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NEW INSIGHTS ON THE CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF RHIZOME ESSENTIAL OIL FROM VIETNAMESE *Cyperus stoloniferus* Retz. (Cyperaceae)

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ABSTRACT

Cyperus stoloniferus Retz. is a perennial grass-like plant widely distributed in Vietnam. The composition of essential oil isolated from rhizomes has been investigated by a combination of chromatographic (Column chromatography, Gas chromatography in combination with retention indices) and spectroscopic techniques (Mass spectrometry, ^{13}C NMR spectroscopy). Cyperene (15.8%), cyperotundone (12.9%) and α-cyperone (11.2%) were the major components. The composition of essential oil sample from this species in Vietnam differed substantially from that previously reported in China. *C. stoloniferus* rhizome essential oil displayed weak antibacterial activity against gram-negative bacteria, gram-positive bacteria, and yeasts.

Keywords: Chemical composition, cyperene, α-cyperone, cyperotundone, *Cyperus stoloniferus*, rhizome oil.

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INTRODUCTION

Genus *Cyperus* (Cyperaceae) is a large group containing about 700 species, distributed throughout all continents in both tropical and temperate regions. Some *Cyperus* species are used in folk medicine. For instance, the roots of Near East species were a component of kyphi, a medical incense of Ancient Egypt (Fadel, 2020).

Cyperus stoloniferus Retz. is known under various synonyms: *Cyperus arenarius* Hance ex C.B. Clarke, *Cyperus bulbosus* E. Camus, *Cyperus bulboso-stoloniferus* Steud., *Cyperus conjunctus* Steud., *Cyperus lamprocarpus* Nees, *Cyperus littoralis* R. Br., *Cyperus mayeri* Kuk., *Cyperus spadiceus* Lam., *Cyperus stoloniferus* sensu Phamh (Nguyen, 2002). It is a perennial grass-like plant with long, creeping stolons hardening in age into a woody rhizome, forming stout, irregular tubers. The floriferous stems can be 15–50 cm tall. Umbels are simple, contracted or subcapitate, with the rays about 2.5 centimetres long. Spikelets are terete, 8- to 20-fid, shortly spicate, with the glumes closely packed. It is fairly common on coastal sands of dunes and beaches, occasionally also found in saline, muddy locations. The plant is considered useful as a sand binder on dunes (Dai et al., 2010).

The plant is harvested from the wild for its roots, which are used medicinally and as a perfume. Indeed, the rhizomes and tubers are aromatic. They are used in perfumery and to scent coconut oil. The tuber is used in India as a stomachic and is considered to be a good heart stimulant (Ekundayo et al., 1991).

In Vietnam, *C. stoloniferus* is a widely distributed species with no known major threats. It is known under various national names, such as Cói gấu biển, Cú biển, Cói củ, Cỏ gấu biển, Hương phụ biển, and Hải dương phụ. *C. stoloniferus* is used as a substitute for *Cyperus rotundus* but has in general a stronger effect. It belongs to the family of wild-sourced plant species used in traditional medicine, which are exploited in large quantities. Indeed, the estimated traded quantity of *C. stoloniferus* is around 50 tons/year (Nguyen et al., 2008).

Concerning secondary metabolites from *C. stoloniferus*, various flavonoids, flavanones, stilbenes, an aurone and a xanthone have been isolated and their structure elucidated (Nguyen et al., 2013; Malik, 2015; Nguyen et al., 2015). Concerning volatiles, to the best of our knowledge, only two studies were published, about ten years ago:

- The first essential oil sample, of Chinese origin, contained mainly substituted hexahydronaphtalenones namely 2(3H)-naphthalenone,4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methylethylidene), that may be regarded as a keto-aristolochene, according to terpene nomenclature, 19.48% and 2(1H)-naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl), that may be considered as an isomer of a-cyperone, 16.59%, beside the quite surprising bis(2-ethylhexyl) phthalate, (29.39%, major component) (Yang et al., 2012).

- The second oil sample, of Vietnamese origin, contained mainly α-cyperone (32.37%), accompanied by β-selinene (7.34%), caryophyllene oxide (3.68%) and a not fully characterized methanoazulen derivative (6.1%) (Tran et al., 2012).

