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Artigo

COMPOSITION AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS FROM *Piper aduncum, Piper arboreum* AND *Piper tuberculatum*

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The composition of essential oils from leaves, stems and fruits of *Piper aduncum*, *P. arboreum* and *P. tuberculatum* was examined by means of GC-MS and antifungal assay. There was a predominance of monoterpenes in *P. aduncum* and *P. tuberculatum* and of sesquiterpenes in *P. arboreum*. *P. aduncum* showed the richest essential oil composition, including linalool. The essential oils from fruits of *P. aduncum* and *P. tuberculatum* showed the highest antifungal activity with the MIC of 10 µg as determined against *Cladosporium cladosporioides* and *C. sphaerospermum,* respectively. This is the first report of the composition of essential oils from *P. tuberculatum*.

Keywords: *Piper*; essential oil composition; antifungal activity.

INTRODUCTION

Several *Piper* species have been used in traditional medicine to treat many diseases including gynaecological maladies, vaginitis, intestinal disorders, psychotropic, antimicrobial, antioxidant and cytotoxic effects[1,2]. The chemical studies carried out on Brazilian Piperaceae species have revealed the occurrence of pyrones, lignoids and chromenes besides various amides which showed potent insecticide and antifungal properties[1,3-9]. The volatile components from aerial parts of Piperaceae species have been subjected to a number of investigations and are variable mixtures with predominance of monoterpenes (C_{10}) and sesquiterpenes (C_{15}) although diterpenes (C_{20}) and phenylpropanoids have also been detected[10].

The essential oil from *Ottonia anisum*, *Piper amplum, P. arboreum, P. dilalatum, P. goesii, P. hispidum, P. hoffmanseffianum, P. nigrum, P. gaudichaudianum*, *P. guineense, P. molliconum*, *P. regnellii, P. cernuum* and *Peperomia blanda* showed biological activity included strong molluscicidal activity against *Biomphalaria glabrata*, cytotoxic, fungistatic, insecticide and antibacterial activies[11-14]. Among the phenylpropanoids, the occurrence of safrole in *P. hispidinervium* was quite relevant since it has been indicated as a new source for synthesis of heliotropine and piperonylbutoxide, fragrance and insecticide synergists, respectively[15].

In this paper, we wish to describe the composition of essential oil in leaves, stems and fruits of *Piper aduncum*, *P. arboreum* and *P. tuberculatum*. Additionally, their antifungal activity as evaluated against the fungi *Cladosporium sphaerospermum* and *C. cladosporioides* by direct bioautography[10]. Furthermore, this is the first analysis of essential oil from *P. tuberculatum*.

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EXPERIMENTAL PART

Plant material

Piper aduncum L. and *P. arboreum* H. B. K. stems, leaves and fruits were collected at Reserva da Ripasa, Ibaté - SP and in the Institute of Chemistry (UNESP) in Araraquara – SP, Brazil. These specimens were identified by Dr. I. Cordeiro (Instituto de Botânica, SP). The voucher specimens (Cordeiro-PA0 and Cordeiro-1936) have been deposited at Herbarium of Instituto de Biociências – USP, São Paulo - SP, Brazil. *P. tuberculatum* Jacq. stems, leaves and fruits were collected in the Institute of Chemistry (UNESP) in Araraquara - SP, Brazil, and identified by Dr. G. E. D. Paredes (Universidad Nacional Pedro Ruiz Gallo, Peru). The voucher specimen (Kato-163) has been deposited at Herbarium of Instituto de Botânica, São Paulo – SP, Brazil.

Extraction of essential oil

Plant material (100 g of each *Piper* species) was subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The oil layers obtained were dried over anhydrous Na_2SO_4. The yields were averaged over three experiments and calculated on the basis of dried weight material.

