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Chemical composition and antimicrobial activities of the essential oil of (Tunisian) *Chrysanthemum trifurcatum* (Desf.) Batt. and Trab. flowerheads

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Abstract

The chemical composition of essential oil isolated from the flowerheads of *Chrysanthemum trifurcatum* (Desf.) Batt. and Trab. var. *macrocephalum* (viv.) Beg. (Asteraceae) by hydrodistillation was analysed by GC and GC/MS. A total of 56 compounds representing 97.48% of the oil were identified: limonene (20.89%), γ-terpinene (19.13%), 1,8-cineole (10.64%), β-pinene (8.77%), α-pinene (5.32%), 2-hexenal (4.85%), 4-terpenyl acetate (3.42%), β-myrcene (2.31%), germacrene-B (2.01%), β-spathulenol (1.62%), longifolene (1.39%), α-cadinol (1.39%), α-thujene (1.23%) and β-bourbobene (1.06%) were found to be the major components. Essential oil of flowerheads of *C. trifurcatum* was tested for antibacterial activity against eight strains, using a microdilution method, and for cytotoxicity and antiviral activity against *Herpes simplex* virus type 1 using a neutral red incorporation method. The oil showed a great potential of antibacterial effect against *Staphylococcus epidermidis* and *Bacillus subtilis*, in the inhibition range of 64–66% and IC_{50} ranging from 62.5 to 125 μg/ml. On Vero cells, the CC_{50} of the oil was 735.9 μg/ml and it did not exhibit a significant antiviral activity. ***To cite this article: A. Ben Sassi et al., C. R. Chimie 11 (2008).***

Résumé

La composition chimique de l'huile essentielle isolée à partir des capitules floraux de *Chrysanthemum trifurcatum* (Desf.) Batt. et Trab. var. *macrocephalum* (viv.) Beg. (Asteraceae) par hydrodistillation est analysée par GC et GC/MS. Un total de 56 composés représentant 97,48% de l'huile ont été identifiés : limonene (20,89%), γ-terpinene (19,13%), 1,8-cineole (10,64%), β-pinene (8,77%), α-pinene (5,32%), 2-hexenal (4,85%), 4-terpenyl acetate (3,42%), β-myrcene (2,31%), germacrene-B (2,01%), β-spathulenol (1,62%), longifolene (1,39%), α-cadinol (1,39%), α-thujene (1,23%) et β-bourbobene (1,06%) sont les composés majeurs. L'huile essentielle des capitules floraux de *C. trifurcatum* a été testée pour son activité antibactérienne contre huit souches en utilisant la méthode de dilution, pour sa cytotoxicité et son activité antivirale contre le virus de l'*Herpes simplex* de type 1 en

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utilisant la méthode d'incorporation du rouge neutre. L'huile a montré un important effet antibactérien vis-à-vis de *Staphylococcus epidermidis* et de *Bacillus subtilis*, avec un pourcentage d'inhibition de 64–66% et une IC_{50} variant de 62,5 à 125 μg/ml. La CC_{50} de l'huile vis-à-vis des cellules Vero est de 735,9 μg/ml, et son activité antivirale est non significative. ***Pour citer cet article : A. Ben Sassi et al., C. R. Chimie 11 (2008).***

Keywords: Antibacterial activity; Antiviral activity; Cytotoxicity; *Chrysanthemum trifurcatum*; Essential oil composition; GC/MS; Tunisia

Mots-clés : Activité antibactérienne ; Activité antivirale ; Cytotoxicité ; *Chrysanthemum trifurcatum* ; Composition de l'huile essentielle ; GC/MS ; Tunisie

1. Introduction

Chrysanthemum trifurcatum (Desf.) Batt. and Trab. var. *macrocephalum* (viv.) Beg. (Asteraceae), spreading in Tunisia, is a herb with small yellow flowers. In Tunisian traditional medicine, the flowerheads of *C. trifurcatum* were used to treat constipation, intestinal transit problems and to fight feminine pain after delivery [1]; no other utilizations of this plant were reported previously [2].