- Concerning the compositions of essential oils isolated from plants with names recognized as synonyms of *C. stoloniferus*, it could be pointed out that the chemical composition of *C. arenarius* from Iran was dominated by cyperene (21.9 %) and cyperotundone (12.5 %) (Feizbakhsh et al., 2013). In contrast, the composition of *C. bulbosus* varied drastically from the previous ones, being dominated by caryophyllene oxide (26.3%) and humulene oxide (24.1%) (Komai et al., 1994). Similarly, *Cyperus tuberosus* from Thailand contained mainly d-cadinene (30.4%) and a-copaene (18.2%) (Komai et al., 1994) while the main components of *C. tuberosus* essential oil were a-humulene and (*E*)-b-caryophyllene (Ekundayo et al., 1991).

In the course of our ongoing work on the characterization of Vietnamese aromatic and

medicinal plants through the composition of their essential oils (Bazzali et al., 2016; Tran et al., 2019; Tran et al., 2022), we investigated an oil sample isolated from the rhizome of *C. stoloniferus*, collected in the North-Western Vietnam.

MATERIALS AND METHODS

Plant material and essential oil isolation

Rhizomes of *C. stoloniferus* were collected in October 2022, in Nam Thang commune, Tien Hai district, Thai Binh province (Fig. 1). The plant has been authenticated by Tran Huy Thai. A voucher specimen has been deposited at the herbarium of the Institute of Ecology and Biological Resources (IEBR), Ha Noi, under the reference HN-YNTV 85. Rhizomes (3,340 g) were dried in the shade and then submitted to hydrodistillation using a Clevenger-type apparatus, for 20 hours, providing 10.5 g of essential oil.

Figure 1. Map of North Vietnam. Thai Binh province in red. Tien Hai district in green. The sampling collection is dot black in Nam Thang commune

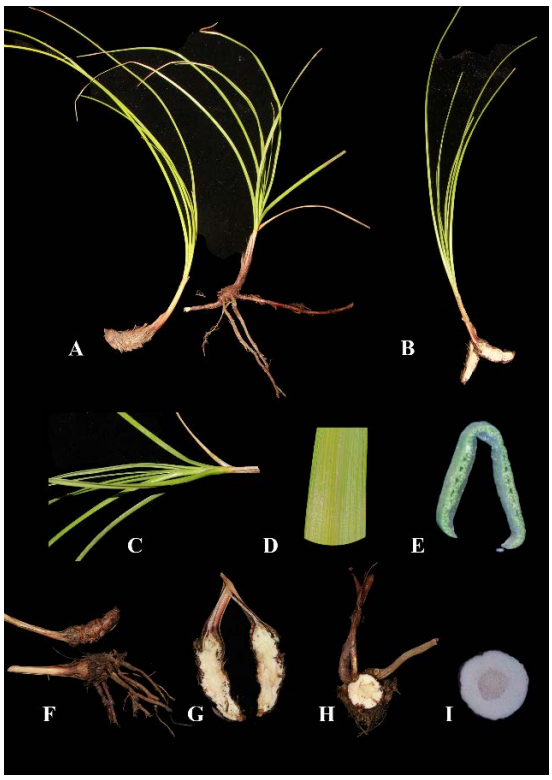

Figure 2. A, B: Whole plant; C: Leaf-bearing stem; D: Leaf, abaxial view; E: Cross section of the leaf; F: Tuber; G: Longitudinal section of the tuber; H, I: Cross section of the tuber

Column chromatography

The rhizome oil (4.124 g) from *C. stoloniferus* was submitted to column chromatography (SiO_2, 50 g, 200–500 µm) leading to five fractions (pentane/ Et_2O, mg): F1 (100/0, 1040), F2 (98/2, 636), F3 (95/5, 1147), F4 (90/10, 820), F5 (0/100, 326).

An aliquot of fraction F2 (495 mg) was again chromatographed (SiO_2, 13 g, 70–200 µm) leading to 9 sub-fractions (pentane/Et_2O, mg): F2.1 (100/0, 100), F2.2 (99.5/0.5, 85), F2.3 (99/1, 74), F2.4 (99/1, 83), F2.5 (98.5/1.5, 75), F2.6 (98.5/1.5, 34), F2.7 (98/2, 11), F2.8 (0/100, 50), F2.9 (0/100, 21).

