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Short Report

Essential Oil Composition of *Croton palanostigma* Klotzsch from North Brazil

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Os óleos essenciais das folhas, ramos finos, galhos, cascas do caule e frutos de *Croton palanostigma* foram analisados por CG e CG-EM. Os componentes principais determinados no óleo das folhas foram linalol (25,4%), (*E*)-cariofileno (21,0%), metileugenol (17,2%) e β-elemeno (6,0%); no óleo dos ramos finos foram α-pineno (41,4%), limoneno (29,0%), sabineno (11,5%) e β-pineno (5,7%); no óleo dos galhos foram metileugenol (24,1%), (*E*)-metilisoeugenol (15,3%), α-pineno (11,2%) e (*E*)-cariofileno (8,5%); no óleo das cascas do caule foram α-pineno (31,6%), metileugenol (25,6%) e (*E*)-metilisoeugenol (23,7%); e no óleo dos frutos foram linalol (42,7%), metileugenol (16,3%) e β-elemeno (6,4%). Análise estatística mostrou que as folhas e os frutos apresentam significante similaridade entre si, assim como os galhos e as cascas do caule. Adicionalmente, o óleo obtido das cascas do caule possui elevada atividade larvicida sobre *Artemia salina* (CL_{50}, 3,71 ± 0,01 μg mL^{-1}).

The essential oils of leaves, twigs, branches, trunk bark and fruits of *Croton palanostigma* were analyzed by GC and GC-MS. The main compounds found in the oil of the leaves were linalool (25.4%), (*E*)-caryophyllene (21.0%), methyleugenol (17.2%) and β-elemene (6.0%); in the oil of the twigs were α-pinene (41.4%), limonene (29.0%), sabinene (11.5%) and β-pinene (5.7%); in the oil of the branches were methyleugenol (24.1%), (*E*)-methylisoeugenol (15.3%), α-pinene (11.2%) and (*E*)-caryophyllene (8.5%); in the oil of the trunk bark were α-pinene (31.6%), methyleugenol (25.6%) and (*E*)-methylisoeugenol (23.7%); and in the oil of the fruits were linalool (42.7%), methyleugenol (16.3%) and β-elemene (6.4%). Statistical analysis showed that the leaves and fruit, and the branches and trunk bark, have significant similarities between them. In addition, the trunk bark oil has high brine shrimp larvicidal activity (LC_{50}, 3.71 ± 0.01 μg mL^{-1}).

Keywords*: Croton palanostigma*, Euphorbiaceae, essential oil composition, α-pinene, linalool, methyleugenol, limonene, brine shrimp larvicidal activity.

Introduction

Croton is a genus of Euphorbiaceae comprising about 1300 species widespread in Africa, Asia and South America. Many species are used in the traditional medicine of these continents, especially to treat cancer, diabetes, hypercholesterolemia, malaria and ulcers, among other diseases.[1] *Croton palanostigma* Klotzsch (syn. *C. benthamianuns* Müll. Arg.)[2] is a medium-sized tree which is native to the Amazon region, known as "marmeleiro" (Brazil)[3] and "sangre de drago" or "sangre de grado" (Peru, Colombia, Venezuela, Guyanas and Bolivia).[4]

Phytochemical studies with the trunk bark of *C. palanostigma* furnished aparisthman and cordatin, two furan diterpenes with a clerodane skeleton that show anti-ulcer activity similar to cimetidine, a drug used for the treatment of peptic ulcers.[5] Previously, these results were reported for the species *Aparisthmium cordatum* Baill., now identified as *C. palanostigma*.[6] The species *C. palanostigma* produces a red viscous sap that was reported to have gastroprotective and gastrointestinal anticancer activities.[7] The chemical studies from another red sap of *Croton* spp led to the isolation of the alkaloid taspine,[8] the

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dihydrobenzofuran lignans 3',4-*O*-dimethylcedrusin[9] and 4-*O*-methylcedrusin[10] and proanthocyanidins.[11]

Recently, Salatino and coworkers[1] have reported the study of the essential oils of about thirty species of *Croton*. The results indicate that some of these oils are rich in terpenoids and phenylpropanoids and others are rich only in terpenoids.[1]

In the work reported here, the essential oils of the leaves, twigs, branches, trunk bark and fruits of *C. palanostigma* were analyzed by GC-FID and GC-MS. Statistical analysis was performed to determine the similarities of chemical composition of the various plant parts. In addition, a brine shrimp lethality bioassay was carried out to investigate the toxicity of the trunk bark oil.

Experimental

Plant processing

The specimen *C. palanostigma* was collected in the locality of Terra Alta, Municipality of Castanhal, Pará, Brazil, in March 2006. The plant was identified by Dr. Ricardo Secco, a specialist on Euphorbiaceae of the Museu Paraense Emílio Goeldi, Belém, Brazil. A voucher of *C. palanostigma* (MG 182.822) was deposited in the herbarium of Museu Paraense Emílio Goeldi. The moisture contents of leaves, twigs (diameter of approximately 1.5 cm), branches (diameter of approximately 3.5 cm), trunk bark and fruits were calculated after phase separation in a Dean-Stark trap (2 h, 5 g) using toluene. All parts of the plant from *C. palanostigma* were dried separately at room temperature (5-7 days) and submitted to hydrodistillation (3 h, 100 g each) using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and their percentage contents were calculated on basis of the plant dry weight.