Aromatic and medicinal plants produced a wide variety of volatile terpene hydrocarbons and their corresponding oxygenated derivatives were known as essential oils. In fact, essential oils have been widely used in traditional medicine. Among others, antibacterial, antifungal, immunomodulatory, antiinflammatory, and antirheumatic activities have been described [3–5]. Hitherto, there is only few information on the effects of essential oils on viruses or viral infections. Recently, the anti-herpes activity of several essential oils of different plant sources as well as of various constituents of essential oils was demonstrated [6–9].

A series of studies has demonstrated the potential medicinal effect of essential oils from various *Chrysanthemum* species, i.e. essential oils from air-dried and processed flowers of *C. indicum* possessed significant antimicrobial activity effect [10], essential oil of *C. boreale* exhibited antibacterial activity against Gram positive and Gram negative bacteria [11], volatile fraction of *C. viscidehirtum* aerial parts exhibited activity against some bacterial strains, in particular *Salmonella typhi* and *Proteus mirabilis* [12], essential oil of flowerheads of *C. coronarium* possessed antifungal activity against agricultural pathogens [13]. Concerning the antiviral activity of *Chrysanthemum* species, only *C. cinerariaefolium* was reported to have an antiviral effect against the *Herpes simplex* virus which has been attributed to pyrethrins [14].

To the best of our knowledge, the chemical composition of essential oil of *C. trifurcatum* has not been studied yet, as well as its antimicrobial properties. The aim of this work was to investigate the chemical composition, antibacterial and antiviral activities of essential oil from *C. trifurcatum* flowerheads growing in Tunisia.

2. Material and methods

2.1. Plant material

C. trifurcatum was identified according to the flora of Tunisia [15] by the botanist Dr. Fethia Harzallah-Skhiri (Institut supérieur de biotechnologie de Monastir, Tunisia). Fresh flowerheads of *C. trifurcatum* were collected from Zeramdine in the center of Tunisia in May 2006. A voucher specimen was deposited in our laboratory for future reference.

2.2. Essential oil extraction and analysis

2.2.1. Essential oil extraction

Fresh flowerheads of *C. trifurcatum* were submitted to hydrodistillation for 5 h using a Clevenger type apparatus. The obtained essential oil was dried with anhydrous sodium sulphate and stored at 4 °C before use. The yield based on fresh weight of the sample was calculated.

2.2.2. Essential oil analysis

The composition of the oil was analysed by GC and GC/MS. The analytical GC was carried out on an HP5890-series II gas chromatograph (Agilent Technology, California, USA) equipped with Flame Ionization Detectors (FID) under the following conditions: the fused silica capillary column, apolar HP-5 and polar HP Innowax (30 m × 0.25 mm ID, film thickness 0.25 μm). The oven temperature was held at 50 °C for 1 min, then programmed at rate of 5 to 240 °C/min and held isothermal for 4 min. The carrier gas was nitrogen at a flow rate of 1.2 ml/min; injector temperature: 250 °C, detector temperature: 280 °C; the volume

injected: 0.1 µl of 1% solution (diluted in hexane). The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

GC/MS was performed in a Hewlett-Packard 5972 MSD system. An HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 1.2 ml/min. Oven temperature was programmed (50 °C for 1 min, then 50–240 °C at 5 °C/min) and subsequently held isothermal for 4 min. Injector port: 250 °C, detector port: 280 °C, split ratio 1:50. Volume injected: 0.1 µl of 1% solution (diluted in hexane). Mass spectrometer: HP5972 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation. The components were identified by comparison of their mass spectra with those in the Wiley 275 GC–MS library and those in the literature [16], as well as by comparison of their retention indices with literature data [16–20]. Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes (relative to C_9–C_{28} on the HP5 and HP-20M columns).

