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Chemical Composition and Anti-Leishmania Major Activity of Essential Oils from Artemesia spp. Grown in Central Tunisia

Ahmed Mathlouthi [1]*, Mouna Belkessam [1], Mouna Sdiri [1], Mohamed Fethi Diouani [2], Abdelaziz Souli [1], Safia El-Bok [3], Mossadok Ben-Attia [1]

[1] Environment Biomonitoring Laboratory (LR01/ES14), Sciences Faculty of Bizerta, University of Carthage, 7021 Zarzouna, Tunisia
[2] Laboratory of Epidemiology and Veterinary Microbiology (LR11-IPT03), Pasteur Institute of Tunis, University of Tunis El-Manar, Tunis-Belvédère 1002, Tunisia
[3] Laboratory of Biodiversity, Biotechnologies and Climate Change, Faculty of Sciences of Tunis, Tunis El-Manar University, 2092 Tunis, Tunisia

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Abstract: Natural products are a very important source of bio-molecules for drug to treat infectious diseases. New antileishmanial agents are timely needed, due to leishmania toxicity, drug-resistant strains and high costs treatments. In this study, we report the chemical characterization and antileishmanial activity of oil extracts from three medicinal plants belonging to genus Artemisia (Asteracea), that grow in central Tunisia. The chemical composition of volatile compounds had been elucidated by gas chromatography-mass spectroscopy analysis. Chemical analysis revealed that essential oils were composed of 34 compounds: α-thujone (29.3 %), chamazulene (39.2 %) and β-pinene (32 %), were the main constituents for the essential oils of *Artemesia herba alba*, A*rtemesia absinthium* and *Artemesia campestris*, respectively. The hydrodistilled essential oils from these three species of Artemisia showed significant anti-leishmanial activities against Leishmania major. Oils from *A. herba alba, A. absinthium* and *A. campestris* exhibited IC_{50} values of 1.20 ± 0.043 µg/mL, 1.49 ± 0.05 µg/mL and 2.20 ± 0.11 µg/mL respectively against promastigotes of Leishmania major. Among three oils tested, *A. campestris* exerted a remarkable antileishmanial activity and it has the lowest cytotoxicity effect compared to amphotericin B. Overall, it was proved that our investigated essential oils possess potential antileishmanial properties and could be used as a promising alternative treatment for leishmaniasis disease in the future.

Key words: *Artemisia herba alba*; *Artemisia absinthium; Artemisia campestris*; essential oils; Leishmania major.

Introduction

Neglected tropical diseases (NTDs) are a diverse group of tropical infections caused by a variety of pathogens such as viruses, bacteria, protozoa and helminths. Leishmaniasis was recognized by The World Health Organization as NTD and caused by parasites that belong to genus *Leishmania* [1], which are chronic endemic diseases in 98 countries worldwide. This protozoa parasite affects 12 million people with a rate of 1.5 to 2 million new cases each year [2], and 350 million in risk of infection [3]. Moreover, NTDs such as rabies, African sleeping sickness, and visceral leishmaniasis are considered to be lethal diseases

*Corresponding author (Ahmed Mathlouthi)
E-mail: < metlouthiahmad@gmail.com >

(especially in Asia, Africa, and Latin America) and many of them are chronic in nature [4,5]. Based on clinical forms in humans and parasite species, leishmaniasis was manifested in three principal types: potentially fatal visceral leishmaniasis, self-healing cutaneous leishmaniasis and progressive mucocutaneous type, which could be caused by more than 20 species of *Leishmania* [6].

The reservoirs of the disease are rodents or dogs beside other wild animals. Its transmission in the Old World (i.e. Africa, Asia and Europe) is carried out by *Phlebotomus* spp. (family *Psychodidae*), which is dipteran insects that are present in the Mediterranean basin, temperate and tropical regions. The lifecycle of Leishmania is composed of two stages. *Leishmania* exists in two forms: an extracellular flagellated form named promastigote and an intracellular form called the amastigotes. The promastigote form is 15 to 20 µm length, who lives in the digestive tract of mosquitoes which transmit the disease infecting human and other vertebrates. The amastigote is an intracellular form, with a diameter of approximately 2.3 µm, hosted by the mononuclear phagocytes of the mammalian vertebrates like the macrophages [7]. Generally, pre-diagnosis is based on clinical symptoms and epidemiological criteria, while infection prognosis is assessed by molecular biology tools as DNA detection or anti-Leishmania antibodies in clinical samples [8-9]. According to previous study in Tunisia, two species were responsible for the visceral and cutaneous form of leishmaniasis in human, *L.infantum* and *L. major* respectively [10], *Phlebotomus papatasi* is the principal vector of *L. major* in Tunisia, this strain is the main agent causing cutaneous leishmaniasis in the Old World [11].