An aliquot of fraction F3 (1095 mg) was again chromatographed (SiO_2, 35 g, 70−200 µm) leading to 16 sub-fractions (pentane/ Et_2O, mg): F3.1 (100/0, 5), F3.2 (99/1, 14), F3.3 (99/1, 30), F3.4 (97/3, 91), F3.5 (97/3,

95), F3.6 (97/3, 57), F3.7 (97/3, 89), F3.8 (97/3, 86), F3.9 (97/3, 94), F3.10 (97/3, 93), F3.11 (97/3, 87), F3.12 (97/3, 85), F3.13 (96/4, 99), F3.14 (96/4, 56), F3.15 (95/5, 63), F3.16 (0/100, 22).

Sub-fraction F3.7 (89 mg) was again chromatographed (SiO_2, 4 g, 70−200 μm) leading to 6 sub-fractions eluted with a gradient of solvents (pentane/Et_2O, mg): F3.7.1 (98/2, 6), F3.7.2 (98/2, 20), F3.7.3 (98/2, 14), F3.7.4 (98/2, 16), F3.7.5 (98/2, 14), F3.7.6 (97/3, 2).

An aliquot of fraction F4 (721 mg) was again chromatographed (SiO2, 35 g, 70−200 μm) leading to 10 sub-fractions, F4.1–F4.10 (pentane/Et_2O, mg): F4.1 (100/0, 21), F4.2 (98/2, 7), F4.3 (96/4, 30), F4.4 (94/6, 121), F4.5 (94/6, 154), F4.6 (94/6, 168), F4.7 (93/7, 80), F4.8 (92/8, 53), F4.9 (91/9, 15), F4.10 (0/100, 18).

Sub-fractions F4.5–F4.8 have been mixed and an aliquot (541 mg) has been chromatographed (SiO_2, 30g, 70–200 μm) leading to 13 sub-fractions F4B1–F4B13 (pentane/ Et_2O, mg): F4B1 (100/0, 4.5), F4B2 (98/2, 2.5), F4B3 (97/3, 6), F4B4 (96/4, 4), F4B5 (94/6, 83), F4B6 (94/6, 57), F4B7 (94/6, 62), F4B8 (94/6, 63), F4B9 (94/6, 88), F4B10 (93/7, 24), F4B11 (92/8, 10.5), F4B12 (91/9, 3.5), F4B13 (0/100, 43).

Lastly, an aliquot of fraction F5 (249 mg) was chromatographed (SiO_2, 7.5 g, 70–200 μm) leading to five sub-fractions, F5.1–F5.5 (pentane/Et_2O, mg): F5.1 (95/5, 6), F5.2 (90/10, 37), F5.3 (80/20, 63), F5.4 (80/20, 47), F5.5 (0/100, 74).

Gas Chromatography (GC) analysis

Analyses were carried out using a Clarus 500 Perkin Elmer Chromatograph (France), equipped with a flame ionization detector (FID) and two fused-silica capillary columns (50 m × 0.22 mm, film thickness 0.25 μm), BP-1 (poly-dimethyl siloxane) and BP-20 (poly-ethylene glycol). The oven temperature was programmed from 60−220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60; injected volume: 0.5 μL. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes (C8–C29) with linear interpolation (Target Compounds» software from Perkin Elmer).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Samples were analyzed with a Perkin Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL (Courtaboeuf, France), equipped with a DB-1 (polydimethylsiloxane) fused-silica capillary column (60 m × 0.22 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermal at 230 °C for 45 min; injector temp.: 250 °C; ion-source temp.: 150 °C; carrier gas: He (1 mL/min); split ratio: 1/80; injection volume: 0.2 μL; ionization energy: 70 eV. The electron ionization (EI) mass spectra were acquired over the m/z range of 35–350 Da.

Nuclear Magnetic Resonance (NMR) analysis

All ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 Fourier transform spectrometer (Bruker, Wissembourg, France) operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in $CDCl_3$, with all shifts referred to internal TMS. The following parameters were used: pulse width = 4 μs (flip angle 45°); relaxation delay D1 = 0.1 s, acquisition time = 2.7 s for 128K data table with a spectral width of 25,000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3,000 for each sample (Essential oil and fractions of chromatography, 40 mg when available, in 0.5 mL of $CDCl_3$).

Identification of individual components

Identification of the individual components was carried out: (i) by comparison of their GC retention indices (RI) on polar and apolar columns, with those of reference compounds (König et al., 2001; Babushok et al., 2011); (ii)

on computer matching against commercial mass spectral libraries (König et al., 2001; Adam, 2007; NIST, 2014); (iii) on comparison of the signals in the ^{13}C-NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi et al., 1995; Ouattara et al., 2014; El Hafidi et al., 2023). This method allows the identification of individual components of the essential oil at content as low as 0.4–0.5%.