2.3. Antibacterial activity

2.3.1. Bacterial strains

Bacterial strains were obtained from the collections of the Pasteur Institute in Paris, France. Microorganisms were as follows: five Gram positive bacteria (*Staphylococcus epidermidis* CIP 53124, *Staphylococcus hoemolyticus* CIP 8156, *Staphylococcus hominis* CIP 8157, *Staphylococcus simulans* CIP 8164 and *Bacillus subtilis* CIP 5265) and three Gram negative bacteria (*Escherichia coli* CIP 54117, *Hafnia alvei* CIP 5731 and clinical strain *P. mirabilis*). Organisms were maintained in nutrient agar (Sigma) at 37 °C. Overnight cultures were prepared in Mueller Hinton Broth (Sigma) and adjusted to approximately 10^8 cfu/ml.

2.3.2. Microdilution method

Antibacterial evaluation of the essential oil was performed in 96-well plates. Samples of cultures grown overnight (10^8 cfu/ml) were incubated with oil dissolved in ethanol 99% (at a concentration of 1, 10, 100 and 500 µg/ml) for 24 h at 37 °C. All inhibition assays were carried out in triplicate. Growth was monitored by measuring OD_{600} with a Packard Spectracount microplate spectrophotometer and the percentage of inhibition (*%I*) was calculated for each concentration [21]:

$$\%I = (\mathrm{ODc} - \mathrm{ODt})/\mathrm{ODc} \times 100$$

where ODc was the mean optical density of the bacterial control cultures and ODt was the mean optical density of the bacterial cultures treated with oil at different concentrations. Ethanol used for solubilizing the oil was used as control. Control was performed for every assay and showed no inhibition of the microbial growth. Ampicillin was used as reference antibiotic.

2.4. Determination of cytotoxicity by cell viability

2.4.1. Cell strain

African green monkey kidney cells (Vero, ATCC CCL-81) were grown in Eagle's Minimum Essential Medium (MEM, Eurobio), supplemented with 8% Fetal Calf Serum (FCS, Eurobio) and 1% of antibiotics PCS (10 000 IU Penicillin/ml, 25 000 IU Colimycin/ml, 10 mg Streptomycin/ml; Sigma).

2.4.2. Cytotoxicity assay

To evaluate the cytotoxic activity of the oil, Vero cellular suspensions (3.5×10^5 cells/ml) were cultivated in 96-well culture plates and exposed to increasing concentrations of the oil from 10 to 1000 µg/ml, using 4 wells for each concentration. The plates were incubated at 37 °C in a humidified CO_2 atmosphere (5% CO_2) during 72 h. Each assay was done in triplicate. The cells were examined daily under a phase-contrast microscope to determine the minimum concentration of oil that induced alterations in cell morphology, including swelling, shrinkage, granularity and detachment [22]. Cytotoxicity was tested using the neutral red dye method [23], and optical densities (OD) were measured at 540 nm using a spectrophotometer (SpectraCount™, Packard). The 50% cytotoxic concentration (CC_{50}) was the concentration of the oil that inhibited actively the replication of cells by 50% of untreated ones. Cytotoxicity was also expressed as the percentage of cell destruction (*%D*):

$$\%D = [((\mathrm{ODc})\mathrm{C} - (\mathrm{ODc})\mathrm{Mock})/(\mathrm{ODc})\mathrm{C}] \times 100.$$

(ODc)C and (ODc)Mock were the OD values of the untreated and treated cells, respectively [24].

2.5. Antiviral assays by cell viability

2.5.1. Viral strain

HSV-1 (wild type strain 17, sensitive to acyclovir) was obtained from Pr of Ingrand (Hôpital Antoine-Béclère, Rheims, France). The virus titer was estimated

from cytopatogenicity according to the Reed and Muench dilution method [25], and expressed as 50% infectious doses per milliliter (ID_{50}/ml). The HSV-1 stock had a titer of $2 \times 10^{5.8}$ ID_{50}/ml. The used viral suspension has a multiplicity of infection (MOI) of 0.001 ID_{50}/ml.