It has been proven in previous research that, the intra-macrophagic location of infectious form of leishmaniasis, emerging drug resistance capacity, expensive chemotherapy and unavailability of efficient vaccine for patients, are major challenges that hampered treatments and quick recovery [12-13]. On the other hand it is known that alkaloids quinine and emetine (secondary metabolites obtained from natural extract of different species of the genera *Cinchona* and *Cephaelis*) were the first developed drugs for treating malaria [14]. This makes the exploration of other natural products a promising and exciting challenge to develop the best drugs against the diseases caused by *protozoan* parasites.

The genus *Artemisia* L. (family *Asteraceae*) contains about 200 to over 400 species (depending on the classification system) found throughout the earth northern hemisphere. This genus, which might be divided into two sections *Artemisia* and *Dracunculus* [15], contain several bioactive molecules against several diseases. Such molecules used as antibiotic [16-17], antiviral [18] and antimalarial agents [19]. Recently, several researches have assessed the capacity of *Artemesia* genus against leishmeniasis. In this context, Sen *et al*. (2007) showed the significant antileishmanian activity of artemisinin extracted from three *Artemesia* species; *A. annua, A. indica and A. dracunculus* [20]. The main assessed components were essential oils, which contain secondary metabolites, generally obtained by hydrodistillation of different parts of plants that are volatile compounds composed of terpenes and aromatic fractions [21].

In this study, we aimed to investigate the chemical composition, the *in vitro* leishmanicidal activity and cytotoxicity effect of essential oils purified from aerial parts of three Tunisian medicinal plants. Our research revolved around finding an effective natural remedy for the treatment of Leishmaniasis. The essential oil from *A. herba alba*, *A. absinthium* and *A. campestris* would be tested to evaluate the inhibitory activity against promastigotes of *L. major*.

Materials and methods

Plant material

The leaves from aerial parts of *A. herba-alba*, *A. absinthium* and *A. campestris* were collected in February 2018 in early morning from Sidi Bouzid ; near Bou-Hedma National Park (central Tunisia) in order to keep them fresh. The collection zone is located under the arid bioclimatic stage where the average annual rainfall is close to 200 mm, distributed between autumn and spring. The average annual temperature is 17.2°C. The geographical situations of this station are as follows (latitude 34°35'15"N; longitude 9°1'36"

E and 348 altitude). 20 to 25 samples from each species were collected within a perimeter of 300 m^2. All the collected plant materials were identified by Pr. Mossadok Ben-Attia, Department of Life Science, Faculty of Sciences Bizerte, University of Carthage. A voucher specimen of the aerial part of each plant was deposited at the herbarium of the laboratory (LBE, Bizerta) under following numbers: AA/LBE13-001, AC/LBE 13-002 and AHA/LBE 13-003. The leaves were separated and dried on the shadow during 10 days.

Isolation of the essential oils

The dried vegetal materials (100 g) of each plant were subjected to hydrodistillation for 3.5 h according to the procedure detailed in the European Pharmacopoeia [22]. The hydrodistillation was carried out using the Clevenger instrument. Obtained essential oils were stored in the dark at 4°C until required.

GC-FID and GC-MS analyses

The essential oils were analyzed using a Perkin-Elmer Sigma-115 gas chromatograph equipped with Flame Ionization Detector (FID). The separation was achieved using a HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d, film thickness 0.25 µm). Oven temperature was kept at 40°C, with 5 min initial hold, and then raised to 270°C (with an increase of 2°C/min for 20 min); injection mode splitless (1 µL of a 1:1000 *n*-pentane solution). Helium was used as carrier gas (1.0 mL/min).

The GC-MS analysis was performed on an Agilent instrument (6,850 Ser. II), fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.33 µm film thickness), associate to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy was 2000 V. Mass spectra were scanned in the range 40-500 amu, scan time 5 scans per second. Helium gas was used as carrier (1.0 mL/min); transfer line temperature, 295°C.

The identification of individual compound was carried out by the calculation of Retention Indices (RI) using homologous series of *n*-alkanes (C10–C35) series and a comparison with available mass spectral data (NIST 02, Wiley 275 libraries) and confirmed by comparison of their retention indices with those of reported in the literature [23]. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

***In vitro* leishmanicidal activity**

L. major LV39 clone 5 promastigotes (Rho_SU_59_P)

Parasites were cultured at 26°C in M199 supplemented with 25 mM HEPES pH 7.2, 0.1 mM adenine, 0.0005 % (w/v) hemin, 2 mg/ml biopterin, 0.0001 % (w/v) biotin, 10 % (v/v) heat-inactivated foetal calf serum (FCS).