^{13}C NMR data in $CDCl_3$ of selected components

Isorotundene **22** (δC ppm): 151,18 C12, 107,08 C13, 55,78 C1, 43,91 C11, 41,35 C5, 38,73 C7, 38,26 C4, 34,31 C10, 33,22 C6, 32,33 C14, 31,43 C3, 29,27 C9, 27,51 C8, 25,30 C2, 16,00 C15 (numbering of carbons according to Sonwa et al., 2001).

Cyperen-6-ol **28** (δC ppm): 145,71 C5, 132,31 C4, 72,85 C6, 67,44 C1, 58,31 C7, 42,73 C3, 40,46 C11, 34,87 C10, 28,66 C9, 27,32 C12, 26,39 C8, 26,03 C2, 20,16 C13, 17,78 C14, 14,61 C15 (numbering of carbons according to Clery et al., 2016).

Rotundenol **32** (δC ppm): 155.63 C12, 112.66 C13, 76.96 C11, 52.78 C1, 40.64 C4, 38.55 C10, 37.82 C5, 37.01 C7, 31.89 C6, 30.47 C3, 28.18 C8, 26.91 C14, 24.34 C2, 21.54 C9, 15.58 C15 (numbering of carbons, Fig. 3).

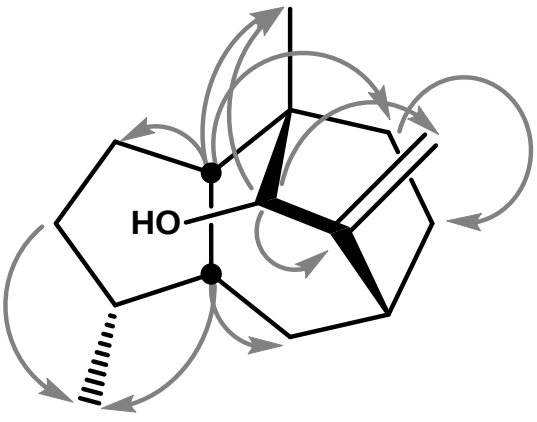

Figure 3. Rotundenol **32.** Arrows indicated the main ^{1}H-^{13}C observed correlations in the HMBC spectrum

Microbial Strains

Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were determined using three strains of Gram-positive test bacteria, including *Staphylococcus aureus* (ATCC 13709), *Bacillus subtilis* (ATCC 6633), and *Lactobacillus fermentum* (VTCC N4), three strains of Gram-negative test bacteria, including *Salmonella enterica* (VTCC), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442), and one strain of yeast, *Candida albicans* (ATCC 10231). The ATCC strains were obtained from the American Type Culture Collection, The VTCC strains were obtained from the Vietnam Type Culture Collection - Vietnam National University, Ha Noi.

Screening of Antimicrobial Activity

Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay (Hadacek et al., 2000; Cos et al., 2006). Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series were prepared from 16,000 µg/mL to 62.5 µg/mL in sterile distilled water in micro-test tubes, from which they were transferred to 96-well microtiter plates. Bacteria grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, and fungi grown in double-strength Sabouraud dextrose broth, were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a negative control. Sterile distilled water and medium served as a positive control. After incubation at 37 ^{o}C for 24 hours, the MIC values were determined at the well with the lowest concentration of agents that completely inhibit the growth of microorganisms. The IC_{50} values were determined by the percentage of microorganisms whose growth was inhibited, based on turbidity measurement data of EPOCH2C spectrophotometer (BioTeKInstruments, Inc Highland Park

Winooski, USA) and Rawdata computer software (Belgium) according to the following equations:

$$\%\,\text{inhibition} = \frac{OD_{control(+)} - OD_{test\ agent}}{OD_{control(+)} - OD_{control(-)}} \times 100\%$$

$$IC_{50} = High_{Conc} = \frac{(High_{Inh\%} - 50\%) \times (High_{Conc} - Low_{Conc})}{(High_{Inh\%} - Low_{Inh\%})}$$

Where: OD: optical density; control (+): only cells in medium without Antimicrobial agent; test agent: corresponds to a known concentration of Antimicrobial agent; control (-): culture medium without cells; $High_{Conc}$/Low_{Conc}: Concentration of test agent at high concentration/low concentration; $High_{Inh\%}$/$Low_{Inh\%}$: % inhibition at high concentration/% inhibition at low concentration. Reference materials: Ampicillin for Gram-positive bacterial strains with MIC values in the range of 0.004 to 1.2 µg/mL, Cefotaxime for Gram-negative bacterial strains with MIC values in the range of 0.07−19.2 µg/mL, Nystatin for fungal strains with MIC value of 2.8 µg/mL to 5.0 µg/mL.