2.5.2. Antiviral assay

To test the antiviral effect of oil, 100 µl of Vero cellular suspension (3.5×10^5 cells/ml) were infected with a virus (HSV-1) suspension (50 µl) with a multiplicity of infection (MOI) of 0.001 ID_{50}/ml without or in presence of different dilutions of the oil (10−1000 µg/ml). Infected cell cultures were cultivated in 96-well culture plates at 37 °C in a humidified CO_2 atmosphere (5% CO_2) during 72 h. Each assay was done in triplicate. After incubation, antiviral activity was evaluated by the neutral red dye method [23]. The antiherpetic compound acyclovir [9-(2-hydroxyethoxymethyl) guanine] was used as reference drug with concentrations ranging from 0.1 to 5 µg/ml. The 50% effective antiviral oil concentration (EC_{50}) was expressed as the concentration of the oil that achieved a protection of 50% of virus infected cells. Optical densities (OD) were measured at 540 nm and the OD was related directly to the percentage of viable cells, which was inversely related to the cytopathic effect (CPE). The linear regression was determined for each assay on the basis of cell controls (0% CPE) and virus controls (100% CPE). Data were expressed as a percentage of cell protection (*%P*):

$$\%P = [((\mathrm{ODt})\mathrm{virus} - (\mathrm{ODc})\mathrm{virus})/((\mathrm{ODc})\mathrm{Mock} - (\mathrm{ODc})\mathrm{virus})] \times 100.$$

(ODt)virus was the OD of the virus infected cell suspensions in the presence of oil; (ODc)virus was the OD of the virus infected cell suspensions (no oil) and (ODc)Mock was the OD of the mock-infected cell suspensions [24].

2.6. Statistical analysis

The percentage of bacterial growth inhibition was calculated for each bacterial strain as described previously and results were expressed as the mean of three replicates. The 50% inhibitive concentrations (IC_{50}) were estimated by regression analysis with Prism software (GraphPad Software, Inc). The results of cytotoxicity and antiviral activity (*%D* and *%P*) were expressed as mean ± s.e.m.

3. Results and discussion

3.1. Chemical composition of the essential oil

Essential oil obtained by hydodistillation of fresh flowerheads of *C. trifurcatum* had a light yellow colour and a pungent odour at room temperature. The yield oil was 0.055% ((v/w), volume/fresh weight). Qualitative and quantitative analytical results by GC and GC/MS are shown in Table 1.

The global chromatographic analysis of this essential oil showed 56 compounds, representing 97.48% of the total oil constituents (Table 1). The oil contains a complex mixture consisting of mainly mono- and sesquiterpene hydrocarbons and oxygenated mono- and sesquiterpenes. It was dominated by monoterpene hydrocarbons (58.72%) and oxygenated monoterpenes (18.65%), while sesquiterpene hydrocarbons and oxygenated sesquiterpenes were only present in small percentage with, respectively, 9.45 and 5.34%. The aldehyde components accounted for 5.63%. The major components in the oil detected were limonene (20.89%), γ-terpinene (19.13%), 1,8-cineole (10.64%), β-pinene (8.77%), α-pinene (5.32%), 2-hexenal (4.85%), 4-terpenyl acetate (3.42%), β-myrcene (2.31%), germacrene-B (2.01%), β-spathulenol (1.62%), longifolene (1.39%), α-cadinol (1.39%), α-thujene (1.23%) and β-bourbobene (1.06%) (Table 1).

To the best of our knowledge, this is the first report of the chemical composition of the essential oil of *C. trifurcatum* flowerheads. Neverthless, some other species in genus *Chrysanthemum* were studied for their essential oil compositions. Limonene, the major component of the oil, was in the same way present in higher quantity in the volatile fraction of *C. viscidehirtum* aerial parts [12]. On the contrary, limonene was present in minor quantity (0.7%) in the essential oil of *C. coronarium* flowerheads [13] which contain mainly camphor (29.2%), α-pinene (14.8%), lynalyl acetate (9.8%), β-pinene (9.5%) and camphene (5.2%) [13]. Moreover, 1,8-cineole was a major component in the essential oil of *C. indicum* flowerheads with 30.41% [10], and in the volatile fraction of *C. boreale* [11]. We can affirm that *C. trifurcatum* from Tunisia (Zeramdine) was the chemotype limonene, γ-terpinene and 1,8-cineole.

