Trypanocidal, trichomonacidal and cytotoxic components of cultivated *Artemisia absinthium* Linnaeus (Asteraceae) essential oil

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Artemisia absinthium *is an aromatic and medicinal plant of ethnopharmacological interest and it has been widely studied. The use of* A. absinthium *based on the collection of wild populations can result in variable compositions of the extracts and essential oils (EOs). The aim of this paper is the identification of the active components of the vapour pressure (VP) EO from a selected and cultivated* A. absinthium *Spanish population (T2-11) against two parasitic protozoa with different metabolic pathways:* Trypanosoma cruzi *and* Trichomonas vaginalis*. VP showed activity on both parasites at the highest concentrations. The chromatographic fractionation of the VP T2-11 resulted in nine fractions (VLC1-9). The chemical composition of the fractions and the antiparasitic effects of fractions and their main compounds suggest that the activity of the VP is related with the presence of* trans*-caryophyllene and dihydrochamazulene (main components of fractions VLC1 and VLC2 respectively). Additionally, the cytotoxicity of VP and fractions has been tested on several tumour and no tumour human cell lines. Fractions VLC1 and VLC2 were not cytotoxic against the nontumoural cell line HS5, suggesting selective antiparasitic activity for these two fractions. The VP and fractions inhibited the growth of human tumour cell lines in a dose-dependent manner.*

Key words: *Artemisia absinthium* - essential oil - activity - *Trypanosoma cruzi* - *Trichomonas vaginalis* - cytotoxicity

The *Artemisia* genus belongs to the family Compositae (Asteraceae) and consists of about 500 species distributed through the world. *Artemisia absinthium* L. is an aromatic and medicinal plant of ethnopharmacological interest (Bora & Sharma 2010, Lachenmeier 2010).The composition and biological effects of the essential oil (EO) and the extracts of *A. absinthium* have been widely studied. Different researches have demonstrated its antimicrobial and antiprotozoal effects against *Leishmania aethiopica*, *Leishmania donovani*, *Leishmania infantum* and *Trypanosoma cruzi* (Juteau et al. 2003, Tariku et al. 2011, Erel et al. 2012, Nasrabadi et al. 2012, Bailén et al. 2013, Bachrouch et al. 2015).

Among the major *A. absinthium* EO components reported are α and β-thujone, myrcene, trans-sabinyl acetate, β-pinene, 1,8-cineole, camphor, cis-epoxyocimene, chrysanthenyl acetate, sabinene, myrtenol, bornyl acetate, artemisiaketone, linalool, hydrocarbon monoterpenes, sesquiterpene lactones (Pino et al. 1997, Jaenson et al. 2005, Kordali et al. 2005, Geszprych et al. 2010, Martín et al. 2011, Erel et al. 2012, Judzentiene et al. 2012, Sharopov et al. 2012, Tehrani et al. 2012, Umpiérrez et al. 2012) and mixtures of some of these components, depending on the plant origin (Chialva et al. 1983, Carnat et al. 1992, Geszprych et al. 2010, Bailén et al. 2013). In fact, the use of *A. absinthium* based on the collection of wild populations can result in variable compositions of the extracts and EOs.

A. absinthium is abundant in the mountains of the Iberian Peninsula, where seven chemotypes have been described (Ariño et al. 1999). Two Spanish populations of wormwood have been domesticated for experimental cultivation in the field and under controlled conditions (Burillo 2009, Martín et al. 2011, González-Coloma et al. 2012a). Based on these results, a long-term field cultivation of selected *A. absinthium* plants has been established for further valorisation of its extracts.

The aim of the present paper is the identification of the active components of the vapour pressure (VP) EO from a selected and cultivated *A. absinthium* Spanish population against two parasitic protozoa with different metabolic pathways, *T. cruzi* and *Trichomonas vaginalis*.