Antipromastigote activity

The leishmanicidal activity was performed in plates (96-well-polystyrene) with an initial inoculum of 1×10^6 parasites/mL incubated with increasing concentrations of essential oils (0, 0.5, 1, 2, 4, 8 and 16 µg/mL) for 72 h at 26°C. The screening of extracts was performed on free-living promastigotes after the incubation period (72 h). Cell fluorescence was measured using Odyssey® Infrared imaging system. The essential oils were diluted in dimethyl sulfoxide (DMSO 0.5 % as the maximal concentration tested for 100 % vitality), and then sterilized using a 0.225 µm membrane filter before testing their antileishmanial activity. Amphotericin B (0.16 µg/mL) a standard antileishmanial drug, was used as a reference drug control.

***In vitro* cytotoxicity of studied oils**

The cytotoxicity potential of *Artemesia* oils was determined in mammalian cells U-937 (U-937 (ATCC® CRL-1593.2™)) based on its influence on cell viability, as determined by the [3-(4, 5-Dimethylthiazol-2- yl)-2, 5-Diphenyltetrazolium Bromide]-MTT microenzymatic assay described by Carol et *al* [24]. The base medium for these cells is ATCC- formulated RPMI-1640 Medium supplemented with fetal bovine serum (10 %) and incubated at 37°C under 5 % CO_2. Cytotoxicity was determined based on percentages of viability after 72 h. Cells (1.10^4) were incubated with deferent concentrations of essential oils or Amphotericin B (5, 10, 20, 40, 80 µg/mL) in 96-well

plates. The absorbance was measured using Odyssey® Infrared imaging system.

Data analysis and determination of IC_{50}

The *in vitro* results of essential oil against *L. major* promastigotes are expressed as mean ±SD of three independent experiments in triplicates. Comparison of the mean values was performed by one-way ANOVA followed by multirange *post hoc* of Tukey's test. This last test was used to compare means between control group versus all other groups and a p-value of 0.05 or less was considered as statistically significant. Statistical analyses were conducted using GraphPad InStat v.5.0a for MacIntosh (GraphPad Software, San Diego, CA, USA).

The median inhibitory concentration, IC_{50}, was evaluated from dose-response curves by nonlinear analysis using the Sigma-Plot statistical software package. Data are displayed as mean ± SD (table 2) of three independent experimental.

Results and discussion

Essential-oil yield

The yield of essential oils is very variable within these three species of the same family, it showed 1.09 %, 0.63 % and 0.57 %, for *A. herba alba*, *A. absinthium* and *A.campestris* respectively. The colors of the essential oils of these three plants are also different. The oils of *A. herba alba* and *A. campestris* were light yellow and blue for *A. absinthium*. There are approximately 3000 essential oils registered in the world, and only 300 among them are marketed, mainly for the aroma and fragrance industry [25]. The yield obtained in this study can be considered average compared to some plants that are exploited industrially as a source of essential oils. Our essential oils had higher yield than *Rosa canina* (0.5 %) [26] and lower than thyme (4 %) [26]. The Chemical variability and the yield of essential oil may be affected by several factors, namely climatic, geographic conditions and growth stage of collected plants [28].

Chemical composition of the essential oils

The GC-FID/GC-MS Analysis of the three *Artemesia* oils was reported in table 1. Our study revealed a high level of chemical polymorphism of the essential oils within this family .Thirty four compounds were identified (Table 1). The Analysis of the oils composition indicated the presence of mono- and sesquiterpenes with highvariable amounts. The oil of *A. herba alba* was mainly composed of oxygenated monoterpenes (77.7 %), followed by monoterpene hydrocarbons (11.3 %), and sesquiterpene hydrocarbons (8.5 %). Our GC/GC-MS analysis indicated the absence of oxygenated sesquiterpenes in this plant. The major constituents of this oil were α-thujone (29.4 %), 1,8-cineole (14.8 %), *trans* pinocarveol (9.9 %), β-thujone (6.3 %) and chrysanthenyl acetate (6.1 %). Previous works studied the chemistry of *A. herba-alba* growed in Tunisia [29,30] showed that, depending on the site of populations, *A. herba-alba* have a high level of variability in chemical composition. Indeed, the oil of this plant from arid regions as Matmata [31] contains oxygenated monoterpenes as a major components which confirms our study. Inversely, this essential oil from southern Tunisia had approximately an equal amount of monoterpenes and sesquiterpenes [28]. Recently, Bellili *et al*.[32] reported that α-thujone (37.9 %) is also the major compound for *A. herba alba* followed by germacrene D (16.5 %), 1,8-cineole (8.4 %) and β-thujone (7.8 %). On the other hand, Younsi *et al*.[33] showed that the essential oil obtained by hydrodistillation containd β-thujone (41.9 %) as major compound followed by α-thujone (18.4 %).

