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Essential Oils from Leaves of *Juniperus thurifera* L., Exhibiting Antioxidant, Antifungal and Antibacterial Activities against Antibiotic-Resistant Microbes

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Citation: Lafraxo, S.; El Barnossi, A.; El Moussaoui, A.; Bourhia, M.; Salamatullah, A.M.; Alzahrani, A.; Ait Akka, A.; Choubbane, A.; Akhazzane, M.; Aboul-Soud, M.A.M.; et al. Essential Oils from Leaves of *Juniperus thurifera* L., Exhibiting Antioxidant, Antifungal and Antibacterial Activities against Antibiotic-Resistant Microbes. *Horticulturae* **2022**, *8*, 321. https://doi.org/10.3390/horticulturae8040321

Academic Editors: Dasha Mihaylova and Aneta Popova

Received: 3 March 2022
Accepted: 6 April 2022
Published: 10 April 2022

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Abstract: The antioxidant, antibacterial and antifungal properties of essential oils (EOs) of *Juniperus thurifera* L., a plant utilized in traditional, herbal medicine, were investigated. The EOs were extracted by use of a Clevenger apparatus and phytochemicals identified by gas chromatography coupled with mass spectrometry (GC/MS/MS). The antioxidant capacity of EOs of *J. thurifera* was determined by 2,2-diphenyl-1-picrylhydrazil (DPPH), total antioxidant capacity (TAC), and ferric reducing antioxidant power (FRAP). Antimicrobial activity of EOs of *J. thurifera* was determined against four fungal strains, *Candida albicans*; ATCC 10231, *Aspergillus niger*; MTCC 282, *Aspergillus flavus*; MTCC 9606 and *Fusarium oxysporum*; MTCC 9913 and four bacterial strains, *Staphylococcus aureus*; ATCC 6633, *Escherichia coli*; K12, *Bacillus subtilis*; DSM 6333, and *Pseudomonas aeruginosa*; CIP A22, by use of the disk diffusion method, and microdilution method used to determine the minimum inhibitory concentration (MIC). EOs of *J. thurifera* consisted of 31 compounds and were dominated by α-thujene (25%), elemol (12%) and muurolol (12%). Antioxidant activity recorded an IC_{50} of 24 ± 0.71 µg/mL (DPPF), EC50 of 0.19 ± 0.01 mg/mL (FRAP), and $9.3 \times 10^2 \pm 38$ mg EAA/g (TAC). The EOs of *J. thurifera* exhibited significant antibacterial activity against all bacterial strains under investigation, especially *P. aeruginosa*; CIP A22 with an inhibition diameter of 28 ± 1.5 mm and MIC of $4.8 \times 10^{-2} \pm 0.001$ µg/mL. EOs of *J. thurifera* also exhibited significant antifungal activity against *C. albicans*; ATCC 10231 and *F. oxysporum*; MTCC 9913 with an activity of 21 ± 2.1 mm, 32 ± 2.3%, and MIC of $9.5 \times 10^{-2} \pm 0.001$ Bioactive molecules found in EOs of *J. thurifera* could be used as an alternative solution to antibiotics available on the market to combat microbial resistance.

Keywords: juniper; natural products; tree; leaves; disease; bioactive compounds; microbial resistance

1. Introduction

Thuriferous juniper (*Juniperus thurifera* L.) is a monoecious, dioecious, conifer tree or shrub with scaly leaves of the cypress family (Cupressaceae) [1]. It plays an important role in the western Mediterranean basin and is regarded as a keystone species of low-temperature-adapted open woodlands, with steppe-like undercover [2]. In Morocco, the area of this species, which constitutes the upper limit of the forest in the Atlas Mountains, has been considerably restricted and, the vast majority of the stands have been degraded by over-exploitation and over-grazing, aggravated by an almost total absence of regeneration. Its current surface area, in the Atlas Mountains of Morocco, is estimated to be 20,000 ha [3,4]. Thuriferous juniper, with its extraordinary resistance, remarkable ability to withstand very severe climatic conditions, and indifference to the physical nature of soils, longevity of as much as 500 years, is unquestionably the predominant tree of the high Moroccan mountains [5]. Development of these natural plant resources is mainly based on extraction of essential oils (EOs), which are high value-added products, employed in the pharmaceutical, cosmetics and food industries [6–8]. The genus *Juniperus* contains essential medicinal plants with a long history of usage in traditional medicine. Its leaves are used to treat diabetes, diarrhoea, and rheumatism as a decoction [9]. Leaves and berries of *Juniperus* are utilized as an oral hypoglycemic medication [10], while leaves are used to treat bronchitis and as a diuretic [9]. Studies of the biological and biotechnological activities of the phytochemical compounds of plants is of interest and the antimicrobial activities of EOs have been reported [6,11–13]. These activities are attributed to oxygenated mono-terpenes [6]. Use of natural molecules to inhibit oxidation of fat, its consequences on health and its economic repercussions have been the subject of several studies [6,14]. Results of several studies of the antioxidant activities of EOs from a variety of aromatic plants have demonstrated that these properties are mainly ascribed to the presence of compounds containing hydroxyl group(s) [15–17]. Recently, the EOs and aqueous extracts of plants have attracted interest because of their richness in natural biologically active constituents including antioxidant, antimicrobial and insecticidal properties.

