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Chemical Composition, Antioxidant Potential and Antimicrobial Properties of the Essential Oils of *Haplophyllum tuberculatum* (Forsskal) A. Juss from Morocco

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ABSTRACT

Essential oils are secondary metabolites of plants which have shown very important biological activities. The chemical composition, antioxidant and antimicrobial activity of essential oils isolated from *Haplophyllum tuberculatum* (Forsskal) A. Juss are the subject of this study. The essential oils was extracted by hydrodistillation, GC/MS and GC-FID were used to determine the chemical composition of the essential oils studied. Two tests were used to assess antioxidant power DPPH (2,2-diphenyl-1-picrilhidrazil) test and TAC (Total antioxidant capacity) assays. Agar diffusion and microdilution were used to examine the antimicrobial activity of these oils. The GC/MS and GC-FID analysis reveal 50 components. The major component was Farnesyl bromide (37.02%) followed by santolina triene (10.26%), terpinene-4-ol (6.13%) and limonene (2.26%). The scavenging capacity of DPPH by essential oils (IC_{50} = 3.2395 ± 0.009 mg/mL) was lower than that of ascorbic acid (IC_{50}= 2.8195 ± 0.008 µg/mL), but the measurement of its TAC revealed the presence of a significant amount of antioxidants in the oils studied (617.95 ± 0.589 mg EAA/g extract). *S. aureus* (bacteria G $^{+}$) appears the most sensitive (24 mm). The values of the MIC are proportional to the values of the diameters of the inhibition zone, the oil was very active on *S. aureus* (MIC = 5.91µg/mL). *E. coli* and *P. aeruginosa* were the least sensitive (MIC = 94.6 µg/mL). The results of this study showed that this oil can be an alternative source of antimicrobials to combat strains with a problem of resistance to conventional antibiotics.

Keywords: *H. tuberculatum*, Essential oils, GC/MS, Antioxidant, Antimicrobial activity.

Introduction

Medicinal plants have been used as a source of remedies since ancient times and the ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in the treatment of various diseases.[1] Interest in the use of herbal drugs has increased in both the traditional and modern medical systems.[2] In addition, several clinical and preclinical studies reveal the biological effects of a wide range of plant-derived compounds.[3] *Haplophyllum tuberculatum* (Forsskal) A. Juss is a species in the rutaceae family. This species is one of 2100 species grouped into 154 genera composed of trees, shrubs and aromatic herbs mainly tropical and subtropical [5] .This family is a potential source of many medicinal substances.[6] *H. tuberculatum* grows on sandy hills and rocky slopes of arid regions, it extends from Morocco to the west of Pakistan,[7] it has shown great variability in its traditional uses depending on geographic and ecological location.[8] According to Hadjadj *et al*.,[9] *H. tuberculatum* was traditionally used as an antiseptic, calming, for infertility, treatment of diabetes, against fever, bloating, diarrhea, flu, scorpion stings, throat inflammation, cough, such as dewormer, tonsillitis, otitis and loss of appetite. In Sudan the *H. tuberculatum* is used as an antispasmodic, to treat allergic rhinitis and gynaecological disorders, and on the cardiovascular System.[10] Essential oils were used in ancient Rome, Greece and Egypt and throughout the Middle and Far East as perfumes, food flavors, deodorants and pharmaceuticals [11]**.** The essential oils which were utilized centuries ago in cosmetics usually show interesting biological features. Essential oils are volatile aromatic concentrated hydrophobic oily liquids which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots.[12] The rutaceae family contains many aromatic and medicinal plants. Multiples studies have been reported on the essential oils of *H. Tuberculatum* species belonging to different regions in the world.[13,14,15] Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use.[16,17] According to Liaqat *et al.*,[18] essential oils were used as antibacterial, antioxidant, antiviral, antifungal, anticancer, anti-inflammatory, antihistamine and anti-diabetic and in food preservation**.** Therefore, the determination of the chemical composition of the volatile oil of *H. tuberculatum* harvest in south-eastern Morocco, the evaluation of its antioxidant and antimicrobial power are the subject of this study.