For *A. campestris* the identified compounds represent 95.1 % of the total essential oil. Our results showed that monoterpene hydrocarbons (83.1 %) were highly present in this species, followed by oxygenated sesquiterpenes (5.0 %), sesquiterpene hydrocarbons (4.2 %) and oxygenated monoterpenes (2.8 %). This oil contains mainly β–pinene (32 %), limonene (17.35 %), α-pinene (11.4 %) and β-terpinene (5.5 %). The chemical composition of the essential oils of *A.campestis* was widely studied in Tunisia. These studies are in line with our work. Indeed, the oil extracted from *A. campestris* from arid region is very rich with monoterpene hydrocarbons and β-pinene was identified as the major compound [34-35]. In contrast, it was reported that the essential oil of this specie contained mainly α-pinene (24 %) as

Table 1. Chemical composition of essential oils (%) from *Artemisia herba alba, A. absinthium* and *A. campestris*

Compound	Ri[a]	Ri[b]	*Artemisia herba alba*	*Artemisia absinthium*	*Artemisia campestris*	Identification
α-Thujene	930	931	-	0.2	0.3	1,2,3
α-Pinene	939	939	1.1	2.4	11.4	1,2,3
Camphene	949	953	2.2	2.2	0.1	1,2,3
Sabinene	969	976	-	0.2	3.1	1,2,3
β-Pinene	980	-	2.3	0.2	32.0	1,3
β-Myrcene	997	991	-	2.0	6.0	1,2,3
α-Phellandrene	1009	1005	-	-	0.3	1,2,3
Limonene	1029	1031	3.2	0.7	17.3	1,2,3
1,8-cineole	1031	991	14.8	0.5	-	1,2,3
α-Ocimene	1042	-	2.1	-	2.7	1,3
β-Ocimene	1054	-	0.4	-	4.4	1,3
γ-Terpinene	1060	1062	-	4.0	5.5	1,2,3
α-Thujone	1110	1102	29.4	0.5	-	1,2,3
β-Thujone	1114	-	6.3	-	-	1,3
Chrysanthenone	1122	1123	1.6	-	-	1,2,3
trans-pinocarveol	1139	1139	9.9	-	-	1,2,3
Camphor	1146	1143	3.7	21.1	-	1,2,3
Terpinen-4-ol	1176	1177	1.6	-	1.9	1,2,3
trans-Verbenol	1149	1144	3.2	-	-	1,2,3
Cuminaldhehyde	1236	-	-	-	0.9	1,3
Chrysanthenyl acetate	1257	-	6.1	9.1	-	1,3
Bornyl acetate	1286	1285	1.1	13.8	-	1,2,3
α-Copaene	1376	1376	1.8	-	-	1,2,3
Aromadendrene	1462	1439	1.6	-	-	1,2,3
Curcumene	1472	-	1.1	-	-	1,3
Germacrene D	1485	1480	1.0	2.3	3.7	1,2,3
Bicyclogermacrene	1486	-	0.7	-	0.3	1,3
Benzyl acetylacetate	1487	-	1.0	-	-	1,3
β-Caryophyllene	1488	-	0.2	0.2	0.2	1,3
β-Selinene	1490	1485	1.1	-	-	1,2,3
Spathulenol	1574	1576	-	-	1.3	1,2,3
Caryophyllene oxide	1585	1581	-	-	0.6	1,2,3
β-Eudesmol	1667	1649	-	-	3.1	1,2,3
Chamazulene	1719	1725	-	39.2	-	1,2,3
Total identified (%)			97.5	98.6	95.1	
Monoterpene hydrocarbons			11.3	11.9	83.1	
Oxygenated monoterpenes			77.7	45.0	2.8	
Sesquiterpene hydrocarbons			8.5	2.5	4.2	
Oxygenated sesquiterpenes			-	39.2	5.0	

Ri[a]: Kovats retention index calculated relative to homologous series of *n*-alkanes (C10–C35) on HP-5 MS column; Ri[b]: Kovats retention index, Adams.

1=Kovats retention index on HP-5 MS column; 2 = Kovats retention index, Adams: 3= Mass Spectrum, NIST, WILEY libraries spectra, from literature and co-injection with authentic compound

a major compound [36].

