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Antioxidant and anticandidal activities of the Tunisian *Haplophyllum tuberculatum* (Forssk.) A. Juss. essential oils

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ABSTRACT

Haplophyllum tuberculatum Forssk. is a medicinal plant growing in Tunisia. It is widely used in traditional medicine against gastro-intestinal problems, fevers, ear infections and rheumatisms. The present investigation evaluated the effects of leaves, stems and leaves + stems essential oils of *Haplophyllum tuberculatum* Forssk. and of their pure compounds on free radicals as well as their anticandidal activities. Screening for the antioxidant activity of the oils, *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol was conducted by DPPH, ABTS and β-carotene/linoleic acid radical scavenging assays. The essentials oils and their compounds were screened for antifungal activity against four *Candida* species: *Candida albicans* ATCC 90028; *Candida glabrata* ATCC 90030; *Candida parapsilosis* ATCC 27853 and *Candida krusei* ATCC 6258. When compared with ascorbic acid as standard, it was found that the essential oils have a significant inhibition in scavenging free radicals, resulting in an important IC_{50}. The pure compounds were inactive against the free radicals. The anticandidal test results showed that leaves, stems and leaves + stems oils strongly inhibited the growth of *Candida krusei* at 30 μg/mL leaves oils and 70 μg/mL for other oils and that moderately of the 3 other *Candida* species. The pure compound, 1-octanol, was active one against the *candida* species, with MIC-values between 0.07 and 1.25 mg/mL.
In all *in vitro* assays, a significant correlation existed between the concentrations of the essential oils, the percentage inhibition of free radicals and of the growth inhibitory of tested *candida* species. The results indicate the essential oils may be applied for treating diseases related to free radicals, potentially to prevent cancer development and as an antifungal agent against *Candida*.

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1. Introduction

Haplophyllum is a genus belonging to the Rutaceae family. It is distributed in different floristic regions: the Irano-Turanian, Mediterranean, Saharo-Arabian, and Sudano-Zambezian regions. It includes 68 species (Navarro et al., 2004; Soltani and Khosravi, 2005; Townsend, 1986). In Tunisia, it is represented by 3 species *Haplophyllum linifolium* (L.) A. Juss (=*Haplophyllum hispanicum* Spach), *Haplophyllum tuberculatum* (Forsk) A. Juss and *Haplophyllum buxbaumii* Poiret (Pottier-Alapetite, 1979).

H. tuberculatum stretches from Morocco to western Pakistan. It is an annual herbaceous plant. The whole plant is covered with large glands containing essentials oils. The leaves are variable in shape, from almost linear to short. The flowers are yellow and variable in size (Pottier-Alapetite, 1979). The plant is traditionally known for its different medicinal properties, for instance against gastro-intestinal affections, intermittent fevers, and rheumatisms. This plant is also an aphrodisiac and is administered against ear infections (Le Floc'h, 1983). It was recommended against nausea and vomiting by taking the leaves' infusion in the morning before breakfast. It is also used for the nervous system, infertility and fever (Said et al., 2002). In northern Oman, the juice is used as a remedy for headaches and arthritis (Al-Burtamani et al., 2005). In Saudi Arabia, *H. tuberculatum* is used to treat malaria, rheumatoid arthritis and gynecological disorders (Al-Yahya et al., 1992). In Southern Tunisia, the fresh plant is used as an antiparasitic against *Enterobius vermicularis* by drinking the infusion of some fresh leaves and it's also used as flavor in the tea.

Abbreviations: DPPH, 1,1-Diphenyl-2-picryl-hydrazyl; ABTS, 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); $K_2S_2O_8$, Potassium persulfate.

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The essential oil of *H. tuberculatum* is anti-bacterial and partially inhibits the growth of *Escherichia coli, Salmonella choleraesuis, Bacillus subtilis* and *Candida albicans* (Al-Burtamani et al., 2005). It also has anti-fungal properties. In fact, the essential oil inhibits the mycelial growth of *Curvularia lunata* and *Fusarium oxysporum* (Al-Burtamani et al., 2005).