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Materials and Methods

Plant material

The aerial parts of *H. tuberculatum* were collected during the second week of February 2019 near the town of Akka in the province of Tata (south-eastern Morocco). The plant has been identified by Pr. Amina

BARI botanist at the Sidi Mohamed Ben Abdellah University, Fez, Morocco. A voucher (RH001190122) sample was deposited at the herbarium of the Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health & Quality of Life, Faculty of Sciences Fez.

Extraction of essential oils

In order to extract the essential oils, the adopted method is hydrodistillation using the Clevenger device.[19] After their fragmentation, 200 g of aerial parts pre-dried in the open air were inserted into a 2 liter flask, 750 mL of water were added to it and the mixture was allowed to boil for 3 h. The essential oils obtained were stored at a temperature of -4°C. The yield (in percentage) was calculated on the basis of the dry weight of the vegetable materials.

Identification of essential oils components

The analysis of essential oils of *H. tuberculatum* leaves was performed by gas chromatography with flame-ionization detection (GC/FID) and gas chromatography coupled with mass spectrometry (GC/MS), equipped with TRIPLUS AS S/N 20063460 in the light of the following experimental protocol, at the City of Innovation in Sidi Mohammed Ben Abdellah University, Fez. Morocco.

Gas chromatography analysis (GC/FID)

The GC (Trace GC ULTRA, S/N 20062969, Thermo Fischer, France) was equipped with flame ionization detector (GC-FID). The column used was, Varian capillary column (CP-Sil 5CB, 50m length, 0.32 mm of diameter and Film thickness 1.25 µm). The column temperature was programmed from 40°C to 280°C for 5°C/min. The temperature of the injector was fixed to 250°C and the detector temperature was 280°C. The debit of the gas vector (nitrogen) was fixed to 1ml/min. The volume of the injected specimens was 1µl diluted in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

Gas chromatography- mass spectrometry (GC/MS)

The different chemical compositions of essential oils was determined by GC (Trace GC ULTRA, S/N 20062969, Thermo Fischer, Trance) coupled with spectrometer (PolarisQ/ S/N 210729, Thermo Fischer, France); with ionisation energy of 70 ev. The utilised column was; Varian capillary column (TR5- CPSIL- 5CB; 50 m length, 0.32 mm of diameter and film thickness 1.25 µm). The column temperature was programmed from 40 to 280°C for 3°C/min. The temperature of the injector was fixed to 260°C and the detector (MS-PolarisQ) temperature was 200°C. The debit of gas vector (Helium) was fixed to 1 mL/min. The volume of injected specimen was 1 µl diluted in hexane with a splitless injection technique; ionization energy 70 eV, in electronic ionization mode; ion source temperature 200 °C; interface temperature was 300 °C and the scanning mass range of m/z 30-650. The constituents of essential oils were identified in comparison with their Kovats index, calculated in relation to the retention time of a series of lineary alkanes (C_4- C_{29}) with those of reference products and in comparison with their Kovats index with those of the chemical constituents gathered by Adams [20] and in comparison their spectre of mass with those gathered in a library of (NIST-MS Search Version 2.0).

Antioxidant activity

Although there are different methods to evaluate the antioxidant potential activity. The most used methods are DPPH (2,2-diphenyl-1-picrilhidrazil) and TAC (Total antioxidant capacity).

Scavenging of the free radical DPPH

The activity of scavenging DPPH radicals by essential oils was measured as described by Blois[21] with slight modifications. For the essential oils, 0.5 mL of various concentrations in methanol was added to 1.5 mL of a freshly prepared 0.004% methanol solution of DPPH. The blank was prepared by replacing the sample with methanol. After shaking, each mixture was incubated in the dark for 30 min at room temperature, and then the absorbance was measured at 517 nm in a Shimadzu 160-UV Spectrophotometer. Ascorbic acid was used as a positive control. The percentage of inhibition of free radicals DPPH was calculated by the following equation:

$$PI\ (\%) = (A_0 - A / A_0) * 100$$

Where; PI: Percentage of inhibition.
A_0: Absorbance of the control (containing all reagents except the test compound).
A: Absorbance of the test compound.
The IC_{50} is calculated graphically by linear regression of the graph.