T. cruzi is the aetiologic agent of Chagas disease, a frequently fatal illness affecting the heart and gastrointestinal systems. An estimated eight million people in Latin America are infected with this pathogen and it is also spreading to the United States of America, Canada and many parts of Europe and the Western Pacific as a result of migratory flows (Rassi Jr et al. 2010). Only two drugs, nifurtimox (NFX) and benznidazole are in use

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against chronic infections and both have limitations, due to the need of a large number of doses over a long time period, side effects and lack of effectiveness against all stages of the disease and all strains of the parasite. Moreover, their lack of efficiency has involved problems in their production and distribution (González-Coloma et al. 2012b).

T. vaginalis is a parasitic protozoa and a major cause of vaginitis, cervicitis and urethritis in women and may cause nongonococcal urethritis, prostatitis and other genitourinary tract syndromes in men. Trichomoniasis is among the world's most common sexually transmitted diseases with an annual incidence of more than 276 million cases per year (WHO 2012). Its clinical manifestations vary from asymptomatic infection to an acute vaginitis. A single drug, metronidazole, is currently available for treating trichomoniasis. However, metronidazole resistant strains have been found in unsuccessfully treated patients and some adverse effects have been described (Dunne et al. 2003, Cudmore et al. 2004).

Natural products could be a source of new drugs. In this paper the antiparasitic effects of a characterised *A. absinthium* VP EO against *T. cruzi* and *T. vaginalis* will be discussed along with the chemical composition of the active fractions. Additionally, their selective cytotoxicity has been tested on several tumoural (A549, H292, HCT116, MCF7, SK-MEL5) and nontumoural (HS5) human cell lines.

MATERIALS AND METHODS

Plant material and cultivation - The individuals for field cultivation were obtained from selected seeds (*A. absinthium* var. candial®) and planted in Ejea de los Caballeros (Zaragoza, Spain) in 2008. A detailed description of the field and the cultivation parameters has been reported (Burillo 2009). Flowering plants were harvested yearly and processed for VP extraction. The material under study is endotoxin free.

EO analysis - Plant material was distilled in an industrial stainless steel VP extraction plant equipped with two 3000 L vessels (ecoaromuz.com). The VP EO extracted was analysed by gas chromatography mass spectrography (GC-MS) using an Agilent 6890N GC (Agilent Technologies, USA) coupled to an Agilent 5973N mass detector (electron ionisation, 70 eV) (Agilent Technologies) and equipped with a 25 m × 0.20 mm i.d. capillary column (0.2 µm film thickness) HP-1 (methyl silicone bonded) (Hewlett-Packard). Working conditions were as follows: split ratio (20:1), injector temperature 260ºC, temperature of the transfer line connected to the mass spectrometer 280ºC, initial column temperature 70ºC, then heated to 270ºC at 4ºC min^{-1}. Electron ionisation mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley Mass Spectral Database (2001). Quantitative data were obtained from the total ion current peak areas without the use of response factors.

Fractionation of the EO - A VP extract of *A. absinthium* (20 g, T2 population, 2011) was submitted to vacuum liquid chromatography on a Si-gel column (40-70 µm, 6 cm diameter, 9 cm length) eluted with a hexane (Hx):dichloromethane (DCM) gradient of increasing polarity (0.5-100% DCM). Nine fractions were obtained and analysed by GC-MS as described. Fraction 8 (690 mg) was further purified by flash chromatography on a 2.5 cm diameter silica cartridge (40-70 µm) eluted with a Hx:DCM (70:30) mixture (isocratic, 18 mL/min flow rate) and by Sephadex LH-20 chromatography [DCM: methanol (MeOH), 1:1] to give (-)-*cis*-chrysanthenol (4) (33.5 mg; 4.9%).

All solvents used were of analytical grade. n-Hx, DCM and MeOH were obtained from Lab-Scan Analytical Sciences (Poland). Silica gel (70-30 mesh) and thin layer chromatography plate (silica gel 60F254) was purchased from Merck Co (Germany). (-)-c*is*-chrysanthenol was isolated from *A. absinthium* EO (VP extract) and *trans*-caryophyllene 80% was purchased from Sigma-Aldrich (USA).