3.2. Antibacterial activity

The essential oil was evaluated for antibacterial activity against pathogenic strains of Gram positive (*S. epidermidis*, *S. hoemolyticus*, *S. hominis*, *S. simulans* and *B. subtilis*) and Gram negative (*E. coli*, *H. alvei* and

Table 1
Composition of essential oil of *C. trifurcatum* var. *macrocephalum* flowerheads

Compound	RI[a]	%	Identification
2-Hexenal	860	4.85	MS, RI
Tricyclene	930	0.27	MS, RI
α-Thujene	935	1.23	MS, RI
α-Pinene	940	5.32	MS, RI
Camphene	952	0.15	MS, RI
Sabinene	976	0.34	MS, RI
β-Pinene	979	8.77	MS, RI
β-Myrcene	991	2.31	MS, RI
Limonene	1032	20.89	MS, RI
1,8-Cineole	1035	10.64	MS, RI
γ-Terpinene	1064	19.13	MS, RI
α-Terpinolene	1089	0.31	MS, RI
1-Octen-3yl-acetate	1096	0.25	MS, RI
Camphor	1145	0.91	MS, RI
Borneol	1169	0.59	MS, RI
Terpinen-4-ol	1180	0.34	MS, RI
p-Cymen-8-ol	1187	0.28	MS, RI
α-Terpineol	1191	0.29	MS, RI
Myrtenal	1193	0.78	MS, RI
Myrtenol	1196	0.47	MS, RI
Fenchyl acetate	1225	0.13	MS, RI
Carveol	1229	0.27	MS, RI
Linalyl acetate	1262	0.17	MS, RI
Bornyl acetate	1289	0.26	MS, RI
Carvacrol	1291	0.40	MS, RI
4-Terpenyl acetate	1340	3.42	MS, RI
α-Cubebene	1352	0.21	MS, RI
α-Terpenyl acetate	1354	0.17	MS, RI
α-Ylangene	1372	0.16	MS, RI
α-Copaene	1379	0.16	MS, RI
β-Bourbobene	1381	1.06	MS, RI
β-Elemene	1390	0.60	MS, RI
β-Cubebene	1401	0.13	MS, RI
Longifolene	1403	1.39	MS, RI
α-Gurjunene	1409	0.17	MS, RI
β-Caryophyllene	1420	0.31	MS, RI
β-Gurjunene	1434	0.31	MS, RI
α-Cedrene	1437	0.32	MS, RI
α-Himachalene	1447	0.22	MS, RI
β-Farnesene	1458	0.16	MS, RI
Germacrene-D	1484	0.23	MS, RI
β-Selinene	1489	0.20	MS, RI
α-Muurolene	1502	0.17	MS, RI
β-Bisabolene	1508	0.12	MS, RI
γ-Cadinene	1516	0.26	MS, RI
δ-Cadinene	1523	0.13	MS, RI
α-Cadinene	1538	0.13	MS, RI
α-Calacorene	1545	0.55	MS, RI
Elemol	1549	0.32	MS, RI
Germacrene-B	1555	2.01	MS, RI
β-Calacorene	1561	0.43	MS, RI
β-Spathulenol	1576	1.62	MS, RI
τ-Cadinol	1640	0.86	MS, RI
τ-Muurolol	1642	0.24	MS, RI
β-Eudesmol	1649	0.68	MS, RI
α-Cadinol	1654	1.39	MS, RI
Monoterpene hydrocarbons		58.72	
Oxygenated monoterpenes		18.65	
Sesquiterpene hydrocarbons		9.45	
Oxygenated sesquiterpenes		5.34	
Aldehyde		5.63	
Total identified		97.48	

[a] RI: retention indices relative to *n*-alkanes on the apolar HP-5 column.