Some biochemical studies of the essential oils (EO) of *H. tuberculatum* aerial part have been published (Al-Burtamani et al., 2005; Al Yousuf et al., 2005; Yari et al., 2000). No studies for the antioxidant activity of the EO were found in the literature. For this reason, in the present study, an attempt was made to explore the possible antiradical properties of the aerial parts and of the individual stems and leaves, by different techniques, which may provide more comprehensive information on these properties.

In our previous work, we analyzed the chemical composition of the essential oils of the separated leaves, stems and leaves + stems from the Tunisian *H. tuberculatum* and we assessed their phytotoxic activities (Hamdi et al., 2017). The present investigation aimed evaluating the antioxidant of leaves (L), stems (S) and the aerial part (leaves + stems; LS) of this species essential oils (LEO, SEO and LSEO, respectively) and assessing their anticandidal activities. Limonene, as a major compound, its isomers and octanol, as a minor compound, were also tested.

2. Material and methods

2.1. Plant material

Plant material from the *H. tuberculatum* Forssk. species was collected at the flowering stage from Medenine in Tunisia. The leaves, stems, leaves + stems and roots were cut into small pieces and weighed before extraction of the volatile compounds (Hamdi et al., 2017).

2.2. Extraction of the essential oils

About 100 g fresh plant parts (leaves, stems, roots and leaves + stems) was subjected to a hydro-distillation for 3 h with 500 mL distilled water using a Clevenger-type apparatus. The LEO, SEO and LSEO obtained were dehydrated by passing through anhydrous sodium sulfate. No volatile oils were obtained from the roots. Then, the essential oils were stored in sealed glass vials in a refrigerator at 4–5 °C, until use (Hamdi et al., 2017).

2.3. Standard compounds

Octanol (for synthesis, Merck), *R*-(+)-limonene (97%, Aldrich) and *S*-(−)-limonene (96%, Aldrich) were provided from the commercial suppliers and dissolved in methanol (Merck). DPPH, ABTS, β-carotene and linoleic acid are provided from Sigma Aldrich.

2.4. Determination of antioxidant activity

Antioxidants are compounds capable to either delay or inhibit free radicals. They are involved in the defense mechanisms of the organism against pathologies related to free radicals. Amongst the most important natural or synthetic antioxidants, vitamin A, vitamin E, vitamin C, vitamin D, β-carotene and flavonoids allow free radicals inhibition because of their molecular structure.

Various methods to evaluate the antioxidant activity have been described. Antioxidant assays can be classified into two main groups, hydrogen atom transfer (HAT) and single electron transfer (ET) assays. Antioxidants are measured by their HAT or ET to probe molecules. In this study, the DPPH and ABTS assays were used. These assays usually are classified as ET reactions, but in fact, both radicals may be deactivated either by radical quashing HAT or by direct reduction through ET mechanisms (Apak et al., 2016; Jiménez et al., 2004).

Lipid peroxidation is the oxidation of lipids, especially of unsaturated fatty acids in cellular membranes mediated by oxidative stress in the cells. The β-carotene bleaching assay is an approach to directly assess the antioxidant activity of compounds toward a lipid substrate (Apak et al., 2016; Laguerre et al., 2007).

These, three above-mentioned methods were applied in this study.

Bellow the protocols of these methods, used to test the antioxidant activity of the essential oils are described. Ascorbic acid (vitamin C) was used as reference compound under the same conditions and with the same concentrations as the tested samples. The inhibition concentration (IC_{50}) value, which is the sample concentration providing 50% inhibition was determined.

2.4.1. DPPH radical-scavenging activity assay

The DPPH assay is known to provide reliable information concerning the antioxidant capacity of specific compounds or extracts. The hydrogen atoms accepting or electron donating ability of the corresponding samples were measured from decolorizing a purple colored methanolic DPPH solution. The effect of the essential oils and their pure components on the DPPH radical was estimated (Shimada et al., 1992). 0.5 mL of each sample was mixed with the same volume DPPH• ethanolic solution (60 μM). The mixture was shaken vigorously and allowed standing for 30 min in the dark at a temperature of 25 °C. The absorbance of the resulting solution was measured at 520 nm with a spectrophotometer. The analyses of the samples were performed in triplicate. A mixture of 0.5 mL DPPH• solution and 0.5 mL methanol was taken as a control. Methanol was used as a blank. The Inhibition of the free radical DPPH in percent (IP%) was calculated as follows:

$$IP\% = 100 \times \left(A_{Control} - A_{Sample}\right)/A_{Control}$$

where $A_{Control}$ is the absorbance of the control, and A_{Sample} of the tested sample.