The VP EO is constituted by apolar substances and the activity assays are performed in aqueous culture media. To the tests, VP, fractions and products are previously dissolved in acetone at an initial concentration of 10 mg/mL. From this initial solution, the different concentrations for assays are prepared using the culture medium of cells and parasites.

Trypanocidal in vitro activity - Trypanocidal activity was assayed on epimastigote forms of *T. cruzi* Y strain, cultured in liver infusion tryptose medium supplemented with 10% heat-inactivated foetal calf serum. Parasites in logarithmic growth phase from an initial culture with 2×10^6 epimastigotes/mL were distributed in 96-well flat-bottom plates. Each well was filled with 90 µL of culture after two days of incubation. VP, factions and compounds were tested at several concentrations (VP at 800, 400, 200, 100, 10 and 1 µg/mL; fractions and compounds at 100, 10 and 1 µg/mL) for 72 h. NFX was used as the reference drug and parasite viability was analysed by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay method (González-Coloma et al. 2012b). Briefly, after 72 h, 10 µL MTT/phenazine methosulfate were added to each well. Incubate 75 min to occur the reduction of MTT and 100 µL sodium dodecyl sulphate were added to dissolve formazan crystals obtained as a result of the reduction of MTT. Once the crystals have dissolved (15-30 min), the plate was read on a spectrophotometer at 570 nm. The activity was calculated as percentage growth inhibition (%GI) as follows: %GI = 100 - [(*Ap* - *Ab*)/(*Ac* - *Ab*)] x 100, where *Ap* being the absorbance of problem wells (treated), *Ac* the absorbance of control wells (not treated) and *Ab* the absorbance of blank wells (culture medium and vehicle only). All assays were carried out in triplicate and were repeated at least three times independently to confirm the results. The concentration that inhibits 50% the growth of the parasites (GI_{50}) as well as the 95% confidence intervals (CIs) were calculated by Probit analysis (SPSS v.20, IBM).

Activity on T. vaginalis - Trichomonacidal assays were carried out against the metronidazole-sensitive *T. vaginalis* JH31A no.4 isolate [American Type Culture Collection, (ATCC)]. The flagellates were cultured in trypticase-yeast extract-maltose modified medium supplemented with 10% heat-inactivated foetal bovine serum (FBS) and antibiotic solutions at 37ºC and 5%

TABLE I

Gas chromatography mass spectrography analysis of the *Artemisia absinthium* vapour pressure (VP) essential oil (T2-11) and its fractions

Compound	VP T2-11	Fraction								
		VLC1	VLC2	VLC3	VLC4	VLC5	VLC6	VLC7	VLC8	VLC9
Yield	-	12.6	4	0.8	7.6	5.3	13.5	4.8	7.8	1.7
Linalool (1)	2	-	-	-	-	-	-	-	17.3	-
cis-epoxycimene (2)	39.8	-	-	-	13.5	63.9	86.6	5.6	-	-
Camphor (3)	4.5	-	-	-	-	1.4	7.2	27	-	-
(-)-*cis*-chrysanthenol (4)	11.9	-	-	-	-	-	-	60.8	63	0.7
(*E*)-3-hexenyl butyrate (5)	1.1	-	-	-	-	-	-	1.7	12.4	-
Chrysanthenyl acetate (6)	5.3	-	-	1	35.7	22.1	0.9	-	-	-
(-)-(5Z)-2,6-dimethylocta-5,7-diene-2,3-diol (7)	2	-	-	-	-	-	-	-	-	67.8
trans-caryophyllene (8)	3.8	29.5	-	-	-	-	-	-	-	-
Germacrene-D (9)	2.3	15.5	-	-	-	-	-	-	-	-
β-selinene (10)	1.1	8.8	-	-	-	-	-	-	-	-
3,6-dihydrochamazulene (11)	5.8	2.5	42.5	7.9	-	-	-	-	-	-
Chamazulene (12)	2.6	1.1	41.4	81.7	-	-	-	-	-	-

data are expressed as relative abundance (%).