Chemical components isolated from *A. absinthium,* recorded approximately an equal amount of oxygenated sesquiterpenes (39.2 %) and oxygenated monoterpenes (45 %). Sesquiterpene hydrocarbons constitute only 2.5 % of the total oil. The major constituents were chamazulene (39.2 %), camphor (21.1 %), bornyl acetate (13.8 %) and chrysanthenyl acetate (9.1 %). Chamazulene was the only representative of oxygenated sesquiterpenes group in the essential oil of *A. absinthium*. Thus, this chemical compound was reported in many studies as a major component in the volatile fraction of *A. absinthium.* In agreement with our study, Riahi and co-workers showed that chamazulene was the major compound in leaves (30.41 %) and flowers (29.9 %) for *A. absinthium g*rowns in Tunisia. These oils showed only very low quantitative variations of chemicals compounds in different parts of this plant, but chamazulene was constantly the dominant compound [37]. More recently, Dhen and co-workers reported that camphor (24.81 %) was the major compound of *A. absinthium*, cultivated in an organic park from the Technical Center of Organic Agriculture (Sahel region of Tunisia), followed by chamazulene (13.17 %) and bornyl acetate (5.89 %) [38].

Oils identified by GC-FID/GC-MS analysis showed a high level of chemical polymorphism. This diversity was recorded even in species from the same family (*Asteraceae*), and in the same specie coming from different habitats in Tunisia. This inter/intra specific diversity would likely be influenced by several factors as stage of growth, climatic and ecological characteristics of the location, edaphic conditions of the region and extraction techniques [39]. Previous data reported in our study showed, that the genus *Artemisia* L is characterized by a wide range of phytochemical variability, which is associated with different geographical origins of the samples, this variability was confirmed through several analyses [40].

Antileishmanial activity

The effect of essential oils from *Artemisia* species was tested on promastigote form of *L. major* viabilities*,* all of our extracts used in this study, were able to reduce drastically the parasite growth and showed a statistical significant reductions in cells survival after 72 hours of exposure to the different essential oils (Fig. 1). Assessments of the antiparasitic activities against promastigotes of *L. major* with essentials oils of three species of *Arte-misia* growing in the center of Tunisia showed that our extracts have promising antileishmanial activity. In fact, we have recorded an important effect on cell viability (Fig. 1, 2). As shown, in oils from *A. herba alba*, *A. absinthium* and *A. campestris* exhibited IC_{50} values of 1.20 ± 0.043 µg/mL, 1.49 ± 0.05 µg/mL and 2.20 ± 0.11 µg/mL, respectively against promastigotes of *L.major* (table 2). Several medicinal plants contained chemical compounds such as mono-terpenoids [41], sesquiterpenes with their derivatives [42], diterpe-noids [43] and triterpenoids [44] were tested

Table 2. Inhibitory concentration (IC_{50}) values of *Artemisia herba alba, Artemisia absinthium* and *Artemisia campestris* essential oils on promastigotes of *Leishmania major* and Cytotoxicity effect on mammalian cells U-937

Substances	IC_{50} [µg /mL] *Leishmania major*	IC_{50} [µg /mL] U-937 Cells
A. herba alba	1.20±0.04	11.24±2,06
A. absintium	1.49±0.05	11.22±1,28
A. campestris	2.20±0.11	21.12±3,61
Controls		
Medium (with 0.5 % DMSO)	NA	NA
Amphotericin B	0.021±0.0017	19.99±1.92

Data are represented as means ± standard deviations of three independent experiments. NA: not applicable