2.4.2. ABTS radical scavenging activity assay

Antiradical activity was also determined using the $ABTS^{+}$• free radical decolorization assay (Re et al., 1999). Briefly, the preformed radical ABTS monocation was generated by reacting aqueous ABTS solution (7 mM) with 2.45 mM ethanolic $K_2S_2O_8$. The mixture was allowed standing for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain an absorbance of 0.7 ± 0.2 at 734 nm and used as control. Extracts (0.0312, 0.0625, 0.125, 0.25, 0.5 and 1 mg/mL) and pure compounds (1 mg/mL) were separately dissolved in ethanol. To estimate the antioxidant activity of the samples, 10 μL was added to 990 μL of diluted $ABTS^{\bullet +}$.

The absorbance was measured spectrophotometrically at 734 nm after 5, 10, 15 and 20 min. The samples/compounds were analyzed in triplicate. Distilled water was used as a blank. The percentage inhibition (PI) of $ABTS^{\bullet +}$ was calculated by the following equation:

$$IP\% = 100 \times \left(A_{Control} - A_{Sample}\right)/A_{Control}$$

where $A_{Control}$ is the absorbance of the control, and A_{Sample} of the tested sample.

2.4.3. β-Carotene/linoleic acid method

The β-carotene bleaching inhibition activity of *H. tuberculatum* Forssk EO was determined (Ikram et al., 2009). Briefly, 2 mL β-carotene solution (1.5 mg β-carotene/2.5 mL chloroform) was added to 20 μL linoleic acid and 200 μL Tween-20 and mixed. The chloroform was removed at 40 °C under vacuum using a rotary evaporator.

Immediately, 50 mL distilled water was added to the dried mixture to form a β-carotene-linoleic acid emulsion. In order to determine the β-carotene bleaching activity of the extract, 5 mL emulsion was added to 500 μL EO (0.0312, 0.0625, 0.125, 0.25, 0.5 and 1 mg/mL) or pure compound (1 mg/mL). The mixtures were incubated in a water bath

at 50 °C for 120 min. The absorbance of the reaction mixture was read at 470 nm. The emulsion without β-carotene was used as blank. Tests were carried out in triplicate. Antioxidant activity of extracts was calculated by using the following equation:

$$IP\% = 100 \times (A_{Control} - A_{Sample})/A_{Control};$$ where $A_{Control}$ and A_{Sample} are the absorbances at 0 and 2 h, respectively.

2.5. Anticandidal activity

The antifungal activities of the oils obtained from the leaves, stems, leaves + stems, *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol were individually tested against 4 Candida strains: *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, obtained from the Microbiology Laboratory of the Monastir University Hospital. The minimal inhibition concentration (MIC) was defined as the lowest concentration of oil that inhibited candidal growth, after incubation at 37 °C for 24 h. These values were determined with the broth dilution method (microdilution using 96-well microplates) (Besbes et al., 2012). A sample was prepared at a concentration of 10 mg/mL by dissolution of the oils in 5% DMSO (dimethylsulfoxide). The final concentrations of the plant samples, tested in triplicate, ranged from 0.009 to 5 mg/mL. The Minimum Fungicidal Concentration (MFC) was defined as the concentration of antifungal agent at which the number of colony forming units was zero and was determined by subculture on blood agar at 37 °C for 18 to 24 h. Thymol and amphotericin B (10 μg/mL) were used as positive controls against candidal strains.

2.6. Statistical analysis

The laboratory biological assays were conducted with triplicates for each sample. The IC_{50} of DPPH, ABTS, and β-carotene bleaching methods values were calculated by linear regression analysis. ANOVA and Duncan tests were performed with SPSS 16.0. The means of the tests values were also evaluated with the Least Significant Differences test at the 0.05 significance level.

