fourteen months of study, seems to have a sub-

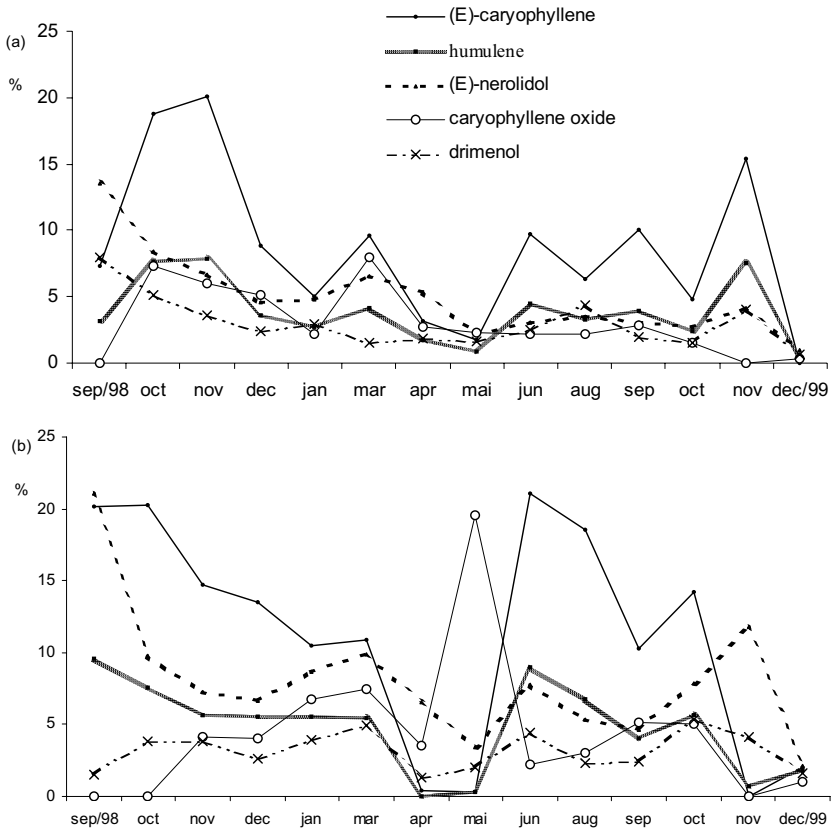

Fig. 1 – Monthly variability of the major sesquiterpene constituents of the *E. grandiflorus* essential oil from the SL (a) and BL (b) population (% of chromatogram oil).

stitutive production (Figures 1 and 2).

This exuberant phytol production is of great value since this compound has been responsible for some recorded important biological activities (Pongprayoon et al. 1992, Rajab et al. 1998) which may indicates *E. grandiflorus* as a potential plant source for phytopharmaceuticals. In conclusion, it is worth to point out that the prominent production of phytol in both populations may characterize this compound as a chemotaxonomic marker for this plant species.

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RESUMO

Análise por cromatografia com fase gasosa e cromatografia com fase gasosa acoplada à espectrometria de massas de óleos essenciais obtidos de folhas de *Echinodorus grandiflorus* ("Chapéu-de-couro") em duas diferentes populações (folhas grandes e folhas pequenas), coletadas mensalmente entre setembro de 1998 e dezembro de 1999, revelou 17 componentes. Fitol foi o constituinte majoritário em ambas populações. Os principais representantes sesquiterpênicos foram (*E*)-cariofileno, α-humuleno e (*E*)-nerolidol.

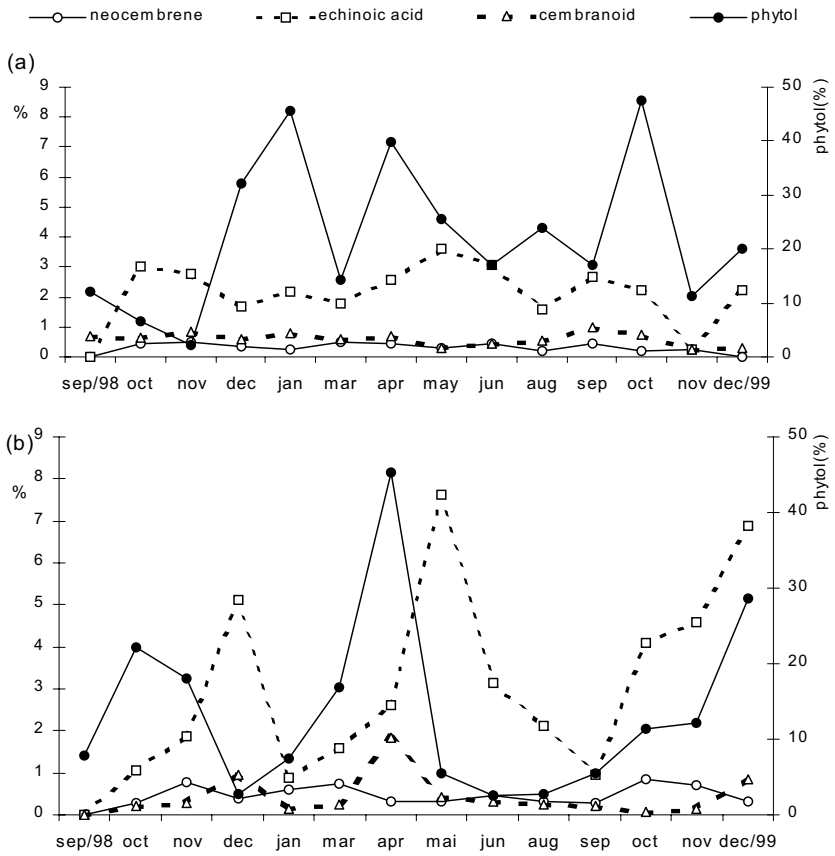

Fig. 2 – Monthly variability of the major diterpene constituents of the *E. grandiflorus* essential oil from the SL (a) and BL (b) populations (% of chromatogram oil).

Palavras-chave: *Echinodorus grandiflorus*, Alismataceae, óleo essencial, sesquiterpenos, diterpenos.

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