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Antifungal activity of *Myrtus communis* and *Zygophyllum album* extracts against human pathogenic fungi

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ABSTRACT: Fungal infections have been increasing in recent years due to a growing number of high-risk patients, particularly immunocompromised hosts. Currently, medicinal plants are known for their properties due to their essential oils and phenolic compounds. They have been empirically used as antimicrobial agents. So the composition of the phenolic extracts and essential oils of *Myrtus communis* and *Zygophyllum album* and their antifungal activity on *Candida albicans*, *Aspergillus fumigatus* fungal strains were studied. In this fact, essential oils from the aerial parts of the plant were obtained by hydrodistillation and analyzed by GC and GC-MS, for the phenolic extracts, several extraction methods with a preliminary phytochemical study were applied. The oils showed high contents of α-pinene and cineol for *M. communis* and verbenone and caryophyllene for the *Z. album*. The MIC and minimal lethal concentration were used to evaluate the antifungal activity against *Candida* and *Aspergillus* strains. Results showed that *M. communis* and *Z. album* essential oil and phenolic extracts exhibited a significant activity against clinically relevant fungi, a significant antifungal activity of the two extracts studied (MCA and ZAM) was observed on *C. albicans* of these, two extracts, MCA was found to be most active with an MFC value of 25 mg/ml versus 100 mg/ml for ZAM. Nevertheless, the essential oils exhibited stronger antifungal activity than the phenolic extracts. The present study indicates that the two medicinal plants have considerable antifungal activity, deserving further investigation for clinical applications.

Keywords: *Myrtus communis*; *Zygophyllum album*; Essential oils; Phenolic extracts; Antifungal activity.

1. INTRODUCTION

Fungal infections have been increasing in recent years due to a growing number of high-risk patients, particularly immunocompromised hosts. *Candida* is the third- or fourth-most-common isolate in nosocomial bloodstream infections in the world [1]. Also, candidiasis caused by *Candida albicans* which is an acute or chronic, superficial or deep infection with a very wide clinical spectrum. Candidiasis occurs mostly in patients who are predisposed to an overgrowth of their yeast flora. Oropharyngeal candidiasis occurs in patients with diabetes mellitus, those receiving antibacterial antibiotics and those infected with HIV [2].

On the other hand, aspergillosis is an infection caused by *Aspergillus*, a common mold (a type of fungus) that lives indoors and outdoors. Most people breathe in *Aspergillus* spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to *Aspergillus*. The types of health problems caused by *Aspergillus* include allergic reactions, lung infections, and infections in other organs.

Despite the introduction of new antifungal drugs, they are limited in number. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies [3]. Medicinal plants have been widely used in folk medicine. It is known that most of their properties are due to their volatile oils and phenolic compounds. Essential oils and phenolic extracts from many plants are known to possess antifungal activity [4], but only limited information exists about activity toward human fungal pathogens. They have been empirically used as antimicrobial agents, but the mechanisms of action are still unknown.

Based on this features the objective of this study was to evaluate the antifungal activity of phenolic crude extracts and essential oils from *Myrtus communis* and *Zygophyllum album* growing in Algeria.

2. MATERIALS AND METHODS

2.1. Plant material

Myrtle (*M. communis* var. *italica* L.) aerial parts were collected at the flowering stage in July 2014 from the Honaine region (North East of Tlemcen-west of Algeria. In the case of *Z. album*; fresh aerial parts were collected in August 2014 from SidiKhouiled region (Sahara of Ouargla).

The sampling was done by a randomized collection of 15–20 shrubs and sub-shrubs in an area of about 200 m^2 each. Myrtle leaves and areal parts of *Z. album* were isolated manually in our laboratory to obtain a weight of 500–700 g of each part. Botanical identification of this species was carried out according to the Africain flowering plants database and by local experts [5].

2.2. Fungal strain

The antifungal activity of different extracts of plants was evaluated against clinical *Candida albicans* (isolated from a patient's oral candidiasis) and *Aspergillus fumigatus* (isolated from lemon juice). The fungal strains were isolated and identified by J. Gabaldon in the Laboratory of Food Science, Environmental Science, Plant Protection and Animal Health, Catholic University of Murcia, Spain by standard microbiology methods and stored in Sabouraud dextrose broth with glycerol at −40 °C.