Essential oil analyses

GC analyses

GC analyses were performed using a Varian CP-3800 gas chromatograph equipped with an auto sampler Varian 8200 and a flame ionization detector using a SPB-5 (30 m x 0.25 mm id,

10 μm film thickness) capillary column. The injector and detector temperature were maintained at 250 and 290 ºC, respectively. The samples (1.0 μL), dissolved in ethyl acetate, were injected in split mode (1:20), using helium as carrier gas at the linear velocity of 50 cm/s. The oven temperature was programmed as follows: 40 ºC (1 min), then 10 ºC/min to 120 ºC, 0.5 ºC/min to 150 ºC and 20 ºC/min to 280 ºC for 5 min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components were determined on the basis of their GC peak areas, without corrections for FID response factors. The retention index was calculated for all constituents using a homologous series of *n*-alkanes[16].

GC-MS analyses

GC-MS analyses were carried out on Shimadzu QP-5000 GC-MS, EI electron impact ion source, 70 eV using DB-5 (30 m x 0.25 μm id, 0.25 mm film thickness, J&W Scientific, USA) capillary column, with similar condition as described in GC programs. Identification of the chemical components was based on the comparison of their mass spectral with the National Institute for Standard Technology – NIST62 library, comparison of calculated retention indexes with literature values and co-chromatography of some constituents with authentic components on the DB-5 capillary column[17].

Table 1. Composition of the essential oils from *Piper aduncum*, *P. tuberculatum* and *P. arboreum*

Compound	RI	*P. aduncum*			*P. arboreum*			*P. tuberculatum*		
		leaf	fruit	stem	leaf	fruit	stem	leaf	fruit	stem
Yield (%)		2.0	2.5	0.5	1.0	0.9	0.4	0.8	0.8	0.3
α-pinene	939	1.7	1.7	7.2	-	1.4	0.4	10.4	28.7	17.3
camphene	953	-	-	-	-	-	-	-	0.4	-
sabinene	976	-	-	-	-	-	-	-	0.8	-
β-pinene	980	2.1	2.4	14.2	-	-	-	12.5	38.2	27.0
myrcene	991	-	1.2	1.1	-	1.5	0.7	-	1.5	1.2
δ-3-carene	1010	-	-	-	2.7	-	18.7	-	-	-
α-terpinene	1018	-	6.8	1.1	-	-	-	-	-	-
p-cimene	1026	-	-	0.7	-	-	0.4	-	-	-
limonene	1031	1.5	2.5	8.7	-	6.3	0.6	4.2	2.4	2.1
1,8-cineol	1033	-	0.6	-	-	-	-	-	-	-
cis-ocimene	1040	3.4	5.6	5.5	0.3	1.8	1.9	-	0.4	-
trans-ocimene	1050	5.0	11.1	13.3	-	1.2	2.9	8.6	9.8	14.5
γ-terpinene	1062	-	12.0	3.3	-	-	-	-	-	-
linalool	1098	31.7	41.2	11.8	-	10.4	-	0.3	-	-
% monoterpenes		45.2	85.1	66.9	3.0	22.6	25.6	36.0	82.3	62.1
geranyl formate	1300	-	-	-	0.3	-	-	-	-	-
δ-elemene	1339	-	-	-	1.3	-	-	-	-	-
α-copaene	1376	0.5	-	-	1.3	-	9.0	-	-	-
β-cubebene	1390	-	-	-	0.9	-	1.3	-	-	-
β-elemene	1391	1.0	-	-	-	5.3	1.3	1.6	-	-
α-gurjunene	1409	0.4	-	-	-	-	-	-	-	-
β-caryophyllene	1418	9.1	1.9	7.6	25.1	6.6	26.5	40.2	14.0	32.1
β-gurjunene	1432	-	-	-	0.7	-	1.4	-	-	-
aromadendrene	1439	0.8	-	-	-	-	-	-	-	-
α-humulene	1454	5.5	1.7	6.3	1.7	-	2.6	2.7	1.4	1.2
β-farnesene	1458	-	-	-	-	-	-	8.3	-	-
seichelene	1460	1.1	-	-	-	-	-	-	-	-
germacrene D	1480	4.2	0.9	-	9.6	49.3	4.7	5.5	-	-
bicyclogermacrene	1494	11.2	-	-	49.5	4.1	21.1	-	-	-
α-muurolene	1499	0.5	-	-	0.5	-	-	-	-	-
germacrene A	1503	-	-	-	-	8.5	0.8	-	-	-
γ-cadinene	1513	1.6	-	-	-	-	-	-	-	-
δ-cadinene	1524	3.0	-	-	0.8	-	2.5	-	-	-
germacrene B	1556	0.9	-	-	-	-	-	1.9	-	-
nerolidol	1564	10.4	6.1	10.6	2.0	1.4	1.0	0.9	-	-
spathulenol	1576	0.9	-	-	0.7	-	-	-	-	-
caryophyllene oxide	1581	-	-	-	-	-	-	2.0	-	-
globulol	1583	0.5	-	-	-	-	-	-	-	-
undecanone	1291	0.4	-	-	-	-	-	-	-	-
% sesquiterpenes		52.0	10.6	24.5	94.4	75.2	72.2	63.1	15.4	33.3
TOTAL		94.2	95.7	91.4	97.4	97.8	97.8	99.1	97.6	95.4