Under the existing restricted and inadequate arsenal of new therapies, the list of microorganisms that are becoming resistant to all commonly used antibiotics is growing, prompting the discovery of alternative classes of medications to prevent significant public health concerns, unconventional therapeutic interventions derived from natural resource exploitation have been intensively investigated [18,19]. Objectives of this study were to describe the chemical composition of the EOs of leaves of *J. thurifera* collected from the Jbel lakraa Massif in the Eastern, Middle Atlas of Morocco and to compare the results to those of previous studies and investigate the antioxidant, antibacterial and antifungal activities of the EOs, so that an evaluation of the economic value of the EOs of *J. thurifera* and their potential as replacements for antibiotics available on the market to combat microbial resistance could be conducted.

2. Materials and Methods

2.1. Extraction of EOs from J. thurifera

J. thurifera was harvested from the mountains (lat: 33.68093368; long: 4.30823143) during October 2021, which was autumn. Specimens were identified by a botanist in the department of biology, Faculty of Sciences-FSDM-USMBA-Fez, and the plant is deposited in the Herbarium under number (FJT/02D20). Leaves were cleaned and subsequently dried at 35 °C for 72 h in a ventilated oven. Dried leaves were crushed with an electric blender, then EOs extracted by hydro-distillation on a Clevenger-type extractor [15]. Briefly, 200 g of the ground leaf material was mixed with 750 mL distilled water (dH_2O) and extracted for about 120 min. Samples were partitioned into hexane (10%). At least three replicates were performed in this study.

2.2. GC/MS/MS Analysis of EOs

Constituents of EOs were identified and quantified by use of gas chromatography (TQ8040 NX; Shimadzu, Tokyo, Japan) attached to a triple quadrupole, tandem mass spectrometer (GC-MS). Chromatography was conducted on an apolar, capillary column RTxi-5 Sil MS column (30 m × 0.25 mm ID × 0.25 µm). Helium was used as carrier gas and the injection volume was 1 µL. Temperatures of the source and the interface were 200 °C and 280 °C, respectively. The chromatographic system was programmed with splitless injection (split opening at 4 min), injection temperature of 250 °C and pressure of 37.1 kPa. Temperature was programmed with an initial temperature of 50 °C for 2 min, ramp 1 was 5 °C/min to 160 °C for 2 min and ramp 2 was 5 °C/min to 280 °C for 2 min. Identification of phytochemicals in EOs was conducted by comparing the obtained retention indices with those of chemical compounds in the literature database [20].

2.3. Antioxidant Activity

2.3.1. DPPH Test

Antioxidant activity was determined by use of the DPPH assay according to previously published method [21]. Briefly, 800 µL of a methanolic solution of DPPH (0.2 mM) was mixed with 200 µL of different dilutions of EOs of *J. thurifera* (0–1 mg/mL), and subsequently incubated in the dark at RT for 30 min. Absorbances of samples were recorded at 517 nm and compared to those of a control consisting of 800 µL of DPPH solution. Samples, positive controls, quercetin or BHT were prepared under the same operating conditions. Decay of absorbance was measured with a spectrophotometer and percent inhibition (I%) calculated (Equation (1)).

$$I\ (\%) = [(T0 - Tx)/T0] * 100 \quad (1)$$

By performing kinetics of this activity, concentrations corresponding to 50% inhibition (IC_{50}), expressed as µg/mL, were determined, where the least IC_{50} corresponds to the greatest efficiency of EOs.

2.3.2. TAC Test

Antioxidant activity was determined by placing 100 µL of EOs at various concentrations after adding 1000 µL of a reagent composed of 0.6 M H_2SO_4, 28 mM Na_2PO_4 and 4 mM $(NH_4)_2MoS_4$. Then, the tubes were tightly closed and incubated at 95 °C for 90 min. After cooling, absorbances were measured at 695 nm. The negative control consisted of 100 µL of methanol after the addition of 1000 µL of the above reagent [22]. The samples and controls were incubated under the same conditions. The obtained results were represented in mg ascorbic acid equivalents per gram (mg EAA/g).