CO_2. Assays were carried out in glass tubes containing 10^5 trophozoites/mL. After 5-6 h of seeding, the VP, factions and compounds were added to log-phase growth cultures at several concentrations (500, 250, 100, 75, 37.5 and 18.75 µg/mL). The tubes were incubated for 24 h at 37ºC and 5% CO_2. The trichomonacidal activity was obtained by a fluorimetric method using resazurin (Sigma-Aldrich) as previously described (Escribano et al. 2012). The experiments were performed at least two times in triplicate. GI_{50} values as well as the 95% CI were calculated by Probit analysis (SPSS v.20, IBM).

Cytotoxicity assays - The A549, NCI-H292 (adenocarcinoma and squamous non-small cell lung cancer, respectively), HCT116 (colorectal carcinoma), MCF7 (luminal breast adenocarcinoma), SK-MEL-5 (melanoma) and HS5 (bone marrow stromal) human cell lines were employed to determine the toxicity of the VP and fractions. HS5 was used as nontumour control cells. Cell lines were purchased from LGC Promochem, SLU-ATCC (Spain). All cell lines were propagated in RPMI-1640 medium, supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM glutamine (Lonza Verviers). Cells were grown at 37ºC in a humidified atmosphere with 5% CO_2 and were in the logarithmic growth phase at the initiation of the experiments. For the determination of the activity, cells diluted in 100 µL/well of complete cell culture medium were plated in 96-well flat-bottom plates and allowed to attach for 24 h. Growth medium was removed from the wells and replaced by medium containing the VP or fractions at concentrations of 100 and 250 µg/mL for another 72 h. The anticancer drugs paclitaxel (a cyclic diterpene) and cisplatin (drug based on the platinum) were tested as reference products. All experimental points were set up in four wells and all were confirmed in at least three independent experiments. Viable cells were determined using the WST-1 assay (Roche, Germany) according to the manufacturer's protocol.

RESULTS AND DISCUSSION

Table I shows the composition of the VP and its fractions. c*is*-epoxyocimene was the major component (40%) followed by (-)-*cis*-chrysanthenol (12%), dihydrochamazulene (6%) and chrysanthenyl acetate (5.3%). Camphor (4.5%), *trans*-caryophyllene (4%) and chamazulene (3%) were also present. Linalool, (-)-(*5Z*)-2,6-dimethylocta-5,7-diene-2,3-diol, germacrene-D, *β*-selinene, (E)-3-hexenyl butyrate were found in low amounts ranging between 1-2.3%.

Two chemotypes have been described from the Iberian Peninsula (Ariño et al. 1999); *cis*-epoxyocimene type and a *cis*-epoxyocimene + chrysanthenyl acetate type. The cultivated Spanish wormwood population VP used in this work showed *cis*-epoxyocimene + *cis*-chrysanthenol chemotype. Similarly, the population that originated this selected germplasm showed a similar VP composition (Bailén et al. 2013).

The antiparasitic effects of the VP and its fractions are shown in Tables II, III. The VP was active on *T. cruzi* up to a concentration of 200 µg/mL, showing GI of 100% at 800 µg/mL, 96% at 400 µg/mL and 83.6% at 200 µg/mL (GI_{50} 144.6 µg/mL), in agreement with the bioactivity reported for the parent population EO (Bailén et al. 2013). This VP also showed a trichomonacidal effect (Table III) with GI of 99.1% at 500 µg/mL, 87.4% at 250 µg/mL and 53.7% at 100 µg/mL (GI_{50} 87.2 µg/mL).