Antifungal assay

The microorganisms used in the antifungal assays *Cladosporium sphaerospermum* (Penzig) SPC 491 and *C. cladosporioides* (Fresen) de Vries SPC 140 have been maintained at the Instituto de Botânica, São Paulo, SP, Brazil. For the antifungal assay – 10.0 μL of solutions corresponding to 400, 200, 100, 50, 10 and 1 μg of essential oils were applied to pre-coated TLC plates. TLC plates were developed with $CHCl_3$ for all of the essential oils and dried for complete removal of solvents. The chromatograms were sprayed with a spore suspension of *Cladosporium sphaerospermum* or *C. cladosporioides* in glucose and salt solution and incubated for 72 h in darkness in a moistened chamber at 25 °C. Clear inhibition zone appeared against a dark background indicating the minimal amount of the essential oils required for it. Nystatin was used as positive control[18,19].

RESULTS AND DISCUSSION

The hydrodistillation of different tissue of *Piper aduncum*, *P. arboreum,* and *P. tuberculatum* gave higher yields, calculated on basis a dry weight, for leaves (2.0, 1.0 and 0.8%) and fruits (2.5, 0.9 and 0.8%) than for stems (0.5, 0.4 and 0.3). The identification of monoterpenes and sesquiterpenes was carried out by automated interpretation of mass spectra of constituents in each oil and also by retention index. A total of 14 monoterpenes and 24 sesquiterpenes identified are shown in order of elution on a DB-5 column (Table 1).

The presence of monoterpenes in fruits and stems was quite remarkable in *Piper adundum* (85.1/66.9%) and *P. tuberculatum* (82.3/62.1%) while in *P. arboreum* the content of monoterpenes were very low (22.6/25.6%) and has been replaced by sesquiterpene whose content ranged from 94.4 to 72.2% in different tissues.

In spite of similarity between of different tissues of the same species, the monoterpenes (α-pinene, myrcene, limonene, *cis*-ocimene, *trans*-ocimene and linalool) and the sesquiterpenes (β-caryophyllene, α-humulene, germacrene D and nerolidol) occur in the three species. In case of *Piper arboreum* only *cis*-ocimene was detected in all tissues. Nevertheless, linalool which has not been detected in previous studies with leaves of *P. arboreum*[11,20], in our study it appeared as major monoterpene (10.4%) in the fruits. Myrcene was not detectable in essential oils from leaves of *Piper aduncum, P. arboreum* and *P. tuberculatum* as well. The monoterpenes were present in high amount in stem oil of *P. tuberculatum* (62.1%), which had β-pinene (27.0%), α-pinene (17.3%) and *trans*-ocimene (14,5%) as the major constituents. The δ-3-carene (18.7%) was the major monoterpene in stem oil of *P. arboreum* in accordance with previous report[12], but it was not detected in *P. aduncum* and *P. tuberculatum*.