P. mirabilis) bacteria. The mean percentage of bacterial growth inhibition and IC_{50} are given in Table 2. Growth inhibition percentages have been calculated for all concentration ranges of the tested oil and we reported here the data (*%I*) corresponding to the concentration 500 μg/ml of oil which shows a bacterial growth inhibition. The oil was active against the tested bacterial strains. However, this activity varies with the kind of bacteria. In fact, at the concentration of 500 μg/ml; essential oil exhibited potent inhibitory effect on the growth of two Gram positive strains: *S. epidermidis* (66%) and *B. subtilis* (64%) with IC_{50}, respectively, for 62.5 and 125 μg/ml; this activity was more pronounced than the standard antibiotic (ampicillin) against those two bacteria. Moreover, this oil was inactive against the other tested microorganisms, with IC_{50} superior to 500 μg/ml. Our results indicated that Gram positive bacteria were more sensitive to the essential oil than Gram negative ones. This is in agreement with observations made by other authors that Gram positive bacteria were more susceptible to essential oils than Gram negative ones [26,27].

Our results were in accord with reported ones. In fact, essential oils of many *Chrysanthemum* species

Table 2
Antibacterial activities (*%I* and IC_{50} in μg/ml) of the essential oil of *C. trifurcatum* flowerheads

Microorganisms	Essential oil		Ampicillin	
	%I	IC_{50}	*%I*	IC_{50}
Staphylococcus epidermidis CIP 53124	66	62.5	20	>500
Staphylococcus simulans CIP 8164	20	>500	32	>500
Staphylococcus hominis CIP 8157	47	>500	32	>500
Staphylococcus haemolyticus CIP 8156	23	>500	85	1.25
Bacillus subtilis CIP 5265	64	125	30	>500
Escherichia coli CIP 54117	33	>500	92	25
Hafnia alvei CIP 5731	20	>500	82	1.25
Proteus mirabilis	27	>500	85	1.25

%I: Growth inhibition percentages with 500 μg/ml of essential oil (data were expressed as mean of three replicates); IC_{50}: 50% growth inhibition concentration; CIP: strains from the collections of the Pasteur Institute, France.

were known to exhibit antimicrobial activity against several bacteria and fungi [10,11,12,28]. The antibacterial activity of the oil could, in part, be associated with major constituents such as limonene, γ-terpinene 1,8-cineole, α-pinene, β-pinene and 2-hexenal. Limonene, the most abundant constituent (20.89%) of the oil, was known to exhibit antibacterial activity [29]. One of the major components of this oil, 1,8-cineole, has been previously shown to be active against many organisms [30,31] and it has been known to exhibit antibacterial activity against *Bacillus subtillis*, *Staphylococcus aureus*, *E. coli* and other bacteria [32]. α-Pinene and β-pinene, which were found to be in appreciable amounts in the oil of *C. trifurcatum* flowerheads, have been reported to display antibacterial effects [29,33]. Terpenes were active against bacteria [34]. In our case, the presence of high quantities of monoterpenoid components in essential oil could explain its antibacterial activity against Gram positive bacteria. Research on the antimicrobial actions of monoterpenes suggests that they diffuse into and damage cell membrane structures [35]. In fact it seems, as described previously by Juliani et al. [36], that essential oils containing terpenoids are more active against Gram positive organisms than against Gram negative ones. In addition, the components in lower amount may also contribute to antibacterial activity of the oil, involving probably some type of synergism with the other active compounds.

3.3. Cytotoxicity and antiviral activity

Essential oil of *C. trifurcatum* flowerheads was investigated against HSV-1 infected Vero cells. Results are summarized in Fig. 1. Assessment of cytotoxicity is clearly an important part of the evaluation of a potential antiviral agent because a useful oil should be selective for virus specific processes with no or only few effects on cellular metabolism. In Vero cells and after 72 h exposure, cytotoxicity of the oil varies within concentrations. In fact, at higher tested doses, the oil exhibited a potent percentage of cell destruction. At a concentration of 1000 μg/ml, oil destructed cells by 63% (Fig. 1). At lower doses, the oil was tolerated by Vero cells and its 50% cytotoxic concentration (CC_{50}) was 735.9 μg/ml. In literature, many essential oil components were known to exhibit cytotoxic activity. β-elemene has been reported to have stronger cytotoxicity against tumor cells [37,38]. α-Cadinol has been reported to have toxicity against human colon adenocarcinoma cell line HT-29 [39], whereas both these sesquiterpene compounds were present in small percentage in the flowerheads' oil of *C. trifurcatum*.