Determination of total antioxidant activity

200 µl of the essential oils (diluted 10 times in methanol) was combined with 2 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), and the medium was incubated at 95 °C for 90 min. The optical density was measured at 695 nm in a Shimadzu 160-UV Spectrophotometer with a blank containing 200 µL of methanol in place of the extract. Ascorbic acid was used as standard, and the total antioxidant capacity was expressed in milligrams of ascorbic acid per gram of dry extract (Mg EAA/ g of extract).[22]

Antimicrobial activity evaluation

Growing medium

For the disc diffusion method, the Muller Hinton Agar MHA medium was used for bacteria, and for yeast the Yeast Pepton Glucose YBG medium was used. For the microdilution technique, the broths used are: YPG Broth for yeasts and Muller Hinton MHB Broth for bacteria. All media were autoclaved at 120°C for 20 min.[23]

Tested strains and inoculum standardization

The strains used in this work were conserved in -80°C, *Escherichia coli* ATCC29213 (G-), was obtained from the Hassan II Hospital Center, Fez, Morocco; *Pseudomonas aeruginosa* CIP 82.118 (G-), *Klebsiella pneumoniae* CIP A22 (G-), and *Staphylococcus aureus* ATCC6633 (G+), were obtained from the Microbiology Laboratory, Faculty of Medicine and Pharmacy, Fez, Morocco and the yeasts; *Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* ATCC9763 were obtained from the Faculty of Medicine and Pharmacy, Fez, Morocco. The microbial inoculum was prepared by the direct suspension method from 2-3 colonies of a fresh 24-hour culture which were aseptically collected and suspended in 0.9% sterile saline solution, turbidity was adjusted to 0.5 McFarland.[24] The bacterial suspensions contain approximately 1-2 x 10^8 CFU/mL, while the yeast suspension contains approximately 1-5 x 10^6 CFU/mL. The McFarland standard was prepared with a mixture of 99.5 mL of a 0.36N sulphuric acid (H_2SO_4) solution with 0.5 mL of a 0.048 M dehydrated barium chloride ($BaCl_2*2H_2O$) solution. The absorbance of the solution was verified with a UV-Visible spectrophotometer (Selecta, E. U) at λ= 625nm.[25]

Disc diffusion method

The plates containing the agar medium MH and YPG were inoculated with 1 mL of the bacterial and fungal suspensions, then the plates were dried for 10 min and after wards 6 mm sterile discs were deposited on the agar surface of the plates previously impregnated with 10 µL of the product to be tested; the essential oils of *H. tuberculatum*, ampicillin 1.67 mg/disc (AMP antibiotic), streptomycin 0.02 mg/disc (STR antibiotic) and fluconazole 5 mg/disc (FLU antifungal). Finally, the plates were incubated at 37°C for the bacteria and 30°C for the yeasts for 24 h. After incubation, the growth inhibition zones were determined in mm.[26,27]

Determination of the minimum inhibitory concentration (MIC)

For the MIC evaluation, we used the microdilution technique in 96-well microplates according to the standards of the Standards. N. C. for C. L.[28] First, the essential oil was diluted in DMSO. The AMP, STR, FLU and the microbial suspensions in 0.5 McFarland were diluted in the culture medium. Then, 50 µL of the culture medium was deposited

in each well of the microplate; MH for bacteria and YPG for yeast, except the first well (negative growth control), and 100 µL of the essential oil diluted was deposited in all wells except the last well (positive growth control). Then, microdilutions were made by transferring 50 µL from the first well to the second and so on (diluting the substance by a factor of ½ in each well). Finally the inoculation was carried out by depositing 50 µl of the microbial suspension whose turbidity was verified in the wells. The microplate was incubated under agitation for 24 h at 37°C for the bacteria and 30°C for the yeasts. To read the results, 20 µL of the 2,3,5-triphenyltetrazolium chloride (TTC) revealer, BIOKAR company, was added to all the wells, giving a pinkish coloration where there is growth due to the activity of the dehydrogenases after incubation for 2 hours.[24, 29]

Statistical analysis

The results were given as the average ± SD for three replicates for each sample. The IC50 of DPPH, TAC values were calculated by linear regression analysis using Microsoft Excel.