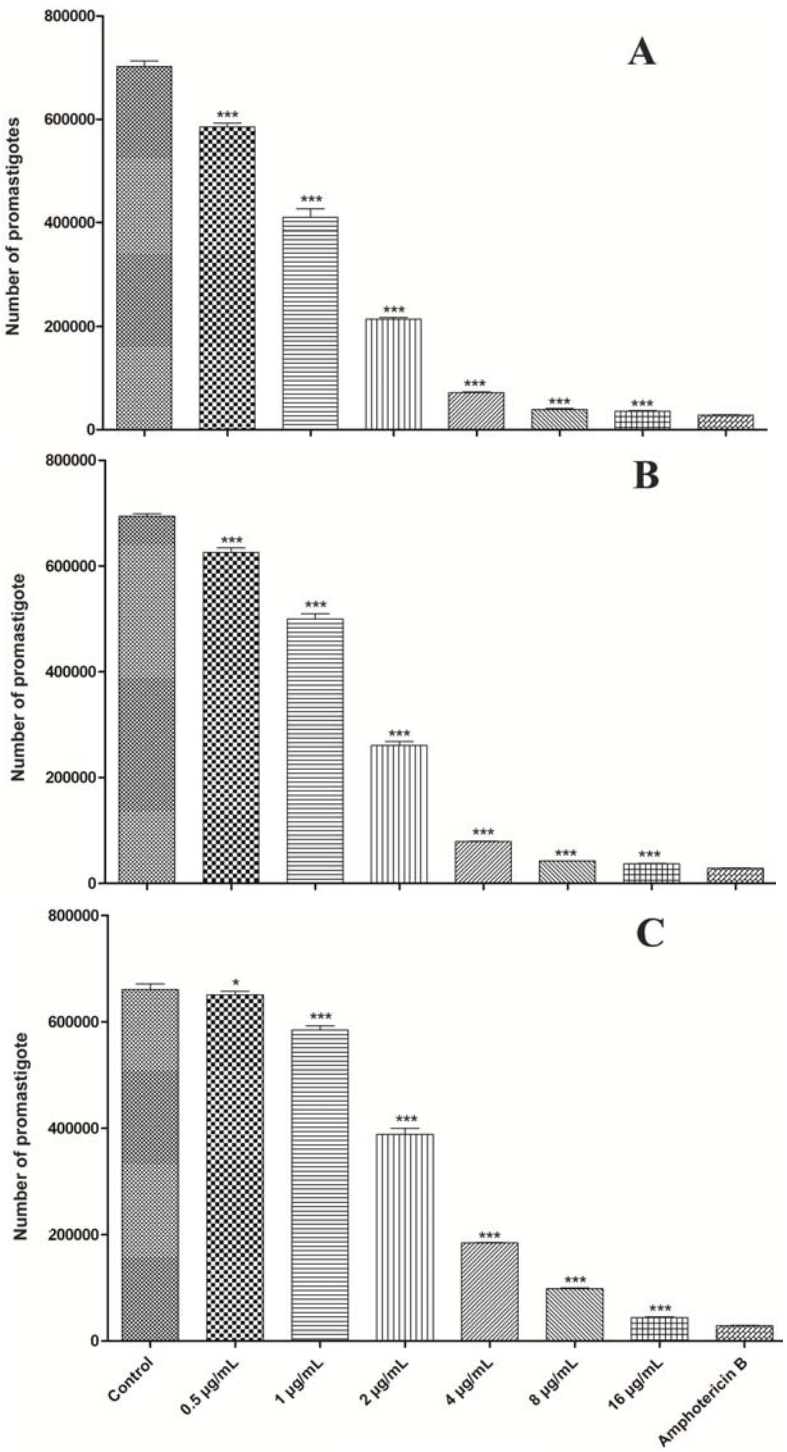

Fig. 1. *In vitro* efficacy of essential oils of *Artemesia herba alba* (A), *Artemesia absinthium* (B) and *Artemesia campestris* (C) against *L. major* promastigotes. Amphotericin B (0.16 µg/mL) as a positive control exhibited complete inhibition of parasite multiplication. Results are expressed as mean ±SD of three separate experiments. Comparison among the experimental groups was analysed by one way ANOVA followed by tukey's post test. *p* values of 0.05 or less were considered as statistically significant. *$p < 0.05$ significant versus control group. ***$p < 0.001$ significant versus control group