Among the VP fractions, VLC1 and 2 were the most active on *T. cruzi* (nearly 100% mortality at 100 µg/mL) (Table II). Seven of the nine VLC fractions (1, 2, 4-8)

TABLE II

Trypanocidal activity of *Artemisia absinthium* vapour pressure (VP) essential oil (T2-11), fractions VLC1-VLC9, *trans*-caryophyllene and (-)-*cis*-chrysanthenol

VP, fractions and compounds	Concentration (µg/mL)					
	800	400	200	100	10	1
VP T2-11	100 ± 1.7	96.0 ± 11.2	83.6 ± 18.0	33.0 ± 7.2	5.8 ± 5.2	4.7 ± 4.9
VLC1	-	-	-	97.8 ± 4.6	0.6 ± 1.8	0.5 ± 1.1
VLC2	-	-	-	94.7 ± 8.4	1.5 ± 2.3	1.3 ± 2.2
VLC3	-	-	-	30.0 ± 5.4	0.9 ± 1.7	0.0 ± 0.0
VLC4	-	-	-	46.7 ± 15.3	2.0 ± 3.8	4.5 ± 4.2
VLC5	-	-	-	26.8 ± 15.9	1.0 ± 1.3	0.5 ± 1.8
VLC6	-	-	-	8.4 ± 6.4	1.6 ± 2.0	3.3 ± 4.3
VLC7	-	-	-	10.1 ± 7.6	0.0 ± 0.0	4.8 ± 5.9
VLC8	-	-	-	41.9 ± 10.1	11.1 ± 5.6	4.5 ± 3.7
VLC9	-	-	-	6.5 ± 8.1	0.8 ± 1.4	1.4 ± 2.3
trans-caryophyllene (8)	-	-	-	99.4 ± 1,9	11.6 ± 14.1	5.4 ± 5.5
(-)-*cis*-chrysanthenol (4)	-	-	-	3.5 ± 8.3	0.0 ± 0.0	0.4 ± 0.7
NFX	-	-	-	99.9 ± 0.2	100.0 ± 0.5	20.8 ± 2.8

nifurtimox (NFX) is included as drug reference. Data are expressed as percentage of growth inhibition ± standard deviation.

showed trichomonacidal effect (> 70%) at 500 µg/mL, but only VLC1 maintained significant activity (81.6%) at 100 µg/mL. VLC4 and VLC8 were also active at 250 µg/mL (Table III).

Fractions VLC1 and 2 were not cytotoxic against nontumour cell line HS5, suggesting selective antiparasitic activity for these two fractions (Table IV).

Fraction VLC1 is characterised by *trans*-caryophyllene (29.5%), germacrene D (15.5%) and *β*-selinene (8.8%). Fraction VLC2 contained dihydrochamazulene (42.5%) and chamazulene (41.4%). The main component of VLC8 is (-)-*cis*-chrysanthenol (63%) (Figure, Table I).

trans-caryophyllene showed a remarkable activity against *T. cruzi* (Table II) with GI of 99.4% at 100 µg/mL (GI_{50} 39.2 µg/mL). This compound also showed significant trichomonacidal effect (Table III) with GI of 100% at 500 µg/mL, 99% at 250 µg/mL, 80.3% at 100 µg/mL and 53.7% at 75 µg/mL (GI_{50} 68.7 µg/mL). The antiparasitic effects of VLC1 against *T. cruzi* and *T. vaginalis* can be partially attributed to *trans*-caryophyllene.

The activity of VLC2 on *T. cruzi* cannot be attributed to chamazulene since fraction VLC3, containing 81.7% chamazulene, was inactive. Therefore, dihydrochamazulene or the mixture of both compounds could be responsible for this effect. Furthermore, neither VLC2 nor VLC3 showed trichomonacidal activity at 100 µg/mL, suggesting a selective activity (alone or in synergy) of dihydrochamazulene on *T. cruzi*.

(-)-*cis*-chrysanthenol also showed activity against *T. vaginalis* with GI of 100% at 500 µg/mL, 96.3% at 250 µg/mL, 54.5% at 100 µg/mL and 45.4% at 75 µg/mL (GI_{50} 87.2 µg/mL) (Table III). The effects of VLC8 against *T. vaginalis* at high concentrations can be attributed to (-)-*cis*-chrysanthenol.