Results and Discussion

Yield and chemical composition of essential oils

After the hydrodistillation of the sample, an essential oils yield of 0.25% was obtained relative to the weight of the dry matter. This value is almost equal to that obtained by Sriti et *al.*[8] (0.24%) who studied a sample of *H. tuberculatum* of Tunisian origin. A lower yield than that obtained was reported in the study by Al-Burtamani et *al.*[30] (0.21%), and the study by Bergheul et *al.*[31] (0.101%), but El-naggar et *al.*[32] reported that *H. tuberculatum* collected in different places in Egypt presented a yield varied between 0.31% and 0.65%. And according to Sriti et *al.*,[8] this variation in the yield of essential oils can be attributed to environmental factors, stage of maturity, genetic factors, or major effects of geographical and ecological variations between habitats.

The analysis of the essential oils of *H. tuberculatum* by GC/MS and GC/FID allowed the detection of 50 compounds (Figure 1, Table 1), of which the sesquiterpenes represent 48%, the latter dominate over the oxygenated monoterpenes and the monoterpene hydrocarbons which represent a set of 46 %. Farnesyl bromide (37.02%) was the majority compound followed by santolina triene (10.26%), terpinene-4-ol (6.13%) and limonene (2.26%). The characteristic of the oils studied is its richness in sesquiterpenes, the thing which makes it different from other essential oils studied in other regions which are in their majority rich in oxygenated monoterpenes and monoterpene hydrocarbons. Thus, studies conducted on *H. tuberculatum* collected in Tunisia:[33] they showed that the essential oils of this plant was rich in monoterpenoids (20.7% for monoterpenic hydrocarbons, 46.7% for oxygenated monoterpenes and 0. 5% for sesquiterpene hydrocarbons), while on the other hand the study showed the richness of the essential oils of this plant in trans-p-menth-2-en-1-ol (18.23%), β-phellandrene (14.46%), piperitone (14.33%), cis-p-menth-2-en-1-ol (13.66%) and α-phellandrene (4.36%).[8]

Other studies were carried out on *H. tuberculatum* collected in Egypt[32], they revealed the richness of essential oils of five samples of this plant in monoterpene hydrocarbons in one group (3-carene (12. 6%) and oxygenated monoterpenes in 4 groups (cis-p-Menth-2- en-1-ol (7% -12.3), trans- p-Menth-2- en-1-ol (9.7% -14.1) and cis-piperitol (5.9% -10.4%). Thus, studies have shown the richness of this plant in compounds of monoterpene hydrocarbons and oxygenated monoterpenes (*α*-pinene (37.1%), cis-sabinol (8.0%), linalool (6.6%) and camphene (4.2%).[34] Other work reported on *H. Tuberculatum in* other regions, represents a chemical composition different from that of this study, like in Iran[35] where the major compound is limonene (27.3%) and α-pinene (21.9%), in United Arab Emirates [36] with α-phellandrene (10.7-32.9%) , in Turkey [37]linalool and *β*-caryophyllene.

The chemical composition of the essential oils of *H. tuberculatum* studied differs from that reported in previous studies. Among the factors influencing these differences are those attributed to geographical area and environmental factors.

Antioxidant activity

Evaluation of DPPH radical scavenging capacity by the *H. tuberculatum* essential oils showed a lower inhibition rate (IC_{50} = 3.23mg/mL) than that obtained using the positive control (ascorbic acid: IC_{50} = 2.81µg mL) (Fig. 2). These results are consistent with those of Debouba et *al.* [38] who reported that Antioxidant analyzes have shown that the essential oils of *H. tuberculatum* has a low iron-reducing power and a moderating activity for free radicals scavenging. In other works, the highest antioxidant activity was also exhibited by essential oils of *H. tuberculatum* at flowering and fruiting stages.[39]

Unlike the results obtained in the DPPH test, the measurement of the total antioxidant capacity revealed the presence of a significant amount of antioxidants in the oil studied (617.95 ± 0.589 mg EAA/g extract), this capacity is expressed in number of equivalents of ascorbic acid from a calibration curve ($y = 1.5033x + 0.0936$ $R^2 = 0.9931$).