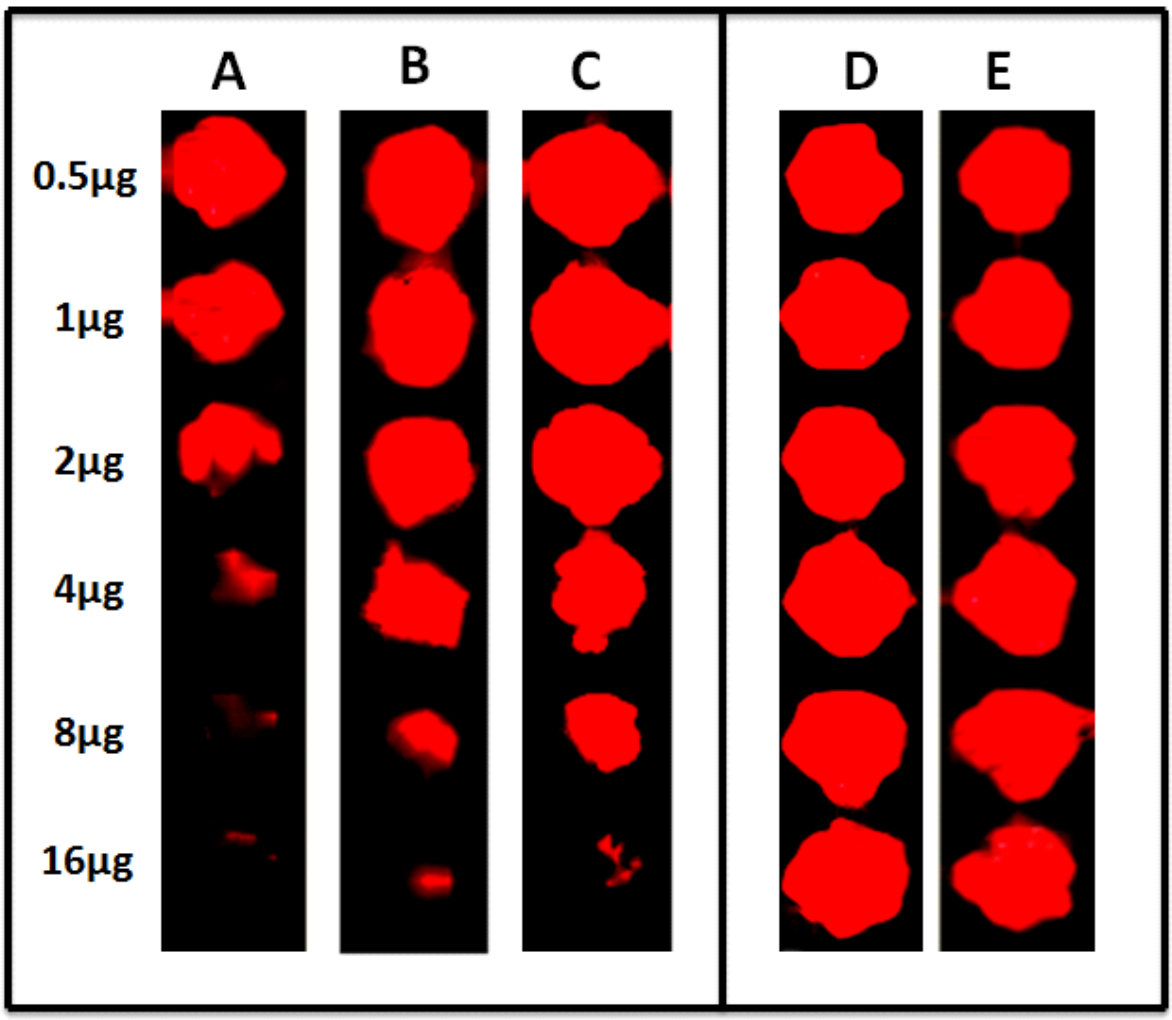

Fig. 2. Cells fluorescence determination for the *in vitro* antileishmanial activity of essential oils: (A) *Artemesia herba alba*; (B) *Artemesia absinthium*; (C) *Artemesia campestris*; (D) control (no drug); (E) medium with 0.5 % DMSO

for their leishmanicidal activity. The current work revealed the abundance of studied plants with oxygenated monoterpenes and oxygenated sesquiterpenes, which were found to be corroborated by previous studies. Thus, the notably evolution of medicinal plants uses according to folk remedies, favored the discovery of new secondary metabolites with strong antiprotozoal activity. Indeed, artemisinin a sesquiterpene lactone produced by *Artemisia annua* was among the first compounds that showed a significant anti-protozoal activity [45].

The first case of cutaneous leishmaniasis was described more than 130 years ago in Tunisia by Deperet and Boinet, 1984 in the region of South West (Gafsa) [46]. Since this date, many researchers have studied the role of medicinal plant extracts to fight this protozoal disease [47-36]. Seen that *Artemisia* is a heterogeneous genus consisting of approximately 500 species growing in the temperate areas throughout the world but usually in Asia, Europe, and America [48], the study of the leishmanicidal activity of this genus has been evaluated in Tunisia and other countries worldwide. In fact the essential oil of *A herba alba* from Morocco showed an antileishmanial activity against *L. major* with an IC_{50} of 2 µg/mL [49], which is in accordance with our data. The essential oil of *A. absinthium* from Ethiopia showed a concentration-dependent inhibitory effects on the growth of of *L. donovani* (MIC 0.1565 µl/ml,) and *L. aethiopica* (MIC 0.1565 µl/ml) promasti-