3. Results and discussion

3.1. Antioxidant activity

In order to estimate the efficiency of these EO as antioxidants, the antioxidant activity related to the composition of the essential oils of *H. tuberculatum* Forssk. was determined by three different methods: the DPPH radical scavenging, the ABTS free radical scavenging and the β-carotene/linoleic acid method. Table 1 summarizes the results of the antioxidant assays and the IC_{50} values.

3.1.1. DPPH radical scavenging activity

The DPPH free radical is stable and it is widely used to evaluate the free radical scavenging ability of natural compounds. The DPPH radical-scavenging activity is conceivably due to a hydrogen-donating ability. The color of the reaction mixture changes from purple to yellow, resulting in a decreased absorbance (Archana et al., 2005). The DPPH radical scavenging capacities of leaves, stems and leaves + stems essential oils of *H. tuberculatum* (Forssk.) A. Juss were compared to ascorbate, with a maximal inhibition percentage of 87.5 ± 0.72% at the concentration of 1 mg/mL.

The essential oils from leaves, stems and leaves + stems presented important activities with inhibition percentages ranging from 34.10% to 81.00%, from 37.3 to 65.1% and from 23.00 to 72.20% for 0.03 and 1 mg/mL of LEO, SEO and LSEO, respectively. The activity increased with increasing concentration of the oil samples. All samples have a moderate IC_{50} with 0.15, 0.30 and 0.14 mg/mL for LEO, SEO and LSEO, respectively, compared to ascorbic acid as reference. LEO and LSEO are significantly more active than SEO. *R*-(+)-limonene showed a moderate antioxidant potential against the DPPH radicals (IC_{50} = 0.94 mg/mL), while, *S*-(−)-limonene and 1-octanol had no activity against these radicals (IC_{50} > 1 mg/mL) relative to the ascorbic acid (IC_{50} < 0.03 mg/mL).

3.1.2. ABTS radical scavenging activity

The ABTS method gives a measure of the antioxidant activities of the essential oils, by determining the reduction of the radical cation, expressed as the percentage of inhibition (PI) of the absorbance at 734 nm. The decolorization of the $ABTS^{\bullet +}$ cation reflects the capacity of an antioxidant to donate electrons or hydrogen atoms in order to inactivate this radical species (Re et al., 1999).

In this study, the essential oils from the three sample types presented a significant activity after 20 min compared to the standard (Table 1; results after 5, 10 and 15 min not shown). The inhibition percentage was important and ranging 29.3 to 83.9; 23.0 to 69.6 and 7.9 to 64.4% for LEO, SEO and LSEO, respectively. The LEO (IC_{50} = 0.14 mg/mL) and LSEO (IC_{50} = 0.10 mg/mL) are less active than the SEO (IC_{50} = 0.04 mg/mL). Thus, SEO is more interesting than the other oils. The pure constituents, *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol showed a moderate activity against the $ABTS^{\bullet +}$ radicals (IC_{50} > 1 mg/mL) compared to ascorbic acid (IC_{50} < 0.03 mg/mL).

3.1.3. β-Carotene/linoleic acid method

The free radical linoleic acid attacks the highly unsaturated β-carotene and the presence of antioxidants hinders the extent of β-

Table 1
Antioxidant activities of leaves, stems and leaves + stems EO of *H. tuberculatum* and their compounds against DPPH, ABTS and β-carotene/linoleic acid scavenging tests.