The study of the *in vitro* antioxidant activity of essential of *H. tuberculatum* by the DPPH method and the measurement of TAC has shown that this oil has antioxidant activity. However, an evaluation of the antioxidant activity *in vivo* will be shown to be effective against free radicals, and some studies may be cited,[40] have calculated the antioxidant activity of *H. tuberculatum* essential oils by the determination of glutathione in the blood of diabetic rats induced by alloxan, and they showed that the reduced level of glutathione in diabetic rats was greatly restored by the essential oils of the aerial parts and flowers of *H. tuberculatum* compared to the positive control (vitamin E), so it could be considered a powerful antioxidant. In another study, Eissa et *al.*[34] showed that, under conditions of oxidative stress, this oil inhibited the production of ROS caused by H_2O_2, and thus protect the cells of the human astrocytoma U373-MG against these ROS.

Antimicrobial assay

Determination of the inhibition area

Measurement of the inhibition diameter showed the presence of an inhibition zone in all strains tested with essential oils (Figure 3). *S. aureus* appeared the most sensitive with a diameter of (24 mm), followed by the two yeasts (20 mm for *S. serevisiae* and 15 mm for *C. albicans*) and the Gram-negative bacteria were the least sensitive to the essential oils studied (Table 2). When compared with the use of standard antibiotics, he found that all bacterial strains were resistant to the Ampicillin and Streptomycin used, with the exception of S. *aureus* sensitive to Streptomycin. While the application of fluconazole to the yeasts gave slightly larger inhibition zones than those obtained with *H. tuberculatum* essential oils. Studies [30] showed that 10 µL (25 mg) pure oil partially inhibited the growth of *E. coli* ATCC 10031 (17.6 ± 0.3 mm), *C. albicans* ATCC 10231 (17.6 ± 0.3 mm), but the oils were ineffective against *K. pneumoniae* ATCC 27853 and *P. aeruginosa* ATCC 27853. Comparing these results with those we obtained, we can explain this difference by the variation in the chemical composition of the essential oils used.

Determination of the minimum inhibitory concentration (MIC)

The minimal inhibitory concentration of essential oil of this plant studied is more important on *S. aureus* (5.91 µg/mL), while, *E. coli* and *P. aeruginosa* were the least sensitive with a value of order of 94.6 µg/mL for each. The bacterial strains were resistant to ampicillin, but only *P. aeruginosa* was resistant to streptomycin (Table 3), this bacterium is known to develop resistance to many antimicrobial agents,[40] but appears to be sensitive to essential oils of *H. tuberculatum*. Both yeasts (*C. albicans* and *S. serevisiae*) were more sensitive than Gram-negative bacteria to the oils studied. In a study performed,[41] the microorganism most sensitive to essential oils from the aerial parts of *H. tuberculatum* was *E. coli* (CBDN 010052) inhibited with MICs of 3.9 µg/mL, *S. aureus* (CBDN 010028) was also inhibited by 7.81 µg/mL, but the essential oils were inactive against *C. albicans* (CBDN 05036) and *P. aeruginosa* (CBDN 010043).

There is no study confirming the antimicrobial effect of farnesyl bromide, the main constituent of essential oils studied (37%), but Bonifait *et al.*[42] reported that, the 2′,6′-dihydroxy-4′-

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geranyloxyacetophenone, oxyprenylated secondary metabolites extracted from plants belonging to the Rutaceae family, was synthesized by selective prenylation of 2,4,6-trihydroxyacetophenone at the 4′position with geranyl and farnesyl bromide, respectively. they demonstrated that the 2′,6′-dihydroxy-4′-geranyloxyacetophenone showed stronger antimicrobial activity against pathogens (*Streptococcus mutans, Streptococcus sobrinus, Prevotella intermedia, Porphyromonas gingivalis* and *C. albicans*).