2.3.3. FRAP Test

Reducing power was recorded by placing 200 µL of sample at several concentrations, into 500 µL of 0.2 M phosphate buffer (pH = 6.6), followed by 500 µL of 1% $K_3Fe\ (CN)_6$ in dH_2O. Mixtures were subsequently placed into a water bath and incubated at 50 °C for 20 min. Next, about 500 µL trichloroacetic acid (TCA, 10%) was added followed by centrifugation. A 500 µL aliquot of the supernatant was transferred to another tube followed by the addition of 500 µL of dH_2O and 100 µL of freshly-prepared $FeCl_3$ (1%) in dH_2O. Similarly, a blank without sample was included by replacing EOs of *J. thurifera* with methanol. Absorbances of reaction media were recorded at 700 nm and compared to the methanol blank, which allowed calibration of the apparatus (UV-VIS spectrophotometer). Positive controls were a solution of the standard antioxidants BHT and quercetin [23].

2.4. Antimicrobial Activity of EOs of J. thurifera

2.4.1. Microbial Strains Tested

Antimicrobial activity of *J. thurifera* EOs against four fungal strains, *Candida albicans*, ATCC 10231; *Aspergillus niger*, MTCC 282; *Aspergillus flavus*, MTCC 9606 and *Fusarium*

oxysporum, MTCC 9913 and four strains of bacteria, *Staphylococcus aureus*, ATCC 6633; *Escherichia coli*, K12; *Bacillus subtilis*, DSM 6333 and *Pseudomonas aeruginosa*, CIP A22. The fungal and bacteria strains were provided by Sidi Mohammed Ben Abdellah University (Fez, Morocco) and Hassan II University Hospital (Fez, Morocco), respectively.

2.4.2. Assessment of Antimicrobial Activity

The antimicrobial activity of *J. thurifera* EOs was determined by use of the disc diffusion method [24]. Petri dishes containing Mueller–Hinton (MH) and Malt Extract (ME) culture media were inoculated with the four bacterial strains and *C. albicans*, respectively, by the double-layer method, from cultures freshly grown in MH and ME medium, decimal dilutions were made in sterile saline (0.9%) until turbidity of 0.5 McFarland (10^8 CFU/mL) was reached, 100 µL were added to tubes containing 5 mL of soft agar (0.5% agar), then the inoculated tubes were spread in Petri dishes containing MH and ME medium. For *A. niger*, *A. flavus*, and *F. oxysporum* the antifungal activity was determined by the direct confrontation method in the ME medium. Sterile 6 mm Whatman paper discs were positioned into the centre of the petri dish and then impregnated with 20 µL of *J. thurifera* EOs, and also with conventional antimicrobial drugs; streptomycin and erythromycin for bacterial strains and fluconazole for fungal strains according to the methodology of the European Committee for Antimicrobial Susceptibility Testing (EUCAST). Then, bacteria- and fungi-inoculated dishes were incubated at temperatures of 30 °C and 37 °C optimal for the bacterial and fungal strains and *C. albicans*, respectively. Inhibition diameters and percentages of inhibition were calculated 18–24 h post inoculation (hpi) for the bacterial strains and after 24–48 hpi for *C. albicans*, and 7 days post inoculation for *F. oxysporum*, *A. niger* and *A. flavus* [24,25].

2.4.3. Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentrations (MIC) of *J. thurifera* EOs against the four bacterial and four fungal strains were determined by use of the microdilution as previously described [25]. Briefly, a sterile 96-well microplate was used and 50 µL of sterile MH or ME medium was added for bacterial and fungal strains, respectively. Serially diluted EOs of *J. thurifera* at a volume of 100 µL prepared in 10% (*v*/*v*) DMSO was pipetted into the first row. This was followed by the addition of 30 µL of microbial strains. Plates were incubated for 24 h, 48 h or 7 d for bacteria, *C. albicans* and fungi (*Fusarium oxysporum*, *A. niger*, *A. flavus)*, respectively; at 37 °C or 30 °C [25,26]. Each well received 20 µL of water 2,3,5-triphenyl tetrazolium chloride solution (0.2%) to visualize bacterial growth. MIC was defined as the least concentration that did not create a red colour [26].

2.5. Statistical Analyses

Results were expressed as means of triplicates ± SD (standard deviation). Shapiro–Wilks test was employed to determine the normality of distribution, while the *t*-test was used to check for homogeneity of variances. Analysis of variance (ANOVA) was performed, with Tukey's HSD test, as a post hoc test for multiple comparisons. Differences were considered significant at probability level (p) < 0.05.

3. Results and Discussion

3.1. Identification of Chemicals Comprising EOs of J. thurifera by GC/MS

The yield of EOs of 0.96%, from leaves of *J. thurifera* provided was greater than that reported previously [27]. Essentially, all of the mass of the EOs of *J. thurifera* (99.99%) was accounted for by 31 phytochemical compounds (Table 1 and Figure 1). Previously, 99.46% of the mass of EOs of *J. thurifera* was reported to be accounted for by 24 compounds [28]. The phytochemical composition of the EOs of *J. thurifera* is dominated by α-thujene (25%), elemol (12%) and muurolol (12%) (Figure 2). The chemical composition of EOs of *J. thurifera* was quantitatively and qualitatively different from that reported previously [27]. In another recent study β-pinene (36%) were determined to be the predominant compounds in EOs of *J. thurifera*, whereas in this study β-pinene accounted for only 1.9% of the mass of EOs.