2.3. Essential oil isolation

The plant samples were separate water distilled in a Clevenger type apparatus for 3 h (time fixed after a kinetic survey during 30, 60, 90, 120, 150, 180 and 210 min) so 100 g of leaves and flowers of the plant was boiled. When the temperature stabilizes, the distillate was collected. Then, the mixture was placed in a separating funnel and three successive washes of cyclohexane were achieved. After agitation, the organic phase was recovered. The concentration of the organic phase was achieved by a rotary evaporator to obtain the essential oil. According to the method recommended by the French standard method [6], all experiments were done in triplicates and results were expressed based on dry matter weight. The essential oil was stored at +4°C after the calculation of the extraction yield.

2.4. Polyphenols extraction

The plant materials were dried at ambient temperature and stored in a dry place before use. The plant was washed well with water, dried at room temperature in the dark, and then ground in an electric grinder to give a coarse powder. In this study, samples were extracted by decoction (10%), maceration with ethanol (80%) and by extraction with solvents of increasing polarity (dichloromethan and methanol/soxhlet) methods [7, 8].

2.5. Essential oil analysis

This analysis was carried out at the THERA-FRE-CNRS Group 3517, Faculty of Pharmacy, University of Picardie, Amiens, France by P. Sonnet. The chromatographic analysis of the HE was carried out with a SHIMADZU gas chromatograph (QP2010SE) coupled to a mass spectrometer (HP 5973).

The stationary phase is a capillary column (SGE of type BPx5 C) of 50 mm in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm while the temperature of the column is programmed to 50°C at 250°C at a temperature of 4°C min^{-1}. The carrier gas is helium whose flow rate is set at 1.5 ml min^{-1}. The mode of injection is in the split mode (leakage ratio: 1/70), that is to say, 1 µl of the substance to be analyzed was produced using a micro-syringe. The device is connected to a computer system managing a NIST 98 mass spectrum library and piloted by an "HP Chem Station" software to monitor the evolution of chromatographic analyzes. The identification of the compounds was obtained by comparing retention times, retention indices and mass spectra with those of the studies carried out on the plants of the same family.

2.6. Phytochemical screnning by colometer method

All plant extract were tested for the presence of different families of compounds according to methods previously described by [9, 10].

2.7. Evaluation of the antifungal activity by diffusion method on agar

It is a method that was proposed and standardized in 2004 by CLSI [11]. The recommended culture medium is Sabouraud, supplemented with 2% glucose and 0.5 mg/ml of methylene blue, which produces visible zones of inhibition [12].

The Petri dishes containing Sabouraud agar added with 2% glucose are inoculated aseptically by swab, and then dried in the vicinity of the flame. Discs of 6 mm in diameter are prepared using glass microfibre filters and sterilized by autoclaving at 120°C for 20 minutes. The latter is then impregnated with 10 µl of the various substances (200 mg/ml for ME and 500 µl/ml for EO) and placed on the agar previously inoculated with the strain to be tested. The disks are prepared extemporaneously. The dishes were left for 15 minutes at room temperature before being incubated in an oven at 35°C for 20 to 24 hours. After incubation, a clear zone or halo appears [13].

2.8. Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

A microplate method, as previously described [14], was used with slight modifications to determine minimal inhibitory concentration (MIC) values of plant extracts. Plant extracts were serially diluted, ranging from 1/2 up to a 1/100 dilution from the crude extract. In each well, 100 µl of each extract dilution was mixed with 100 µl of the fungal spore suspension (2×10^6 spores ml^{-1} in fresh PDB). The microplateswere incubated for 2-3 d at 27°C with daily monitoring. All experiments were done in triplicate. The MIC readings were performed by a spectrophotometer with a microplate reader at 595 nm. MICs values were calculated by

comparing growth in control wells and the extracted blank, which consisted of inoculated plates. The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition of more than 90% at 48 h, as compared to the control.

The *in vitro* fungicidal activity (MFC) was determined described by Espinel-Ingroff et al. [15]. After 72 h of incubation, 20 µl was subcultured from each well that showed no visible growth (growth inhibition of over 98%), from the last positive well (growth similar to that for the growth control well), and from the growth control (extract-free medium) onto PDA plates. The plates were incubated at 27°C until growth was seen in the growth control subculture. The minimum fungicidal concentration was regarded as the lowest extract concentration that did not yield any fungal growth on the solid medium used.

All extraction and evaluation of antifungal activity technicals were carried out at the Laboratory of Research Bioconversion, Engineering Microbiology and Health Safety, University of Mascara, Algeria.

2.9. Statistical analysis

Results obtained were subjected to statistical analysis using one-way analysis of variance. All data were the average of three experiments.

3. RESULTS AND DISCUSSION

3.1. Extraction yields

3.1.1. Essential oils

According to the results shown in figure 1, there was a clear difference between the two extraction yields of *M. communis* (0.52 ± 0.03%) and *Z. album* (0.05 ± 0.8%).