The part of essential oils that exercise antimicrobial activity consists essentially of oxygenated terpenoids (alcohols and phenolic terpenes), while certain hydrocarbons also exhibit antimicrobial effects.[43] However, all these prevents only a combined action (synergy) of different majority and minority compounds at the origin of this essential oils.

Table 1: The relative percentage, chemical composition and the retention indices of identified compounds in the essential oils obtained from the aerial parts of *H. tuberculatum* collected from south-eastern morocco using GC-FID and GC-MS

Peak	Compound name	*RT (min)	**RI	Relative percentage (%)		Method of identification
				Area	Height	
1	α-pinene	12.74	934	0.89	0.88	RI, GC-MS
2	linalool	14.13	1085	0.17	3.51	RI, GC-MS
3	cis-ocimene	14.35	1021	0.13	0.79	RI, GC-MS
4	3-carene	14.61	1006	0.23	0.38	RI, GC-MS
5	santolina triene	16.12	1503	10.26	4.56	RI, GC-MS
6	andrographolide	16.55	1576	0.16	0.66	RI, GC-MS
7	camphor	17.43	1125	0.13	0.12	RI, GC-MS
8	Farnesene	17.81	1485	0.21	0.45	RI, GC-MS
9	alpha-elemol	18.77	1545	0.08	0.80	RI, GC-MS
10	borneol	18.95	1151	0.92	1.38	RI, GC-MS
11	α-terpinene	20.10	1050	0.13	4.58	RI, GC-MS
13	1.3-cineole	20.65	1016	0.18	1.39	RI, GC-MS
14	isoborneol	20.89	1042	0.08	0.56	RI, GC-MS
15	eugenol	21.60	1325	0.23	1.54	RI, GC-MS
16	caryophyllene	22.19	1430	0.24	1.12	RI, GC-MS
17	terpinene-4-ol	23.15	1162	6.13	4.58	RI, GC-MS
18	myrcene	23.35	982	0.08	0.56	RI, GC-MS
19	trans-p-mentha-2,8dienol	23.59	1125	0.08	0.78	RI, GC-MS
20	ç-elemene	25.24	1020	0.22	0.56	RI, GC-MS
21	thujopseme	25.58	1100	0.21	0.65	RI, GC-MS
22	caryophyllène oxide	25.99	1230	0.19	0.26	RI, GC-MS
23	naphthalene	26.61	1001	0.22	0.31	RI, GC-MS
24	humulen-(v1)	26.78	1024	0.08	2.61	RI, GC-MS
25	thujone	27.16	1101	0.20	0.80	RI, GC-MS
26	germacrene B	27.68	1472	0.94	3.10	RI, GC-MS
27	isoaromadendrene epoxide	28.22	999	0.12	0.16	RI, GC-MS
28	α-phellandrene	28.51	998	0.15	0.76	RI, GC-MS
29	sesquiphellandrene	29.17	1201	0.02	0.24	RI, GC-MS
30	azulene	29.56	887	0.19	0.26	RI, GC-MS
31	bornyl acetate-	30.10	1261	0.21	0.56	RI, GC-MS
32	farnesyl bromide	31.32	1025	37.02	3.58	RI, GC-MS
33	spathulenol	31.87	785	0.08	0.88	RI, GC-MS
34	1.8-cineole	32.86	1117	0.16	0.58	RI, GC-MS
35	epizonarene	33.12	689	0.02	0.17	RI, GC-MS
36	taus.-cadinol	33.44	756	0.18	0.28	RI, GC-MS