Table 1. Phytochemical compounds identified by GC/MS/MS in EOs of *J. thurifera*.

Peak	Retention Time	Name	KI		Area (%)
			Calculated	Literature	
1	7.666	Sabinene	975	969	0.95
2	7.886	α-Pinene	939	948	3.09
3	9.031	α-Thujene	930	897	24.98
4	9.487	β-Pinene	979	972	1.94
5	10.096	3-Carene	1011	948	3.39
6	10.554	o-Cymene	1024	1042	4.45
7	10.707	Limonene	1029	1018	0.37
8	11.583	γ-Terpinene	1059	998	0.71
9	11.927	Sabina ketone	1120	1041	1.10
10	12.416	Terpinolene	1088	1052	0.52
11	12.798	Linalool	1090	1082	3.66
12	12.855	Thujanol	1138	1041	1.05
13	13.565	Dihydro carveol	1193	1109	0.44
14	15.258	Limonen-10-ol	1289	1137	12.39
15	15.647	α-Terpineol	1017	1143	0.92
16	17.170	Linalool acetate	1257	1272	1.97
17	18.925	Decadienal	1293	1274	0.38
18	19.176	p-Methylacetophenone	1446	1113	0.42
19	19.865	β-Terpineol	1144	1333	1.04
20	23.828	α-Muurolene	1500	1440	0.66
21	24.199	Selinene	1498	1435	1.02
22	24.317	γ-Cadinene	1513	1469	2.79
23	25.106	Elemol	1549	1522	12.20
24	26.748	β-Oplopenone	1607	1540	0.85
25	27.565	α-Eudesmol	1607	1626	2.43
26	27.830	Cadinol	1640	1580	1.81
27	27.886	Muurolol	1642	1580	11.58
29	28.403	Ethyl pentanoat	901	1068	0.52
30	31.385	Geranyl propanoate	1477	1536	1.32
31	32.545	Cymene	1426	1569	1.04
		Total			99.99%

3.2. Antioxidant Activity

When antioxidant activities of EOs of *J. thurifera* were evaluated by three methods (Figure 3a), the percentage of inhibition of free radical (DPPH) was directly proportional to the concentrations of the EOs of *J. thurifera*. For a concentration of 13 µg/mL of EOs of *J. thurifera*, the percentage of DPPH inhibition was 85 ± 0.24% and for a concentration of 27 µg/mL the percentage of inhibition was approximately 91 ± 0.17% (Figure 3a). Antioxidant capacity was determined from the IC_{50}, which is the concentration necessary to reduce 50% of the DPPH radical. The smaller the IC_{50} value, the greater the antioxidant activity of a compound [29]. Free radical activities of EOs of *J. thurifera*, BHT and quercetin revealed that IC_{50} of EOs of *J. thurifera* is of the order of 23.6 ± 0.71 µg/mL,

14.2 ± 0.14 µg/mL and 15.9 ± 0.56 µg/mL (Figure 3b), respectively. Evaluation of antioxidant capacity by use of the FRAP method revealed that the effective concentration (EC-50) is in the range of 0.19 ± 0.01 mg/mL (EOs of *J. thurifera*), 3.6 × 10^{-2} ± 0.003 mg/mL (BHT) and 2.8 × 10^{-2} ± 0.002 mg/mL (quercetin) (Figure 4a). Total antioxidant capacity (Figure 4b) of EOs of *J. thurifera* was 9.3 × 10^{2} ± 38 mg EAA/g versus 5.3 × 10^{2} ± 22 mg EAA/g (BHT) and 6.6 × 10^{2} ± 46.67 mg EAA/g (quercetin).