Sample	DPPH			ABTS			β-Carotene		
	PI (%)		IC_{50}*	PI (%)		IC_{50}*	PI (%)		IC_{50}*
	0.03	1		0.03	1		0.03	1	
LEO	34.1 ± 0,69	81.00 ± 0,84	0.15^a	29.30 ± 0,51	83.9 ± 0,14	0.14^a	38.4 ± 0,13	81.9 ± 0,03	0.16^a
SEO	37.30 ± 0,08	65.1 ± 0,01	0.30^b	23.05 ± 0,6	69.6 ± 0,14	0.04^b	44.2 ± 0,22	85.45 ± 0,11	0.08^b
LSEO	23 ± 0,21	72.2 ± 0,21	0.14^a	7.9 ± 0,08	64.45 ± 2,71	0.10^a	29.5 ± 1,71	70.80 ± 0,71	0.22^c
R-(+)-limonene	nd	52.31 ± 0.12	0.94^c	nd	42.44 ± 0.58	>1	nd	50.88 ± 0.08	>1
S-(−)-limonene	nd	39.33 ± 0.25	>1	nd	23.92 ± 0.26	>1	nd	33.49 ± 0.11	>1
1-octanol	nd	0.67 ± 0.75	>1	nd	0.65 ± 0.87	>1	nd	7.97 ± 0.42	>1
Ascorbic acid	56.26 ± 0,36	87.5 ± 0,72	<0.03	51.33 ± 0,85	79.45 ± 0,90	<0.03	53,58 ± 0,07	89,00 ± 0,22	<0.03

PI = percentage of inhibition.
nd: not determined.
The letters (a–c) indicate significant differences between the different concentrations of a given oil according to the Duncan test (p < 0.05). Results with the same letter are not significantly different.
* mg/mL.

carotene-bleaching by neutralizing the linoleate free radical and other free radicals formed.

At 0.03 mg/mL to 1 mg/mL, the total antioxidant activities were 38.4 to 81.9%; 44.2 to 85.45% and 29.5 to 70.8% for LEO, SEO and LSEO, respectively. SEO was the most active one with an interesting IC_{50} (0.08 mg/mL) followed by LEO (0.16 mg/mL) and then LSEO (0.22 mg/mL). The pure constituents, *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol showed a weak activity against the linoleic acid radical (IC_{50} > 1 mg/mL) compared to ascorbic acid (IC_{50} < 0.03 mg/mL).

According to the three methods, all samples showed a moderate antioxidant effect against free radical damage compared to ascorbic acid. *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol had few effect against the radicals.

The significant results of the EO antioxidant activities may be due to the chemical composition and the higher contents of hydrocarbon and oxygenated monoterpene compounds (Hamdi et al., 2017). Previous studies showed that the terpenes exhibited an antioxidant activities (Wu et al., 2013).

The major compounds of the tested essential oils, which were *cis*-*p*-menth-2-en-1-ol, *trans*-*p*-menth-2-en-1-ol, *cis*-piperitol, *trans*-piperitol, 1-octyl acetate, piperitone and isobornyl acetate, may be responsible for this activity but there are no reports in the literature about antioxidant assays on these components. Moreover, *R*-(+)-limonene, *S*-(−)-limonene and 1-octanol, which exhibited weak antioxidant activities, confirm that the EO antioxidant activities cannot be attributed to these compounds. Limonene had a weak activity (12.6%) in a deoxyribose assay (Emami et al., 2011). Oppositely to our results, Dai et al. (2013) reported that limonene had a high antioxidant effect using the DPPH, superoxide anion, and reducing power assays. The same study claimed that α-pinene had a moderate antioxidant against the DPPH radical, superoxide anion, and reducing power assays (Dai et al., 2013).