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37	himachalene	33.94	895	0.19	0.56	RI, GC-MS
38	ledol	34.18	1205	0.13	0.09	RI, GC-MS
39	octadecadien-1-ol	34.32	958	0.14	0.59	RI, GC-MS
40	alloaromadendrene oxide	34.57	1250	0.15	0.46	RI, GC-MS
41	limonene	35.02	1020	2.26	1.38	RI, GC-MS
42	longifolenaldehyde	35.29	1494	0.08	0.38	RI, GC-MS
43	elemene	37.14	1410	0.18	0.89	RI, GC-MS
44	taus.-muurolol	37.53	1210	0.19	0.17	RI, GC-MS
45	cadinol	38.19	1598	0.12	0.29	RI, GC-MS
46	spathulenol	39.69	1138	0.17	0.86	RI, GC-MS
47	retinal	42.48	1975	0.17	0.89	RI, GC-MS
48	octacosane	45.50	1402	0.17	0.88	RI, GC-MS
49	isoledene	48.77	1015	0.30	1.07	RI, GC-MS
50	p-cymene-8-ol	51.96	1140	0.68	1.15	RI, GC-MS
51	squalene	54.79	1430	0.18	0.87	RI, GC-MS
Total identified constituents (%)			65.79			
Yields (%v/w)			0.25			

*RT: Relative retention time obtained by chromatogram (Figure 1)
**RI: Relative retention indices calculated against homologous series of n-alkanes (C_4-C_{29}) on a TR5- CPSIL- 5CB column
The components are arranged in order to GC-FID elution on Varian CP9103 capillary column

Table 2: Results of the agar diffusion of the *H. tuberculatum* essential oils and standard antibiotics (mm)

		***H. tuberculatum* Essential oils**	**Ampicillin**	**Streptomycin**	**Fluconazole**
	Escherichia coli	10	0	0	NT
Bacteria	*Klebsiella pneumonia*	11	0	0	NT
	Pseudomonas aeruginosa	10	0	0	NT
	Staphylococcus aureus	24	0	9	NT
Yeasts	*Candida albicans*	15	NT	NT	21
	SaccharomyceSerevisiae	20	NT	NT	27

NT: Not tested

Table 3: Mic of the *H. tuberculatum* essential oils and standard antibiotics (µg/ml)

		***H. tuberculatum* essential oils**	**Ampicilline**	**Streptomycine**	**Fluconazole**
	Escherichia coli	94.6	R	25	NT
Bacteria	*Klebsiella pneumonia*	47.3	R	0.3	NT
	Pseudomonas aeruginosa	94.6	R	R	NT
	Staphylococcus aureus	5.91	R	0.62	NT
Yeasts	*Candida albicans*	11.82	NT	NT	40
	Saccharomyce Serevisiae	11.82	NT	NT	20

NT: Not tested; R: Resistant

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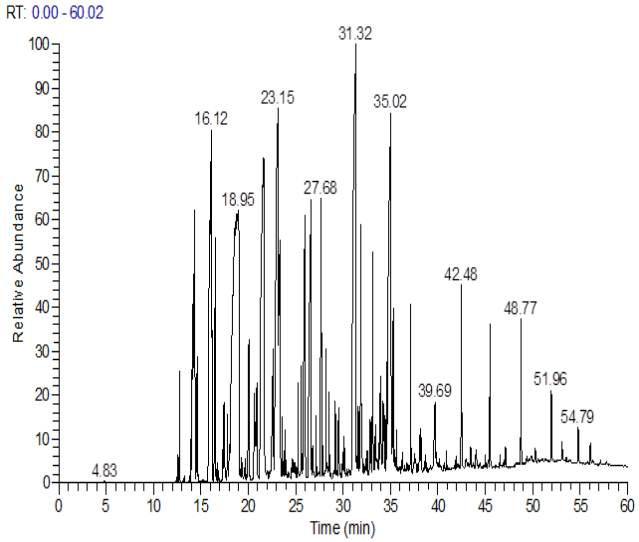