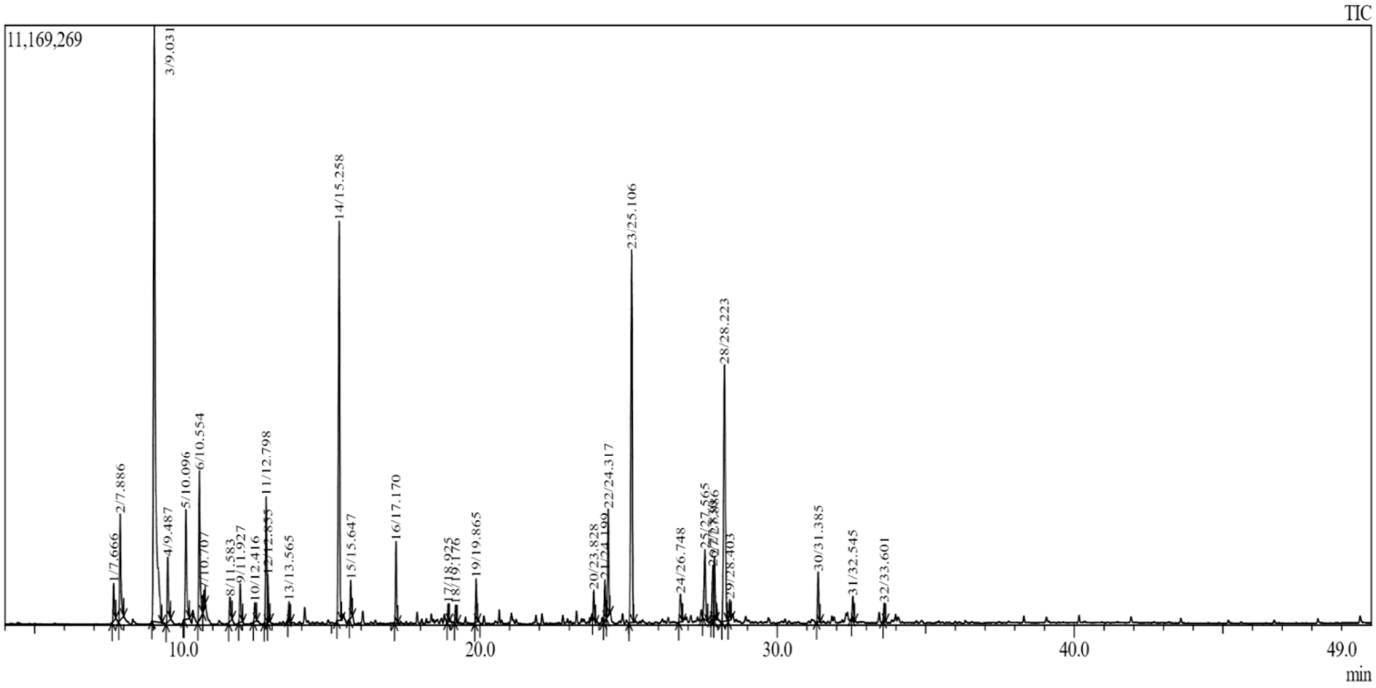

Figure 1. Chromatograph of compounds identified by GC/MS in EOs of *J. thurifera*. Peaks represent absolute abundances, whereas numbers on the x-axis represent retention times in min.

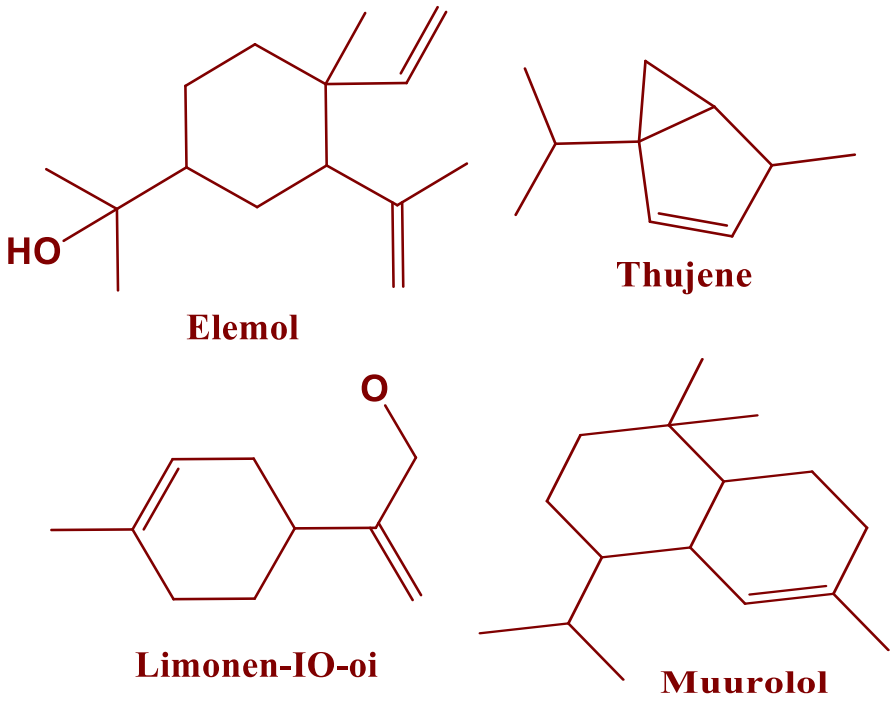

Figure 2. Molecular structures of phytochemical compounds in EOs of *J. thurifera*.