Beta-pinene, α-pinene and sabinene were evaluated in deoxyribose assay and exhibited weak activity with 31.7; 4.1 and 1.3%, respectively (Emami et al., 2011). Sabinene (0.64 μL/mL) showed potential antioxidant activity against the nitric oxide (NO) scavenging assay (Valente et al., 2013). Moreover, camphene decreases lipid peroxidation, inhibits NO release and reactive oxygen species generation in the pre-treated cell (IP = 83.84%) (Tiwari and Kakkar, 2009). Myrcene increased the level of reduced glutathione and the activities of superoxide dismutase, catalase, glutathione peroxidase in rat liver (Ciftci et al., 2011). de Oliveira et al. (2015) studied *p*-cymene as antioxidant and they found that, at 150 mg/mL, it decreases lipid peroxidation to 89.83% and at 100 mg/kg it decreases nitrite content to 68.61%. At 150 mg/kg, it also increases the superoxide dismutase and catalase activities to 63.1% and 182.70%, respectively (de Oliveira et al., 2015). Cumin aldehyde and *p*-cymene increase the activities of superoxide dismutase to 34% and 65%, catalase to 37% and 17% and glutathione peroxidase to 48% and 41%, respectively in the rat liver (Roozi and Boojar, 2014). Moreover, linalool (IC_{50} = 108.90 mg/mL) and valencene (IC_{50} = 150.92 mg/mL) had an antioxidant effect against the radical DPPH (Liu et al., 2012). Linalool had a strong antioxidant activity (IC_{50} = 0.61 μg/mL) by using DPPH radical scavenging (Pripdeevech et al., 2010). Ar-curcumene showed an interesting antioxidant activities on human LDL peroxidation (IC_{50} = 0.06 μg/mL), which at 1.56 μg/mL, the percentage inhibition was 90.58% (Jantan et al., 2012). The antioxidant assays of single pure monoterpenes compounds, as well as the synergistic and antagonistic antioxidant interactions of their mixtures were studied (Ciesla et al., 2016). Therefore, at 1.0 mM and after 60 min of contact with the radicals, α-phellandrene (IP = 58.57%), *p*-cymene (IP = 12.35%), (±)-linalool (IP = 57.57%), α-terpinene (IP = 95.0%) exhibited DPPH scavenging activity. Synergism and antagonism between constituents may also contribute to the antioxidant activity. The mixture 1/1, V/V of α-phellandrene and *p*-cymene (IP = 76.36%), α-phellandrene and (±)-linalool (IP = 61.3%) had a synergetic effect. No significant effect was found by mixing α-phellandrene and α-terpinene (IP = 93.5%), *p*-cymene and α-terpinene (IP = 94.7%), (±)-linalool and α-terpinene (IP = 93.89%). Antagonism was observed in the mixture of *p*-cymene and (±)-linalool (IP = 17.2%) (Ciesla et al., 2016).

Thus, antioxidant activities observed for the *H. tuberculatum* could be attributed to the remaining chemical constituents.

3.2. Antifungal activity

Medicinal plants are not only important to the millions of people for whom traditional medicine is the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals. Plants EO have been a source of a wide variety of biologically active compounds and used extensively as crude oils or their pure and separated compounds for treating various fungal infections (Abad et al., 2007).

In this work, the anti-fungal potential of the Essential Oils, extracted by hydro-distillation from *H. tuberculatum,* grown in Tunisia, and of their pure compounds on *Candida* species were tested. Evaluation parameters included Minimal Inhibitory Concentrations (MICs) and Minimal Fungicide Concentrations (MFC). The data recorded could help preventing and combating some infectious diseases in the short run and guide the production of conventional drugs in the long run.

The LEO was more active against *Candida krusei* MICs = MFC = 0.03 mg/mL (Table 2) than against *C. albicans, grablata* and *parapsilosis* where the MICs were 0.62, 0.31 and 0.15 mg/mL, respectively. The SEO was active against *C. krusei* with MIC = 0.03 mg/mL and MBC = 0.07 mg/mL. The LSEO had a higher activity against *C. krusei* than against *C. parapsilosis*, *C. albicans* and *C. glabrata* with MFC = 0.07; 0.15; 0.62 and 2.5 mg/mL, respectively.

Another technique was used to evaluate the antifungal assays. In fact, using well diffusion assay, the EO from the areal parts of *H. tuberculatum* has partially inhibited the growth of *C. albicans* ATCC 10231. Thus, at 25 mg/mL, EO inhibit *C. albicans* ATCC 10231 with an inhibition zone 17.6 mm. However, at 1 mg/mL, it had no effect on *Alternaria alternata, Stemphylium solani*. The inhibition percentage, at 1 mg/mL, of *Curvularia lunata, Fusarium oxysporium* and *Bipolaris* sp. was 16.3, 3.1 and 9.5%, respectively (Al-Burtamani et al., 2005). Hence, the tested species and the difference methods used to investigate the antifungal activities can explain the obtained results.