When tested against tumour cell lines, the VP and fractions inhibited the growth of human tumour cell lines in a dose-dependent manner (Table IV). We carried out parallel experiments with cisplatin and paclitaxel on cells plated at the same time from a single flask to serve as reference cytotoxicity. Doses used for these two compounds are those that lead to a 50% of GI in most tumour cell lines.

For the fractions tested, the most sensitive cell lines were SK-MEL-5 (melanoma) and HCT116 (colorectal adenocarcinoma). MCF-7 (luminal breast adenocarcinoma) was the less sensitive tumour cell line. VP and VLC-1 were the most active fractions for the majority of cell lines, being also remarkable cytotoxic effects of VLC-5 and VLC-8 fractions. Moreover, VP and VLC-1 also showed activity against A549 and H292 cell lines (adenocarcinoma and squamous nonsmall cell lung cancer). These cytotoxic effects may be due to the presence of *trans*-caryophyllene and/or germacrene D in the VP and fractions. The cytotoxic activity of *trans*-caryophyllene on animal and human tumour cells has been previously described by other authors (el Hadri et al. 2010).

It is noteworthy that these fractions had little or no cytotoxic effect against the nontumour cell line, HS5, suggesting selective cytotoxic activity for these fractions. This would be an important biological effect and could provide new rational basis for the design of new antitumour compounds.

EOs rich in chamazulene showed variable growth-inhibitory effects on human cancer cell lines with GI_{50} values ranging from 14.3 µg/mL on A375 (human malignant melanoma cell line) to 59.8 µg/mL on T98G (human glioblastoma cell line) (Ornano et al. 2013) and strong antifungal properties against dermatophytes and opportunistic saprophytes (Jamalian et al. 2012). Chamazulene has

TABLE III

Trichomonacidal activity of *Artemisia absinthium* vapour pressure (VP) essential oil (T2-11), fractions VLC1-VLC9, *trans*-caryophyllene and (-)-*cis*-chrysanthenol

VP, fractions and compounds	Concentration (μg/mL)					
	500	250	100	75	37.5	18.75
VP T2-11	99.1 ± 0.4	87.4 ± 1.3	53.7 ± 0.5	51.7 ± 3.3	14.5 ± 2.4	0.8 ± 1.2
VLC1	99.3 ± 0.2	98.8 ± 0.2	81.6 ± 0.2	47.6 ± 3.8	10.3 ± 2.0	0.4 ± 0.5
VLC2	100 ± 0.0	44.3 ± 5.6	11.8 ± 4.0	2.7 ± 3.9	1.2 ± 1.7	0.0 ± 0.0
VLC3	35.5 ± 0.1	12.2 ± 2.9	10.3 ± 2.0	0.4 ± 0.5	1.0 ± 1.4	0.0 ± 0.0
VLC4	99.5 ± 0.1	99.4 ± 0.1	39.1 ± 0.2	27.0 ± 3.2	4.0 ± 0.1	0.0 ± 0.0
VLC5	99.3 ± 0.4	43.3 ± 2.6	2.4 ± 3.4	3.7 ± 5.3	0.0 ± 0.0	0.0 ± 0.0
VLC6	71.6 ± 1.3	36.5 ± 1.8	4.7 ± 6.6	2.5 ± 3.6	0.4 ± 0.6	0.0 ± 0.0
VLC7	100.0 ± 0.0	47.9 ± 4.3	27.0 ± 6.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
VLC8	99.9 ± 0.6	92.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
VLC9	4.7 ± 6.7	1.7 ± 2.5	0.0 ± 0.0	1.7 ± 2.4	0.0 ± 0.0	0.5 ± 0.7
trans-caryophyllene (8)	100.0 ± 0.0	99.0 ± 0.2	80.3 ± 7.3	53.7 ± 2.2	34.3 ± 6.0	19.9 ± 8.5
(-)-*cis*-chrysanthenol (4)	100.0 ± 0.0	96.3 ± 1.4	54.5 ± 5.9	45.4 ± 2.5	40.9 ± 0.8	2.1 ± 0.8