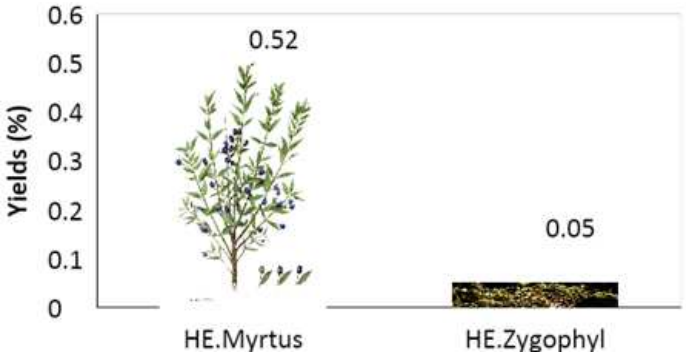

Figure 1. Extraction yields of essential oils of *M. communis* and *Z. album.*

This is confirmed by the work of Wannes et al. [16] on the different parts of *M. communis*, which found the greatest extraction yield of leaf essential oils with 0.61% [17, 18] and the myrtle of Morocco with 0.3% [19]. For *Z. album*, the yield was lower than that of the essential oil of *Z. eurypterum* from Iran [20], but significantly higher than the yield of the essential oil of the same species from the Sahara which is 0.02% [21]. In the laboratory, this performance was influenced by several factors such as the harvest season, the origin of the plant and the method of extraction. Several works also related to drying aromatic and medicinal plants indicate considerable changes, particularly quantitatively at the level of essential oils.

3.1.2. Phenolic extracts

From these results, we find that polyphenolic extracts from *M. communis* leaves have high yields

compared to *Z. album* because yields are higher in leaves than in other parts of the plant. This has been confirmed by Falla et al. [22], especially for aqueous (decoctioned) extract, which has a yield of 24.65%, is close to that obtained by Hosseinzadeh et al. [23] (24.50%) but clearly Greater for the yield of ethanolic extract (7.66%). Other more or less considerable yields have been observed in the aqueous, dichloromethan and methanolic extracts of *Z. album* which are in the order of 15, 14.6 and 14.5% respectively. In contrast, the yield of ethanol extract was the lowest compared to the other registered yields [24].

In general, plant diversity was responsible for the wide variability of physicochemical properties influencing the extraction of polyphenols [25, 26]. Among other things, the solubility of phenolic compounds was affected by the polarity of the solvent used.

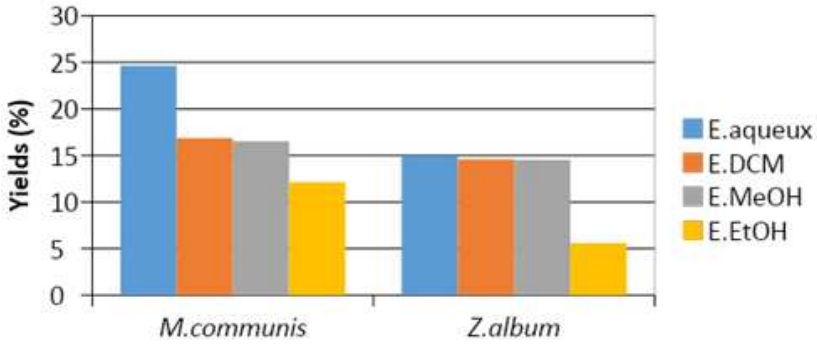

Figure 2. Phenolic extract yields of *M. communis* and *Z. album.*

DCM.E: Dichloromethanic extract; MeOH.E: methanolic extract, EtOH.E: Ethanolic extract.

3.2. Essential oils composition

3.2.1. Myrtus communis composition

Table 1. Constituents of the essential oil of *M. communis.*

No.	Compound	Retention time (min)	Retention index	Percentage (%)
1	Acetate de geranyl	4,192	899	3,09
2	p-Cymene	4,592	936	0,52
3	Limonene	4,830	946	7,75
4	β-Myrcene	6,058	973	1,46
5	α-Murolene	6,282	991	0,68
6	δ-3-Carene	6,717	1010	0,39
7	Nerol	6,847	1020	1,15
8	α-Terpineol	7,237	1022	3,49
9	α-Pinene	7,467	1024	39,02
10	Camphene	7,723	1175	0,82
11	Isobutylisobutyrate	8,699	1185	0,46
12	Sabinene	8,813	1228	0,11
13	1,8-Cineole	9,107	1498	43,58

In Algeria [27-29] by examining the chemical composition of the essential oil of myrtle leaves also found that α-pinene (20-34), 1,8-cineole (15-23.2) and linalool (3,6-10.1) is the main essential composition of myrtle and this oil is also characterized by the lack of myrtenyl acetate. Further research in the Mediterranean

region revealed that α-pinene, 1,8-cineole and limonene are the main constituents of myrtle essential oil [30-33]. The same results have been confirmed by others [34-37].