gote forms. It also showed an important effect on axenic amastigote forms of *L. donovani* (EC_{50} 42.00 nl/ml) and *L. aethiopica* (EC_{50} 7.94 nl/ml). Compared to our findings, the oil of *A. absinthium* from Ethiopia was rich in camphor (27.4 %), which was the major component in this plant [50]. According to Monzote *et al.* [51] the *in vitro* experiments of anti-leishmanial tests showed the capability of the essential oil of *A. absinthium* from Cuba to inhibit the growth of promastigotes (14.4 ± 3.6 µg/mL) and amastigotes (13.4 ± 2.4 µg/mL) of *L. amazonensis* after 72 h of incubation. The major constituent of this oil was the *trans*-sabinyl acetate, which represents up to 36.7 % of the total compounds identified with GC-MS. Contrary to *A. herba alba* and *A. absinthium*, a few studies assessed the antileishmanial activity of *A. campestris* were available. A recent study by a Tunisian group reported that oil from *A. campestris* growing in northern Tunisia exhibit an IC_{50} = 3.24 µg/mL against *L. infantum* after 72 h of exposure [36]. Furthermore, the documented chemical composition from this study was similar to ours, except the α-pinene which was the major compound.

The leishmanicidal reference drug Amphotercin B has been used as positive control in this study. Its IC_{50} value was 0.021 µg/mL ± 0.0017, which is similar to IC_{50} value found against *Leishmania donovani* (0.035 µg/mL ± 0.0006) [52]. In a Brazilian study, the IC_{50} values of Amphotercin B were higher compared to our study, they were 0.6 µg/mL ± 0.36 and 0.7 g/mL ± 0.36 for *L. amazonensis* and *L. braziliensis* respectively [53]. This drug presented high efficiency levels associated with serious side effects. Thus, Amphotercin B and Miltefosine are the most used chemical drugs in leishmaniasis therapy. Nonetheless, these antimony drugs have many side effects in addition to their high costs and their associated development of resistant strain [54].

Literature data indicate that some of the compounds present in our volatile oils possess a high antileishmanial activity. Thus, the IC_{50} of sabinene (126.6 µg/mL), α-pinene (55.3 µg/mL), µ-phellandrene (32.8 µg/mL), β-pinene (200.1 µg/mL), , 4-terpineol (335.9 µg/mL) were reported against promastigotes of *L. major* [55]. These high IC_{50} values obtained with major compounds isolated from oils compared with our data can be explained by the fact that the antileishmanial activity is not only depended on the major compound of studied plants, but also on the synergy effect between components (crude extracts) of oils and studied strain of *Leishmania* spp.

Our results corroborate with those reported in literature which has revealed that *Artemesia* genus demonstrates highly potential leishmanicidal agents. This activity remains important despite the instability of the major compound of these plants (depending on origins, climate and growth stages).

Cytotoxicity

The cytotoxicity of our oils was tested against mammalian cells U-937. The IC_{50} values were 11.24 µg/mL for *A. herba alba*, 11.22 µg/mL for *A. Absinthium* and 21.12 µg/mL for *A. campestris*. Compared to the positive control Amphotercin B, the essential oil exhibits moderate or even low cytotoxicity compared to this reference drug (19.99 µg/mL). The essential oils discussed above, have shown a strong *in vitro* antileishmanial activity with an acceptable toxicity profile particularly in *A.campestris* which has a lower cytotoxic effect compared to Amphotercin B. In accordance with our finding, Essential oils, used in traditional Colombian medicine coming from *Salvia aratocensis*, *Turnera diffusa* and *Lippia americana* was shown to have a partially or non toxic effect on Vero and THP-1 mammalian cells with CC^{50} values from 30 to 100 µg/mL [56].The high cytotoxicity effect observed in *A.herba alba* and *A. Absinthium* is explained by the ability of some essential oils to pass easily through cell membranes which increases its permeability and induced apoptosis [57-58] causing damaging to proteins and lipids [59] and block many synthetic metabolic pathway [44].

The results of this study can be considered as an initiation to the research of new drugs which possess a strong antileishmanial activity and acceptable cytotoxicity in order to develop natural antiprotozoal drugs against leishmaniasis which possess a low cytotoxicity with the final aim of substituting chemical drugs considered expensive and toxic.

Conclusions

Drug discovery and development for therapeutics of various diseases including antimicrobial, antioxidant, and antibiotic activities still relies on natural ingredients. In leishmaniasis disease, several antileishmanial treatments are derived directly from natural sources. Overall, the present work reported the main antileishmanial activity (*in vitro*) of essential oils extracted from *A. herba alba, A. absinthium* and *A. campestris* harvested from central Tunisia. These essential oils exhibited a potent antileishmanial activity against *L. major* promastigotes after 72 hours of exposure. However, further studies need to be carried in order to understand the mechanistic of the antileishmanial activities. Moreover, isolation of the major constituents of these essential oils, which might be of interest for further bioactivity investigations, either *in vitro* or *in vivo*.

Competing interests

The authors declare no conflict of interest.

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