RESULTS AND DISCUSSION

Chemical composition of *Cyperus stoloniferus* rhizome oil

Air-dried rhizomes from *C. stoloniferus* were submitted to hydrodistillation using a Clevenger-type apparatus and yielded a colorless essential oil (0.31%). The essential oil sample was submitted to GC (in combination of retention indices on two chromatographic columns of different polarity, RIs), GC-MS and ^{13}C NMR analyses, following a computerized method developed at the University of Corsica (Tomi et al., 1995; Ouattara et al., 2014; El Hafidi et al., 2023). It clearly appears that we had in our hands an essential oil with an extremely complex composition. Twenty four compounds have been identified by GC-MS in combination with retention indices. They accounted for 69.3% of the whole composition. Thirteen major components, out of 24, have also been identified by ^{13}C NMR by matching against our in-house spectral library. The structures of the main components of essential oil isolated from rhizomes of *C. stoloniferus* are reported in Figure 4.

In order to improve the number of identified components and the identification percentage, the essential oil was submitted to successive column chromatography (CC) on silica gel. The workflow was described in the "Material and Methods" section.

In fraction F1 (eluted with pentane), six other sesquiterpene hydrocarbons have been identified, they accounted for 0.1−1.3% each in fraction F1, up to 0.6% in the EO, for instance, aromadendrene, α-humulene, selina-4,11-diene and calacorene. In that fraction calamenene **24** was identified by MS, and the trans stereochemistry was ensured by ^{13}C NMR. Indeed, both isomers display superimposable mass spectra and identical retention indices (Blanc et al., 2006);

- The structure of isorotundene was suggested by MS for component **22** (1.3% in fraction F1, 0.5% in EO). The spectrum of that compound was missed in our in-house ^{13}C NMR library. In parallel, the spectrum reported in the literature was recorded in C_6D_6 (Sonwa et al., 2001). Therefore, we recorded the spectrum of fraction F1 in that solvent, and we confirmed the presence of isorotundene **22**.^{13}C NMR data of isorotundene in $CDCl_3$ were reported in the "Material and Methods" section;

- In sub-fraction F3.9, a component with RIs apol/pol = 1598/2156 accounted for 10.0% (1.4% in EO) and was not identified, neither by GC-MS nor by ^{13}C NMR. Despite its low content, the 15 signals belonging to that compound, have been picked up and identified as follows, according to the DEPT spectrum: 4 quaternary carbons, including 2 vinylic carbons; 3 aliphatic methines, including a carbon linked to an oxygen atom;

4 aliphatic methylenes, and 4 methyl groups. From these data, the formula $C_{15}H_{24}O$ was deduced, corroborated by the mass spectrum m/z = 220, and belonging to a tricyclic sesquiterpenol. A search among ^{13}C NMR data belonging to sesquiterpenols isolated from *Cyperus* species suggested the occurrence of cyperen-6-ol, already reported in *Cyperus scariosus* essential oil (Clery et al., 2016). Once again, the ^{13}C NMR spectrum of the reference compound was reported in C_6D_6. Recording the spectrum of sub-fraction F3.9 in C_6D_6 confirmed the identification of cyperen-6-ol **28**. ^{13}C NMR data in $CDCl_3$ of cyperen-6-ol were reported in the "Material and Methods" section;

- Compound **32** accounted for 6.6% in EO, and remained unidentified. Therefore, fraction F3 was chromatographed again giving 13 sub-fractions, F3.1-F3.13. In sub-fraction F3.7, compound **32** accounted for 45.5%. Its content was improved to 60.5% in sub-fraction F3.7.5, which permitted to recording of the full set of 2D NMR spectra (HSQC, HMBC and COSY). The structure of rotundenol was deduced from 2D NMR analysis (Fig. 3) and our ^1H NMR data fitted with those reported by Paknikar et al. (1977). The stereochemistry of the hydroxyl group was ascertained by considering the strong steric effect on carbon C9 (-7.7 ppm);

- Compound **34** (2.5% in Fraction F4, 0.7% in EO) was suggested by GC-MS as dehydro α-cyperone. ^{13}C NMR data of this compound were reported by Naya et al. (1990). All the chemical shift values have been observed in the spectrum of F4 and confirmed the occurrence of this compound.