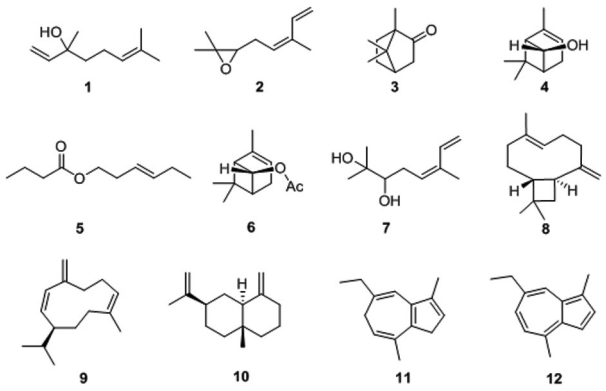
data are expressed as percentage of growth inhibition ± standard deviation

TABLE IV

Cytotoxic effects on A549, H292, HCT116, MCF7, SK-MEL-5 and HS5 cells of *Artemisia absinthium* vapour pressure (VP) essential oil (T2-11) and fractions VLC1-VLC9

VP and fractions	Concentration (μg/mL)	Cell lines					
		A549	H292	HCT116	MCF7	SKMEL-5	HS5
VP T2-11	250	79.5 ± 0.4	76.7 ± 1.1	51.1 ± 1.8	91.3 ± 0.9	60.1 ± 3.1	80.5 ± 3.4
	100	98.6 ± 5.2	82.5 ± 1.0	58.5 ± 3.5	95.7 ± 1.8	78.5 ± 0.9	86.7 ± 0.9
VLC-1	250	60.6 ± 1.5	73.0 ± 0.9	49.6 ± 1.3	95.7 ± 1.8	58.2 ± 0.8	80.8 ± 1.5
	100	74.8 ± 1.9	77.2 ± 3.2	52.1 ± 0.8	91.3 ± 0.9	56.9 ± 3.0	79.1 ± 2.4
VLC-2	250	84.0 ± 4.7	89.2 ± 7.8	59.4 ± 4.2	84.0 ± 9.8	58.8 ± 10.5	91.7 ± 6.8
	100	69.5 ± 10.2	90.4 ± 2.0	59.5 ± 3.6	89.6 ± 2.1	67.7 ± 5.2	83.3 ± 7.4
VLC-3	250	83.6 ± 4.2	97.2 ± 7.1	59.0 ± 1.4	91.9 ± 3.5	57.3 ± 5.7	90.2 ± 5.2
	100	100 ± 9.1	98.1 ± 5.4	63.4 ± 0.9	93.3 ± 0.5	66.3 ± 5.5	84.1 ± 4.3
VLC-4	250	80.6 ± 2.5	73.5 ± 3.1	63.9 ± 1.3	88.7 ± 0.2	57.4 ± 1.0	78.5 ± 2.5
	100	95.9 ± 2.3	79.9 ± 0.8	78.5 ± 0.6	100 ± 0.7	58.8 ± 1.6	86.1 ± 1.8
VLC-5	250	57.7 ± 1.3	78.1 ± 0.6	51.7 ± 1.5	80.1 ± 3.7	54.1 ± 2.1	75.9 ± 1.7
	100	95.2 ± 3.1	79.7 ± 1.3	62.8 ± 3.6	97.3 ± 2.3	53.5 ± 3.9	68.2 ± 1.8
VLC-6	250	100 ± 2.5	87.8 ± 3.9	64.4 ± 2.2	96.9 ± 2.3	59.5 ± 4.2	81.2 ± 2.9
	100	100 ± 2.7	96.8 ± 1.8	75.0 ± 2.7	100 ± 1.1	72.0 ± 1.3	90.7 ± 3.7
VLC-7	250	100 ± 3.4	94.4 ± 1.1	93.1 ± 1.9	93.7 ± 1.6	69.5 ± 8.9	89.1 ± 1.8
	100	100 ± 2.3	100 ± 1.7	93.6 ± 2.3	97.9 ± 1.9	78.4 ± 5.1	95.7 ± 2.1
VLC-8	250	67.7 ± 4.6	82.8 ± 13.6	50.9 ± 2.0	69.1 ± 2.3	54.5 ± 1.9	76.3 ± 3.9
	100	100 ± 2.4	87.2 ± 5.2	65.0 ± 0.6	100 ± 1.7	54.5 ± 2.8	86.6 ± 2.8
VLC-9	250	100 ± 0.2	92.5 ± 6.0	96.8 ± 1.6	97.8 ± 1.7	65.9 ± 6.0	95.6 ± 1.7
	100	100 ± 3.1	97.4 ± 5.0	89.6 ± 3.5	96.1 ± 1.7	79.3 ± 2.1	95.4 ± 2.1
Paclitaxel	85	41.9 ± 2.9	41.8 ± 1.7	40.2 ± 2.8	52.9 ± 3.5	34.9 ± 2.4	27.7 ± 4.8
Cisplatin	300	34.6 ± 1.5	57.3 ± 2.6	25.7 ± 1.7	65.6 ± 1.1	42.6 ± 0.3	22.8 ± 2.4