Oil composition analysis

Qualitative analysis of the volatile compounds was performed on a Thermo DSQII GC-MS instrument, with the following conditions: WCOT DB-5ms (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column; temperature programmed from 60 to 240 °C (3 °C min^{-1}); injector temperature, 250 °C; carrier gas, helium, adjusted to a linear velocity of 32 cm s^{-1} (measured at 100 °C); injection type, splitless (0.1 µL of a 2:1000 hexane solution); the split flow was adjusted to give a 20:1 ratio; septum sweep was a constant 10 mL min^{-1}; EIMS, electron energy, 70 eV; ion source temperature and connection parts, 200 °C. The quantitative data of oils were obtained by peak area normalization using a Focus GC-FID operated under the same conditions, except that the carrier gas that was nitrogen. The retention index was calculated for all volatile constituents using a homologous series of *n*-alkanes.

Brine shrimp bioassay

The brine shrimp (*Artemia salina* Leach) lethality bioassay was carried out to investigate the toxicity of the essential oils of the trunk bark. Brine shrimp eggs were hatched in artificial salt water and used after 48 h using the method of Parra *et al.*[12] Experiments were conducted along with control and different concentrations (1, 5 and 10 µg mL^{-1}) in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. Lethal concentration (LC_{50}) values were obtained from the best-fit line plotting concentration versus percentage lethality.[13]

Hierarchical Cluster Analysis (HCA)

The oils were submitted to the HCA technique taking into account their chemical composition and the major components. HCA examines the distances between the samples in a data set and the information is then represented in a two-dimensional plot (dendrogram). The most similar points were grouped forming the clusters and the process was repeated until all the points are inserted into a unique group.[14,15]

Results and Discussion

The leaves, twigs, branches, trunk bark and fruits of *C. palanostigma* provided oil yields of 0.7, 0.6, 0.3, 2.2 and 0.5%, respectively and their volatile constituents were analyzed by GC-FID and GC-MS. The individual components of the oils were identified by comparison of both their mass spectra and their GC retention data with those of authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparison of mass spectra with those existing in data system libraries or cited in the literature.[16,17] The main compounds found in the oil of leaves were linalool (25.4%), (*E*)-caryophyllene (21.0%), methyleugenol (17.2%) and β-elemene (6.0%). The oil of twigs was dominated by α-pinene (41.4%), limonene (29.0%), sabinene (11.5%) and β-pinene (5.7%). The major constituents identified in the oil of branches were methyleugenol (24.1%), (*E*)-methylisoeugenol (15.3%), α-pinene (11.2%) and (*E*)-caryophyllene (8.5%). The oil of trunk bark was dominated by the volatiles α-pinene (31.6%), methyleugenol

(25.6%) and (*E*)-methylisoeugenol (23.7%). The principal components found in the oil of fruits were linalool (42.7%), methyleugenol (16.3%) and β-elemene (6.4%). The seventy-one constituents identified in the oils of *C. palanostigma* are listed in Table 1.

The essential oils of *Croton palanostigma* are rich in terpenoids and phenylpropanoids. Linalool, α-pinene, limonene, methyleugenol and (*E*)-methylisoeugenol were the main compounds. According to Salatino *et al*,[1] the essential oils of *Croton* species are rich in terpenoids and phenylpropanoids, or only in terpenoids.[1]

Concerning Hierarchical Cluster Analysis (HCA), the resulting dendogram is shown in the Figure 1. It can be observed that the volatile compositions from different parts of *C. palanostigma* are separated into three groups. The first group comprises the samples of leaves and fruits, the second group is represented by the sample of twigs, and the third group is composed of the samples of branches and trunk bark. Based on this classification we can say that the volatile composition of leaves and fruits, as well as, the branches and trunk bark, are similar to each other. Linalool and methyleugenol characterize the first group,