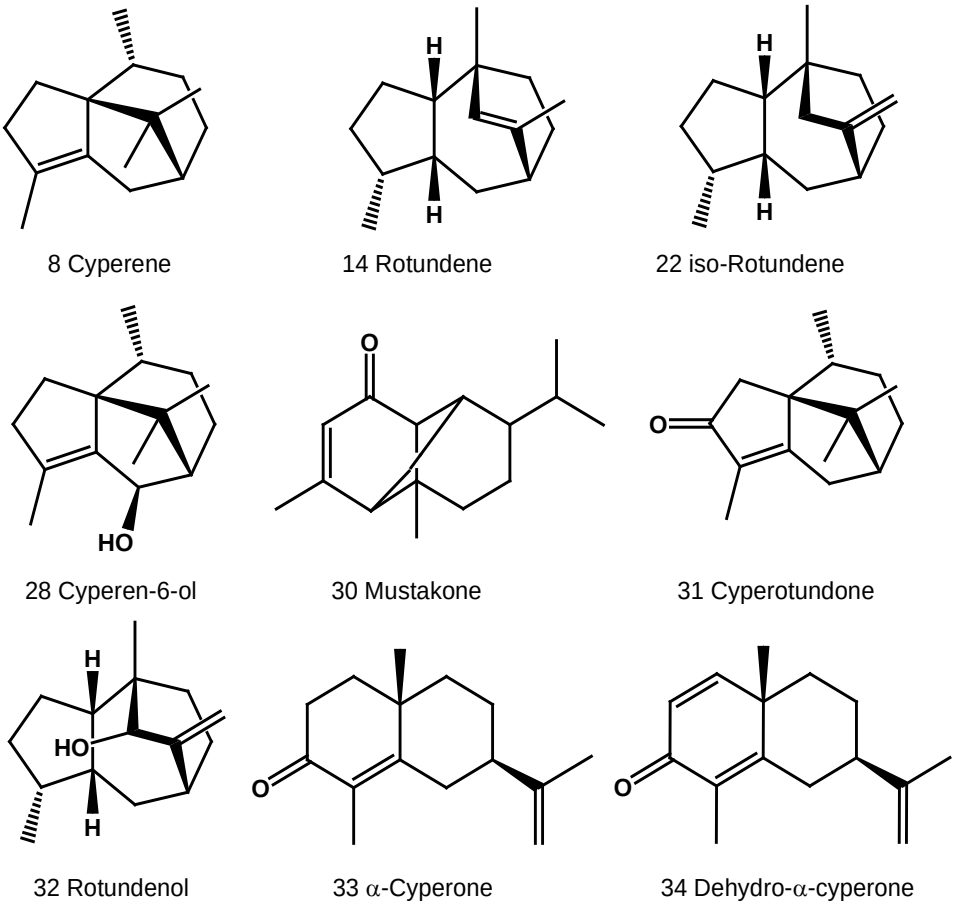

Figure 4. Components of *Cyperus stoloniferus* rhizome essential oil

Table 1. Chemical composition of Vietnamese *Cyperus stoloniferus* rhizome oil

No.	Compounds	RIaL	RIa	RIp	EO	Identification
1	Estragole	1178	1173	1637	0.1	RI, MS
2	Cyprotene[a]	1321	1323	1438	0.6	RI, MS
3	Eugenol	1339	1329	2172	0.9	RI, MS, ^{13}C NMR
4	Cyperadiene[a]	1361	1363	1507	1.0	RI, MS
5	Methyleugenol	1376	1371	2003	0.1	RI, MS, *^{13}C NMR*
6	α-Copaene	1375	1378	1492	1.5	RI, MS, ^{13}C NMR
7	β-elemene	1388	1390	1590	0.7	RI, MS, ^{13}C NMR
8	Cyperene	1399	1404	1528	15.8	RI, MS, ^{13}C NMR
9	(E)-β-Caryophyllene	1419	1420	1528	0.3	RI, MS
10	Norotundeneb	1421	1424	1628	0.1	RI, MS
11	Patchoula-2,4-diene[b]	1434	1432	1673	0.3	RI, MS
12	Aromadandrene	1439	1449	1611	0.4	RI, MS, *^{13}C NMR*
13	α-Humulene	1449	1454	1675	0.1	RI, MS
14	Rotundene[a]	1460	1461	1637	5.2	RI, MS, ^{13}C NMR
15	Eudesma-2,4,11-triene[b]	1471	1469	1736	0.7	RI, MS
16	γ-Gurjunene	1467	1471	1654	0.6	RI, MS, *^{13}C NMR*
17	γ-Muurolene	1473	1473	1688	0.3	RI, MS, *^{13}C NMR*
18	Selina-4,11-diene[b]	1475	1475	1661	0.6	RI, MS, *^{13}C NMR*
19	β-Selinene	1481	1485	1717	5.1	RI, MS, ^{13}C NMR
20	α-Selinene	1489	1494	1722	0.7	RI, MS, ^{13}C NMR
21	Eudesma-2,4(15),11-triene[b]	1495	1496	1802	0.3	RI, MS
22	Isorotundene[b]	1503	1499	1660	0.5	RI, MS, *^{13}C NMR*
23	α-Bulnesene	1501	1502	1715	0.1	RI, MS, *^{13}C NMR*
24	trans-Calamenene	1513	1512	1832	0.4	RI, MS, *^{13}C NMR*
25	δ-Cadinene	1514	1517	1757	0.6	RI, MS, ^{13}C NMR
26	α-Calacorene	1530	1531	1915	0.5	RI, MS, *^{13}C NMR*
27	Caryophyllene oxide	1570	1574	1978	1.0	RI, MS, ^{13}C NMR
28	Cyperen-6-ol[c]	1619	1598	2156	1.4	RI, MS, ^{13}C NMR
29	Patchoulenone[d]	1616	1604	2095	3.1	RI, MS, ^{13}C NMR
30	Mustakone[a]	1658	1657	2248	6.3	RI, MS, ^{13}C NMR
31	Cyperotundone[a]	1679	1676	2265	12.9	RI, MS, ^{13}C NMR
32	Rotundenol [e]	-	1684	2294	1.9	RI, MS, ^{13}C NMR
33	α-Cyperone	1762	1730	2340	11.2	RI, MS, ^{13}C NMR
34	Dehydro α-cyperone [e]	-	1747	2464	0.7	RI, MS, *^{13}C NMR*
	Sesquiterpene hydrocarbons				36.4	
	Oxygenated sesquiterpenes				38.5	
	Phenyl propanoids				1.1	