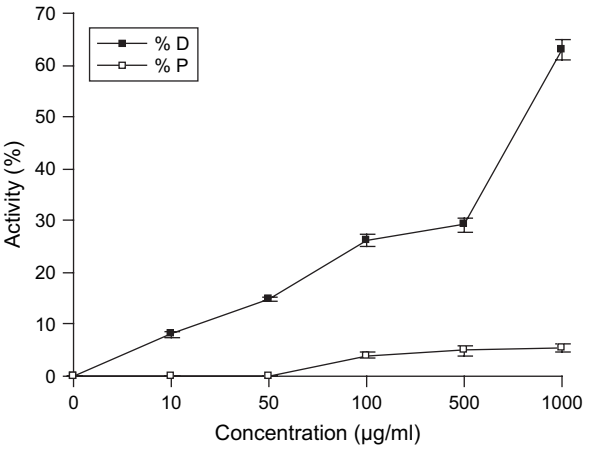

Fig. 1. Cytotoxicity and anti-HSV-1 activity of the essential oil of *C. trifurcatum* flowerheads. This figure shows the antiviral and cytotoxic activities of the essential oil of *C. trifurcatum* flowerheads tested at various concentrations, after incubation for 72 h. Vero cells infected by HSV-1 (MOI 0.001 ID_{50}/ml) were mammalian fibroblastic cells and HSV-1 was *H. simplex* virus type 1. Antiviral activity was expressed as the percentage of protection of virus-infected cells (% Protection *P*: □). Cytotoxic activity was observed as the percentage of destruction of mock-infected cells (% Destruction *D*: ■). The 50% cytotoxic (CC_{50}) oil concentration was 735.9 μg/ml and the 50% effective (EC_{50}) oil concentration was more than 1000 μg/ml. Values are presented as mean ± s.e.m. of three replicates.

Concerning the cytotoxic potential of *Chrysanthemum* species, Ukiya et al. [40] reported that arnidiol, a triterpene isolated from edible *Chrysanthemum* flowers, exhibited remarkable cytotoxic activity against a panel of human cancer cell lines, and authors suggested that this compound can be useful as an anticancer agent. The essential oil of *C. trifurcatum* flowerheads did not exhibit a significant antiviral activity against *H. simplex* virus type 1. The percentage of virus-infected cells protection was lower (4.1% at a dose of 100 μg/ml) (Fig. 1). The reference standard, acyclovir (1 μg/ml), conferred total protection (100%, $EC_{50} = 0.35$ μg/ml) against HSV-1 with a low percentage of cell destruction. In the literature, only *C. cinerariaefolium* has an antiviral activity against *H. simplex* virus, which has been attributed to pyrethrins [14].

4. Conclusion

In our study it has become clear that essential oil of flowerheads of *C. trifurcatum* has a great potential to inhibit the growth of *S. epidermidis* and *B. subtilis* along with other bacteria tested. However, it was not active against the *H. simplex* virus of type 1 and it was generally tolerated by Vero cells. Analysis by gas chromatography (GC) and gas chromatography/mass spectrometry

(GC/MS) demonstrated that this oil contained mainly terpenoid compounds and that it was exceptionally rich in monoterpenes. We have also characterized the *C. trifurcatum* from Tunisia (Zeramdine) as the chemotype limonene (20.89%), γ-terpinene (19.13%) and 1,8-cineole (10.64%). These bioactivities and chemical composition of the essential oil were reported here for the first time. However, it was still necessary to investigate *in vivo* bioactivity and toxicity of this oil and its major constituents.

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