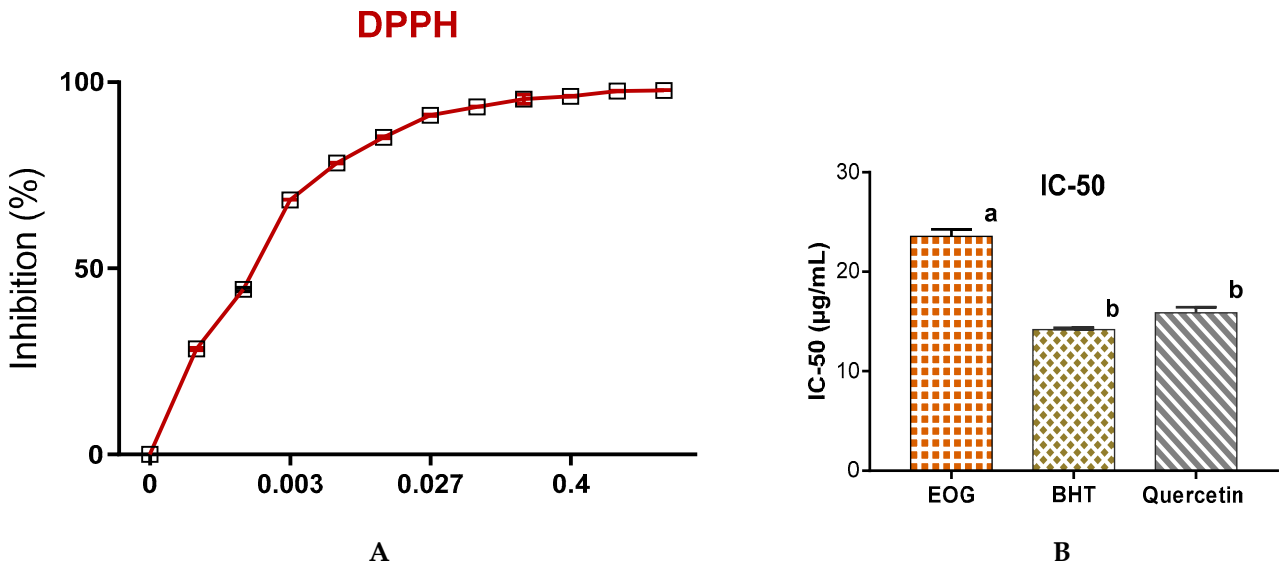

Figure 3. Antioxidant activities of EOs of *J. thurifera* by the DPPH method (**A**) and the concentration of IC-50 (**B**). Bars with the same letters do not differ significantly ($p < 0.05$).

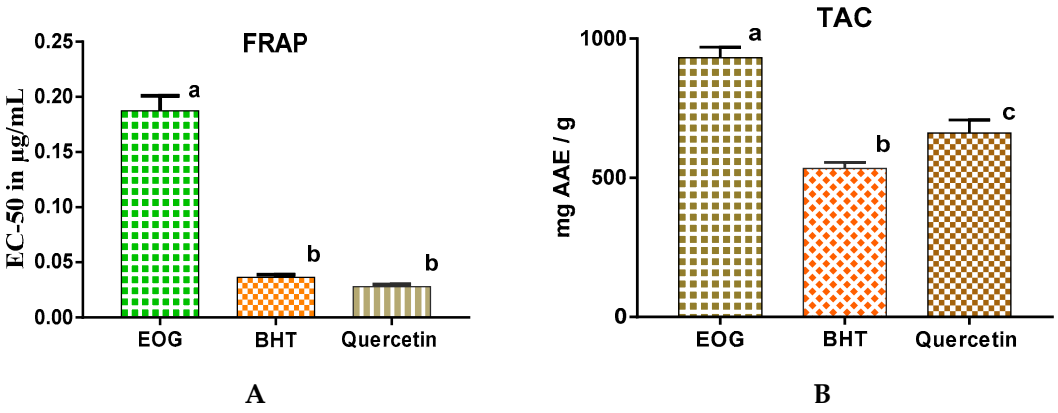

Figure 4. Antioxidant activities of EOs of *J. thurifera* by the FRAP method (**A**) and antioxidant capacity of EOs of *J. thurifera* (**B**). Bars with the same letters do not differ significantly ($p < 0.05$).

Assessment of oxidative stress (OS, oxidation in vivo) has become important since this type of oxidation can be involved in several health effects including rheumatoid arthritis, atherosclerosis, diabetes, aging and cancer [30–32]. Natural antioxidants present in plant extracts and EOs can provide protection against OS by two main mechanisms, namely scavenging reactive oxygen species (ROS) and blocking lipid peroxidation [33,34]. There is a correlation between the antioxidant power of EOs and its phytochemical composition. In this context, it has been previously documented that the antioxidant capacities of EOs are associated with their phytochemical composition via the hydroxyl function present in their constituents, and the richer an oil is in phenolic compounds and terpenes, the more effective its antioxidant capacity [35–40].

The phytochemical profile of EOs of *J. thurifera* (Table 1) revealed that EOs of *J. thurifera* are rich in terpenic compounds, such as thujene, γ-terpinene, cymene and linalool which are known for their antioxidant potentials [41]. Recent studies have shown that sabinene is an antioxidant compound [42]. Similarly, cymene possesses a potent anti-nociceptive behavior although it exhibited lesser antioxidant potential [43].