	Total				76.0	

Notes: Order of elution and percentage of individual components are given on apolar column DB1; RIa, RIp: Retention indices measured on apolar and polar columns, respectively; RIaL: Retention indices on apolar column, from Literature: ref (Babushok et al., 2011), otherwise stated: a) Essaidi et al. (2014), b) König et al. (2001); c) Clery et al. (2016) d) Zoghbi et al. (2006); e) not found; ^{13}C NMR: compounds identified by ^{13}C NMR in the EO, and confirmed in fractions of chromatography, ^{13}C NMR (italic): compounds identified by ^{13}C NMR in fractions of chromatography.

The composition of this essential oil sample is strongly dominated by sesquiterpene hydro-carbons and oxygenated sesquiterpenes. Various components bear uncommon tricyclic structures, such as tricyclo[5.3.1.$0^{1,5}$]undecane (cyperane) and tricyclo[6.2.2.$0^{1,5}$]undecane (rotundane) already mentioned in essential oils from other *Cyperus* species. For instance, cyperene **8**, the major component of the investigated oil sample (15.8%) was reported in EOs from Iranian *Cyperus rotundus*; (37.9%) (Aghassi et al., 2013) and *Cyperus conglomeratus* (27.2%) (Feizbakhsh et al., 2011). Rotundene **14** accounted for 5.0% of *Cyperus papyrus* from South Africa (Lawal et al., 2009); mustakone **30** up to 14.5% of *C. articulatis* from Brazil (Zoghbi et al., 2006). Cyperotundone **31** was present in *C rotundus* essential oil from Nigeria (42.3%) (Olawore et al., 2006) and Tchad (34.9%) (Mahmout et al., 1997). α-Cyperone **33** reached 38.46% in Chinese *C. rotundus* essential oil (Hu et al., 2017). In contrast, various components present at appreciable contents in the Vietnamese sample were scarcely found in essential oils, for instance, norotundene **10** and overall dehydro-α-cyperone **34**, first isolated from the rhizome oil of *Ligularia dentata* (Naya et al., 1990) and, to the best of our knowledge, identified for the first time in an essential oil from *Cyperus* species. Lastly, all unidentified compounds were minor components, among which eight of these accounted for 0.8–1.2%.