The sesquiterpenes were very abundant and structurally diversified in the oils from leaves of the three species. The most general representative was β-caryophyllene accounting up to 9.1/7.6, 25.1/26.5 and 40.2/32.1% in leaves/stems of *Piper aduncum*, *P. arboreum* and *P. tuberculatum*, respectively. Further important sesquiterpene in *P. arboreum* should include bicyclogermacrene (49.5/21.1% in leaves/stems) but, interestingly, germacrene D (49.3%) and germacrene A (8.5%) were more abundant in its fruits.

In summary, the study of volatile components of *Piper aduncum*, *P. arboreum* and *P. tuberculatum* showed the predominance of monoterpenes in fruits and stems while the sesquiterpenes were mostly detected in their leaves. This finding contrast with the occurrence of sesquiterpenes in Piperaceae species. The analysis of fruits resulted in the description of further components including linalool in *P. arboreum* which was absent[11] or detected in very low amounts[20] in previous studies. Such blend of monoterpenes detected in fruits should have attractant properties to the pollinization insects. The linalool, an important compound used in the fragrance industry, was found in significant concentration in the essential oils of fruits and leaves from *Piper aduncum.* Non-oxygenated sesquiterpenes (β-caryophyllene, bicyclogermacrene, germacrene D, and germacrene A) were identified as the major components of the essential oils of the specimens studied. The essential oil of *P. tuberculatum* here described for the first time, had the monoterpene α-pinene (10.4-28.7%), β-pinene (12.5-38.2%), and *trans*-ocimene (8.6-14.5%) and the sesquiterpenes β-caryophyllene (40.2% in leaves), and β-farnesene (8.3% in leaves) as major constituents. In contrast to other Piperaceae species, *e.g. Piper hispidinervium*[2], *P. fulvescens*[21] and *P. sanctum*[22], no detectable amount of phenylpropanoids was observed. A positive correlation between the production of phenylpropanoid (eg. elemicin, myristicin, and sarisan) and lignans/neolignans such as in case of *P. regnellii* [9] and *P. solmsianum*[12,23] was observed. In *P. aduncum,* which has the antifungal chromenes as major compound, the phenylpropanoid dillapiol has been detected as major constituent in the Amazonian species[4] but not in specimens occurring in southern region of Brazil. In case of *P. tuberculatum* and *P. arboreum*, which have amides as major compounds no phenylpropanoid have been detected as well[6].

The antifungal activity of the essential oils was evaluated by means of direct bioautography on TLC plate[7]. The detection limits of samples (Table 2) were obtained according to methodology described[15]. The essential oils from fruits of *Piper aduncum* and *P. tuberculatum* showed high activity (MIC of 10 μg) against C*ladosporium cladosporioides* and *C. sphaerospermum*, respectively, when compared with the standard nystatin (1.0 μg). Further investigations looking at determinations of bioactive constituents should be carried out.

Table 2. Antifungal activity of the essential oils from leaves, stems and fruits of *Piper* species against *C. sphaerospermum* and *C. cladosporioides*

Species	MIC[a] (μg)	
	C. sphaerospermum leaves, stems, fruits	*C. cladosporioides leaves, stems, fruits*
Piper aduncum	50, ni, 50	ni, ni, 10
Piper arboreum	ni, ni, ni	ni, ni, ni
Piper tuberculatum	ni, 50, 10	ni, ni, 50

Positive control: nystatin (1.0 μg); [a] Minimum amount required for the inhibition of fungal growth on TLC plates. ni = no inhibition

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