3.3. Antimicrobial Activity of J. thurifera EOs

3.3.1. Antibacterial Activity of *J. thurifera* EOs

EOs extracted from leaves of *J. thurifera* exhibited antibacterial activity in comparison with the concentration used and which antibiotic was used, streptomycin sulphate or erythromycin especially against *P. aeruginosa* CIP A22 with an inhibition diameter of 27.67 ± 1.53 mm and a MIC of 0.0475 ± 0.00 µg/mL, against *S. aureus*, ATCC 6633 with an inhibition diameter of 20.33 ± 0.58 mm and a MIC of 0.095 ± 0.00 µg/mL, against *E. coli* K12 with an inhibition diameter of 15.67 ± 3.05 mm and a MIC of 0.095 ± 0.00 µg/mL and against *B. subtilis* DSM 6333 with an inhibition diameter of 14.33 ± 1.15 mm and a MIC of 0.095 ± 0.00 µg/mL (Table 2).

Table 2. Antibacterial activity of *J. thurifera* EOs in comparison with the antibiotics streptomycin and erythromycin.

		Staphylococcus aureus **ATCC 6633**	***Escherichia coli*** **K12**	***Bacillus subtilis*** **DSM 6333**	***Pseudomonas aeruginosa*** **CIP A22**
EOs of *J. thurifera*	Antibacterial activity (mm)	20.33 ± 0.58 [a]	15.67 ± 3.05 [b]	14.33 ± 1.15 [b]	27.67 ± 1.53 [c]
	MIC (µg/mL)	0.095 ± 0.00 [a]	0.095 ± 0.00 [a]	0.095 ± 0.00 [a]	0.0475 ± 0.00 [b]
Streptomycin	Antibacterial activity (mm)	11 ± 1.00	Rs	Rs	Rs
	MIC (µg/mL)	1.56 ± 0.00	-	-	-
Erythromycin	Antibacterial activity (mm)	Rs	Rs	Rs	Rs
	MIC (µg/mL)	-	-	-	-
DMSO 10%	Antibacterial activity (mm)	Rs	Rs	Rs	Rs

Mean values (±SD, n = 3) followed by different letters in the same row are significantly different according to a mean analysis (Student t-test) and an analysis of variance (One-way ANOVA; Tukey's test, $p \leq 0.05$). MIC: minimum inhibitory concentration; Rs: resistance.

Antibacterial activity of *J. thurifera* EOs might be due to their chemical composition, *J. thurifera* EOs are rich in terpene compounds, especially thujene, γ-terpinene, cymene, and linalool which are well known for their antibacterial activity [41]. They are also rich with sabinene and cymene which are compounds with antibacterial activity [44]. Results of the study reported here were different from results of a previous study [44], which indicated that extracts of *J. thurifera* L. leaves growing in eastern Algeria were active only against *S. aureus*, ATCC and methicillin-resistant *S. aureus* bacteria and the greatest activity with an inhibition diameter of 14 mm for a concentration of 1 g/mL. However, no inhibition was detected for extracts against *E. coli* ATCC or *P. aeruginosa* ATCC. However, the antibacterial activity of extracts of leaves of *Juniperus phoenicea* L was observed against both Gram-positive and Gram-negative bacteria [45]. *J. thurifera* EOs exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria, especially against *S. aureus*, *E. coli*, and *P. aeruginosa* with inhibition diameters of 31.12 ± 3.11, 13.23 ± 2.59, and 18.27 ± 2.29 mm, respectively [46]. Those results were similar to those observed in the study, the results of which are presented here for *E. coli*, but are the opposite of the results for *S. aureus* and *P. aeruginosa*, for which the greater antibacterial activity might have been due to the different physicochemical composition of the EOs observed in the study reported here, which are consistent with results of several other studies [47], which found *S. aureus* was sensitive to the EOs of *J. thurifera* from Algeria. Furthermore, two strains of *Pseudomonas* proved to be resistant, [28]. In that study, the EOs from twigs of *J. thurifera* collected in the Eastern range of the Middle Atlas Mountains of Morocco exhibited significant antibacterial activity against *E. coli*, *B. subtilus*, *M. luteus*, and *S. aureus.* Similarly, EOs of *J. thurifera* had significant antibacterial activity against *S. aureus*, ATCC 33862 with an inhibition diameter of 27 mm and MIC of 450 µL/mL, against *E. coli*, ATCC 25922 with an inhibition diameter

of 25.6 mm and MIC of 530 µL/mL and against *P. mirabilis*, ATCC 7002 with an inhibition diameter of 18.8 mm and MIC of 930 µL/mL [48].