paclitaxel and cisplatin are included as drugs reference. Data are expressed as viability percentage ± standard deviation relative to vehicle-treated control cells.



Chemical structures of linalool (1), *cis*-epoxycimene (2), camphor (3), (-)-*cis*-chrysanthenol (4), (*E*)-3-hexenyl butyrate (5), chrysanthenyl acetate (6), (-)-(5Z)-2,6-dimethylocta-5,7-diene-2,3-diol (7), *trans*-caryophyllene (8), germacrene-D (9), *β*-selinene (10), 3,6-dihydrochamazulene (11) and chamazulene (12).

been reported to be a potent hydroxyl radical scavenger capable to effectively inhibit lipid peroxidation (Siveen & Kuttan 2011, Ornano et al. 2013) and possess antiinflammatory activity in vivo, by inhibiting the leukotriene synthesis and lipid peroxidation (Safayhi et al. 1994, Rekka et al. 1996). However there are no reports on antiparasitic effects of chamazulene or dihydrochamazulene.

trans-caryophyllene has been reported as antinociceptive (Katsuyama et al. 2013), anxiolytic, antidepressant (Bahi et al. 2014) and antimicrobial (Goren et al. 2011), among other effects. EOs rich in *trans*-caryophyllene and caryophyllene oxide from different plant species have been tested on *T. cruzi*, *T. vaginalis* (Vermani & Garg 2002, Cheikh-Ali et al. 2011, Polanco-Hernández et al. 2012, 2013, Costa et al. 2013, da Silva et al. 2013, Monzote et al. 2014) and other protozoan parasites such as *Trypanosoma brucei*, *Plasmodium falciparum* and *L. infantum* (Nibret & Wink 2010, Gachet et al. 2011, Monzote et al. 2014), with different activity ranges alone or showing synergistic effects with the different EO components (Setzer et al. 2007, Polanco-Hernández et al. 2012, 2013). In our study, *trans*-caryophyllene was effective against *T. cruzi* and *T. vaginalis*, but the activity of fraction VLC1 is not fully explained by its content in *trans*-caryophyllene, suggesting synergistic effects.

In summary, the most interesting compounds are *trans*-caryophyllene (main compound in fraction VLC1), for its demonstrated antiparasitic and cytotoxic activity and 3,6-dihydrochamazulene, because of the activity of fraction VLC2; both compounds present in the *A. absinthium* var. candial® VP EO.

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