The composition of the essential oil investigated in this study differed drastically from the Chinese oil sample, that displayed bis(2-ethylhexyl)phthalate as the major component (29.39%) followed by two substituted naphthalenone, namely 2(3H)-naphthalenone,4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methylethylidene), bearing the aristolochane skeleton (19.48%), and 2(1H)-naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methyl ethenyl), that look as an isomer of α-cyperone (16.59%) (Yang et al., 2012). It could also remain that α-cyperone was the major component (32.37%) of a previously investigated oil sample from *C. stoloniferus* of Vietnamese origin that contained also a not fully characterized methanoazulen derivative bearing the tricyclic skeleton of cyperotundone (Tran et al., 2012).

α-Cyperone also found in the essential oil of *C. rotundus* has the effect of reducing inflammation by destabilization of microtubule fibers in the brain (Azimi et al, 2016). α-cyperotundone isolated from the root bark of *Maytenus retusa* was able to inhibit the in vitro growth of the amoebae at relatively low concentrations (Camen et al., 2010). Cyperene extracted from the essential oil of *Cyperus scariosus* was effective against *Alternaria triticina* with ED_{50} value of 0.19 mg/ml (Amit, 2018).

The antibacterial and anti-yeast activity of Cyperus stoloniferus rhizome essential oil

The essential oil sample was then subjected to microbroth dilution assays to determine the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values using 7 strains of microorganisms: *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus fermentum*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. The results of the assay obtained after 16–24 hours of incubation are presented in Table 2. The essential oil from *C. stoloniferus* showed weak antibacterial activity against 6 out of 7 tested microorganisms, with IC_{50} values ranging from 639.0 μg/mL to 7,428.5 μg/mL and MIC values ranging from 4,000 μg/mL to more than 16,000 μg/mL. Gram-positive bacteria and yeast were more susceptible to the essential oil sample in comparison with Gram-negative ones. Among them, *L. fermentum* and *C. albicans* were the most sensitive to the oil with IC_{50} of 971.0 ± 87.9 μg/mL and 639.0 ± 37.9 μg/mL. *S. enterica* was found to be the least sensitive to the oil.

Table 2. Microbial minimum inhibitory concentrations and median inhibitory concentrations of essential oil of *Cyperus stoloniferus*

Bacteria/Fungi	IC_{50} (μg/mL)	MIC (μg/mL)
Staphylococcus aureus	2,500 ± 104.5	4,000 ± 0.0
Bacillus subtilis	2,578 ± 179.4	4,000 ± 0.0
Lactobacillus fermentum	971.0 ± 87.9	16,000 ± 0.0
Salmonella enterica	>16,000	>16,000
Escherichia coli	3,181.8 ± 197.9	>16,000
Pseudomonas aeruginosa	7,428.5 ± 321.7	16,000 ± 0.0
Candida albicans	639.0 ± 37.9	16,000 ± 0.0

CONCLUSION

In opposition with essential oils from various species of *Cyperus*, particularly *C. rotundus*, whose compositions have been extensively investigated, very little was known about *C. stoloniferus*. The composition of the essential oil sample investigated in this study appeared extremely complex. Combined analysis by chromatographic (GC, successive CC) and spectroscopic techniques (GC in combination with retention indices, MS and ^{13}C NMR) allowed the identification of 34 components, the structure of 25 out of 34 being confirmed by ^{13}C NMR either by comparison with literature data and/or by 2D NMR. Among the major components, it could be cited cyperene (15.8%), cyperotundone (12.9%), α-cyperone (11.2%), mustakone (6.3%), rotundene (5.2%), β-selinene (5.1%). Other components present at appreciable content included patchoulenone (3.1%), rotundenol (1.9%), cyperen-6-ol (1.4%), and dehydro-α-cyperone (0.9%). The compositions of the Vietnamese and Chinese oil samples differed substantially. Lastly, the essential oil from *C. stoloniferus* showed weak antibacterial and antifungal activities.

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