In our study, *R*-(+)-limonene, *S*-(−)-limonene and octanol had an interesting anticandidal activity. *R*-(+)-limonene showed a similar MIC (0.31 mg/mL) against *C. parapsilosis*, *C. albicans* and *C. glabrata* and 0.07 mg/mL against *C. krusei*. Its MFC against *C. albicans* and *C. krusei* was 0.62 mg/mL. Besides, *S*-(−)-limonene was active against *C. krusei* with MIC = 0.15 and MFC = 0.31 mg/mL. Further,

Table 2
Anticandidal activities of the essential oils isolated from the vegetative parts (stems, leaves and leaves + stems) of *H. tuberculatum* and their pure compounds. MIC and MFC are given in mg/mL.

Samples		*Candida albicans* ATCC 90028	*Candida glabrata* ATCC 90030	*Candida parapsilosis* ATCC 27853	*Candida krusei* ATCC 6258
LEO	MIC	0.62	0.31	0.15	0.03
	MFC	1.25	0.62	0.31	0.03
SEO	MIC	0.62	1.25	0.31	0.03
	MFC	1.25	1.25	0.6	0.07
LSEO	MIC	0.31	1.25	0.15	0.03
	MFC	0.62	2.5	0.15	0.07
R-(+)-limonene	MIC	0,31	0,31	0.31	0.07
	MFC	0,62	1.25	1.25	0,62
S-(−)-limonene	MIC	0,31	0,62	0,31	0,15
	MFC	0,62	1.25	1.25	0.31
1-Octanol	MIC	0,15	0,31	0.07	0.07
	MFC	0,31	1.25	0.07	0.15
Thymol	MIC	$0.12 \cdot 10^{-3}$	$0.16 \cdot 10^{-3}$	$0.32 \cdot 10^{-3}$	$0.32 \cdot 10^{-3}$
Amphotericin B	MFC	$0.5 \cdot 10^{-3}$	$0.5 \cdot 10^{-3}$	$0.5 \cdot 10^{-3}$	$0.5 \cdot 10^{-3}$

C. parapsilosis was more sensitive to octanol with MIC = MFC = 0.07 mg/mL.

The correlation between the achieved anticandidal activities of these EO and their chemical composition suggests that the activity may be ascribed to monoterpene compounds present in high percentages in the oils.

No reports about the antifungal activity of the major compounds *cis-p*-menth-2-en-1-ol, *trans-p*-menth-2-en-1-ol, *cis*-piperitol, *trans*-piperitol, 1-octyl acetate and isobornyl acetate were found. The antifungal activity of some minor EO components was studied. Linalool was effective against fifty clinical isolates of *C. albicans*, which were oropharyngeal and vaginal strains (D'auria et al., 2005).

The oils exhibited an interesting anticandidal activity against all the human pathogenic yeasts tested in this study with MICs of 0.03–0.62 mg/mL (Table 2). According to our results, the anticandidal activity of the oils may be attributed to the pure tested constituents: *R*-(+)-limonene, *S*-(−)-limonene and octanol.

4. Conclusion

In the present study, three essential oils of leaves, stems and leaves + stems from *H. tuberculatum* were tested for their antioxidant and anticandidal potentialities. The isomers of the limonene, as major compound, and the minor component, 1-octanol, are also evaluated.

Results showed that the activity increased with increasing concentration of the oil samples. LEO and LSEO showed a similar IC_{50} against DPPH and ABTS radicals which were different from SEO. However, LEO, SEO and LSEO had significant different IC_{50} for the β-carotene/linoleic acid methods. Pure compounds revealed moderate antioxidant activities.

The antifungal assays showed that *H. tuberculatum* essential oils and the pure compounds were active against *C. krusei*, *C. albicans, C. grablata* and *C. parapsilosis*. The antifungal activity suggests that the studied oils and their compounds may be considered as important components with anticandidal properties.

Finally, the differences between our bioassay results and the published work can be explained by the difference in the chemical profile of the EO, which depends on the abiotic factors (edaphic, climatic, season…) of the plant collecting region, the used techniques, etc.

Conflicts of interest

The authors declare that there are no conflicts of interest and no significant financial support for this work that could have influenced its outcome.

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