Figure 1: Chromatogram of *H. tuberculatum*

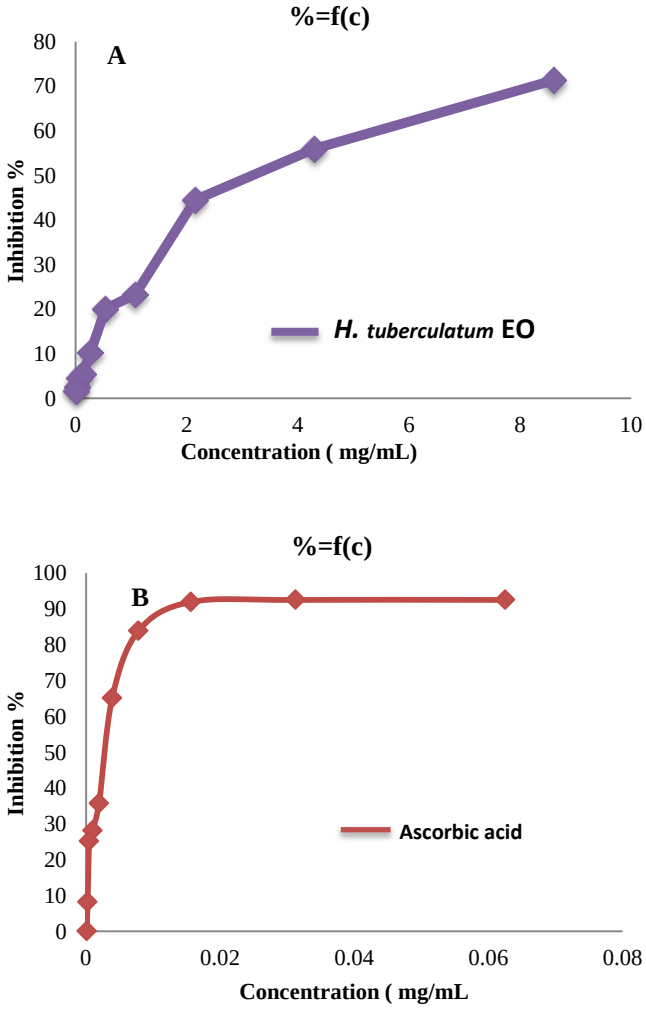

Figure 2: Results of the antioxidant test by the DPPH method for essential oils **(A)** and ascorbic acid **(B)**

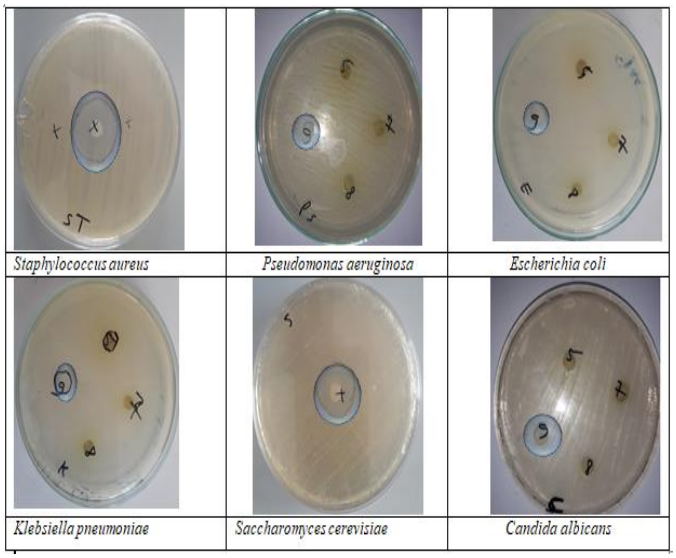

Figure 3: Antimicrobial activity of *H. tuberculatum* essential oils on agar medium

Conclusion

This study revealed the richness of *H. tuberculatum* essential oils in sesquiterpenes and confirmed its antioxidant and antimicrobial properties *in vitro*. The total antioxidant capacity test has shown that these essential oils have an interesting antioxidant capacity which can be one of the most effective solutions against oxidative stress. Its great antimicrobial power has been proven against 6 strains which have been shown to be sensitive to these essential oils. This power is relatively strong, with MIC values varying between 6.25 µg/mL and 100 µg/mL. The results of this study affirmed that these oils can be used as an antimicrobial alternative for strains resistant to conventional antibiotics. This study identified the need for further research on the bioactive mechanisms of natural extracts.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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