3.3.2. Antifungal Activity of *J. thurifera* EOs

When compared with the fungicide fluconazole in the in vitro evaluation of antifungal activity of *J. thurifera* EOs against *A. niger*, *A. flavus*, *F. oxysporum*, and *C. albicans* in the disc diffusion test, these EOs exhibited significant activity against *F. oxysporum*, MTCC 9913 with percent inhibition of 32.47 ± 2.25 and MIC values of 0.095 µg/mL as well as with an inhibition diameter of 21.33 ± 2.08 mm and a MIC value of 0.095 µg/mL against *C. albicans*; ATCC 10231 (Table 3), In addition, *J. thurifera* EOs exhibited antifungal activities against *F. oxysporum* and *C. albicans*. However, *J. thurifera* EOs did not exhibit antifungal activity against *A. niger* or *A. flavus*. The antifungal activity of *J. thurifera* EOs may be mainly due to their chemical composition, *J. thurifera* EOs are particularly rich in thujene, pinene, and limonene which are well known for their antimicrobial activity, especially antifungal activity [16,17].

Table 3. Antifungal activity of *J. thurifera* EOs in comparison with fluconazole.

		Candida albicans **ATCC 10231**	***Aspergillus niger*** **MTCC 282**	***Aspergillus flavus*** **MTCC 9606**	***Fusarium oxysporum*** **MTCC 9913**
EOs of *J. thurifera*	Antifungal activity	21.33 ± 2.08 mm [a]	0.00 ± 0.00% [b]	0.00 ± 0.00% [b]	32.47 ± 2.25% [c]
	MIC (µg/mL)	0.095 ± 0.00 [a]	-	-	0.095 ± 0.00 [a]
Fluconazole	Antifungal activity	Rs	8.20 ± 2.02% [a]	Rs	30.77 ± 0.58% [b]
	MIC (µg/mL)	-	7.125 ± 0.00 [a]	-	3.125 ± 0.00 [b]
DMSO 10%	Antifungal activity	Rs	Rs	Rs	Rs

Mean values (±SD, n = 3) followed by different letters in the same row are significantly different according to a mean analysis (Student t-test) and an analysis of variance (One-way ANOVA; Tukey's test, $p \leq 0.05$). MIC: minimum inhibitory concentration; Rs: resistance.

Several studies have been devoted to the control of pathogenic and phytopathogenic fungi in general, and *A. niger*, *A. flavus*, *F. oxysporum* and *C. albicans* in particular, through the use of various bioactive substances, either natural or synthetic. The results of this study are opposite of those of another study [49], in which sesquiterpenes of *J. thurifera* EOs did not present any antifungal activity against *C. albicans* CECT;1394. Similarly, in another study [28] the EOs of *J. thurifera* twigs collected from the Eastern sector of the Middle Atlas Mountains of Morocco exhibited antifungal activities against *A. niger*, *Penicillium expansum*, and *Penicillium digitatum*. The results of the study presented here are consistent with those focused on substances of bacterial and fungal origin [24], which reported an isolate from *Bacillus* sp. Gn-A11-18 exhibiting antifungal activity of 31.33 ± 0.58 mm against *C. albicans*; ATCC 10231 and a percentage of inhibition of 29.66 ± 0.57% against *A. niger.* Similarly, results of another study [50] showed significant antifungal activity mainly against *Alternaria alternata*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani* and *Verticillium dahlia* with percentage inhibitions ranging from 24 to 92.1%.

4. Conclusions

The results of this study indicated that the EOs extracted from *J. thurifera* had excellent antioxidant and antimicrobial potencies against clinically important drug-resistant microbes. These results are intriguing since they suggest that EOs extracted from *J. thurifera* could potentially be used as an alternative to traditional antioxidant antimicrobial treatments. However, prior to any prospective application of the studied EOs as natural medicines to control microorganisms, evaluation of the potential side effects on non-target organisms along with pre-clinical and clinical works on non-human primates and humans will be required.

Author Contributions: Conceptualization: S.L., A.E.B., A.E.M. and M.B.; formal analysis: S.L., A.M.S. and A.A.; investigation: S.L., A.A.A., A.C., M.A., A.M.S. and A.A.; methodology: A.M.S. and A.A.; supervision: A.M.S., A.A. and A.B.; writing original draft, and editing: M.B., S.L., A.M.S., A.A., M.A.M.A.-S. and J.P.G.; visualization: M.A.M.A.-S.; validation: J.P.G. and M.A.M.A.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Researchers Supporting Project number (RSP-2022R437), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data reported here is available from the authors upon request.

Acknowledgments: The authors extend their appreciation to the Researchers Supporting Project number (RSP-2022R437) King Saud University, Riyadh, Saudi Arabia. Giesy was supported by a Discovery Grant from the Natural Science and Engineering Research Council of Canada, the Canada Research Chair program and a Distinguished Visiting Professorship in the Department of Environmental Sciences, Baylor University, and Waco, TX, USA.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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