Table 1. Essential oil composition (%) of *Croton palanostigma*

Components	RI	Leaves	Twigs	Branches	Trunk bark	Fruits
α-pinene	939	1.3	41.4	11.2	31.6	2.9
sabinene	975	0.8	11.5	2.3	1.0	0.2
β-pinene	980		5.7	1.3	2.3	1.7
myrcene	988			0.2		
α-phellandrene	1003		0.1			
α-terpinene	1017		2.7	0.2		
p-cymene	1025		3.5	0.4		
limonene	1029		29.0	2.7	1.6	
1,8-cineole	1032	2.4		2.0		3.1
(*E*)-β-ocimene	1050		1.5	0.4	1.2	0.7
γ-terpinene	1060		1.3	0.3		
terpinolene	1089		0.4	0.5		
linalool	1097	25.4	0.4	3.0	1.1	42.7
allo-ocimene	1132		0.4	0.1		0.1
trans-limonene oxide	1142	0.1				
(*E*)-myroxide	1145					0.2
borneol	1168	0.1	0.3	0.1	0.2	0.1
terpinen-4-ol	1177	0.1	0.2	0.1	0.1	0.1
α-terpineol	1189	0.8	0.2	0.5	0.2	0.9
methyl chavicol	1197	0.2				
thymol methyl ether	1235		0.2	0.1		
isobornyl acetate	1287	tr	0.1	0.3	0.2	tr
isoascaridole	1302		0.1	0.1		
methyl geranate	1325		0.1	0.1		
δ-elemene	1338	2.0	0.1	0.6		3.1
α-cubebene	1345			tr		
eugenol	1359			tr		0.1
α-ylangene	1375			0.1	tr	tr
α-copaene	1377	0.5	tr	0.9	0.4	0.7
β-boubonene	1389	0.3		0.1	tr	0.3
β-elemene	1391	6.0	tr	2.8	1.1	6.4
methyleugenol	1404	17.2	0.3	24.1	25.6	16.3
(*E*)-caryophyllene	1419	21.0	0.1	8.5	0.8	3.8
β-copaene	1432	0.2	0.1	0.1	0.1	0.2
trans-α-bergamotene	1435	2.9	0.1	3.8	1.9	1.7
α-humulene	1455	3.1	0.1	1.7	0.4	0.8
(*E*)-β-farnesene	1457				0.1	
allo-aromadendrene	1460	0.6		0.4		0.9
γ-muurolene	1480				0.1	
germacrene D	1485	1.8			0.3	1.6

Table 1. continuation

Components	RI	Leaves	Twigs	Branches	Trunk bark	Fruits
B-selinene	1489			0.4		
(*E*)-methylisoeugenol	1492	tr	0.1	15.3	23.7	
bicyclogermacrene	1500	4.2				4.7
β-bisabolene	1504			0.3		
α-bulnesene	1510				0.2	
δ-amorphene	1511			0.2		
γ-cadinene	1514	0.4		0.3		0.5
7-*epi*-α-selinene	1522				0.4	
δ-cadinene	1523	0.2		0.2	0.1	0.2
trans-cadina-1,4-diene	1535			0.1	tr	tr
α-calacorene	1546					0.1
elemicin	1557	0.9		1.4	0.3	1.4
(*E*)-nerolidol	1563	1.1		0.1	0.1	0.3
spathulenol	1578	0.7		0.6	0.1	0.6
caryophyllene oxide	1583	0.7		1.0	0.1	0.1
globulol	1585	0.1		0.1		0.2
viridiflorol	1593	0.2			0.1	0.2
humulene epoxide II	1608	0.2		0.8		
(*Z*)-asarone	1617				0.4	
1.10-di-*epi*-cubenol	1619					0.1
dillapiole	1621	0.1		0.1		0.1
caryophylla-4(12),8(13)-dien-5-α-ol	1639			0.3		
allo-aromadendrene epoxide	1641					0.3
α-muurolol	1646	0.5		0.3		
α-cadinol	1654	1.3		2.1	2.9	1.2
selin-11-en-4-α-ol	1658			1.4		
mint sulfide	1740			0.4		
hexadecanoic acid	1960			0.4		
eicosane	2000			0.7		
heneicosane	2100			0.4		
docosane	2200			0.6		
total		97.4	99.9	96.5	98.7	98.6

tr = trace (< 0.05%), RI = retention index (on DB-5ms column)

while limonene characterizes the second one, and, finally, α-pinene and methyleugenol the last group.

The trunk bark oil of *C. palanostigma* showed a high brine shrimp larvicidal activity (LC_{50}, 3.71 ± 0.01 μg.mL^{-1}).

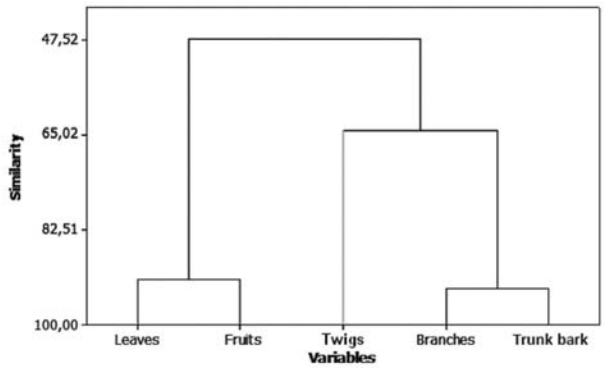

Figure 1. Dendrogram (HCA analysis) for the main compounds identified in the essential oils of *C. palanostigma*.

According to Meyer *et al.*,[12] crude extracts and pure substances are toxic when LC_{50} value < 1000 μg mL^{-1}, that is, the lower the value of LC_{50}, the higher the biological activity. So the trunk bark oil of C. *palanostigma* can be considered highly toxic.

Conclusions

The essential oils of *Croton palanostigma* furnished volatiles belonging to the classes of phenylpropanoids and terpenoids. The HCA analysis showed that the oils from different parts of the plant are dominated by linalool and methyleugenol in the first group (leaves and fruits), limonene in a second group (twigs) and α-pinene and methyleugenol in the third group (branches and trunk bark). The trunk bark oil of *C. palanostigma* showed high brine shrimp larvicidal activity.

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