3.2.2. Zygophyllum album composition

The results shown in Table 2 show that 15 compounds could be identified, representing only 56.95% of our essential oil. This could be explained by the fact that *Z. album* contains more than 60% so there is a high dielectric loss [38]. The majority compounds were damascenone(E)-β (13.05%), geraniol (8.09%) and verbenone (6.76%). The composition of the essential oil of *Zygophyllum album*is marked by the presence of the oxygenated compounds (more than 58%) and the hydrocarbons as the case of caryophyllene (2.12%). Our results are comparable to those obtained by Akhgar et al. [20] and Tigrine-Kordjani et al. [38] on the same species and origin (Sahara de Ouergla) with some minor differences in the percentage of components.

Table 2. Constituents of the essential oil of *Z. album.*

No.	Compound	Retention time (min)	Retention index	Percentage (%)
1	Geraniol	3,366	1071	8,09
2	Liguloxide	3,530	1099	2,40
3	3-Nonen-2-one	3,849	1136	4,50
4	Caryophyllene (trans)	3,950	1203	2,12
5	2-Oxabicyclo [4,4,0] dec-9-ene-1,3,7,7-tetramethyl	4,107	1275	3,06
6	Damascenone(E)-β	4,208	1312	13,05
7	Eicosane	4,364	1379	3,28
8	Tricosane	4,423	1415	1,83
9	Linalooloxide-Cis	4,635	1483	1,34
10	Liguloxide	4,808	1531	2,43
11	Massoya lactone	4,869	1537	1,92
12	Isoamylbenzylether	4,949	1537	2,19
13	Verbenone	5,275	1657	6,76
14	β-Bisabolene	6,575	2002	1,99
15	Nonanal	9,167	2296	1,99

3.3. Phytochemical analysis of phenolic extracts

Table 3. Phytochemical analysis results.

	Myrtus communis				*Zygophyllum album*			
	Aq	EtOH	DCM	MetOH	Aq	EtOH	DCM	MetOH
Alkaloids	+++	-	-	-	-	-	-	++
Free anthracene derivatives	+	-	-	-	-	-	+	-
Anthraquinones	-	-	-	-	-	-	-	-
C-heterosides	+	+	+	+	+	+	-	++
Anthocyans	+++	+	+	+	-	-	+	+
Saponins	-	+++	-	-	+++	+	+	++
Tannins	+++	+	-	+	+	+	+	+
Flavonoids	+++	+	-	+	+	-	+	++

Aq.E: Aqueous extract; EtOH.E: Ethanolic extract; DCM.E: dichloromethanic extract; MtOH.E: methanolic extract.

3.3.1. Myrtus communis

The results of the Kanoun [39] have shown results comparable to our study, confirming that tannins are present with a high-intensity aqueous extract of *M. communis* leaves from the same region of Honaine (Tlemcen), except for the alkaloids which are revealed higher in our extract. Similarly, phytochemical tests [23, 41, 45] have also shown that *Myrtus communis L.* leaves contain tannins, flavonoids, and volatile oils. However, in the two remaining extracts (methanol and dichloromethane), the presence of the main chemical groups was low, this is confirmed by the work of Hyder et al. [42].

3.3.2. Zygophyllum album

These results show that the methanol extract of the plant is very rich in saponins, heteroside, anthocyanins, and flavonoids. There is also a small number of tannins and alkaloids. Similar results were found in the studies of Ayad [43] on secondary metabolites of the methanol extract of *Zygophyllum cornutum* Coss. The work of Ksouri et al. [44] demonstrated the absence of free anthracene derivatives and anthraquinones in the *Z. album* species, which is consistent with our results. On the other hand, the presence of flavonoids in the tested plants is lower compared to that found in the works of Belyagoubi [24] on the same species from Egypt. According to the screnning results and based on the richness on compounds, two phenolic extracts were selected for study; aqueous extract of *Myrtus communis* and methanolic extract of *Zygophyllum album.*

3.4. Antifungal activity

The inhibition diameters are between 7.5 and 14 mm. Contrary to the previous results, *M. communis* exerts an antifungal effect better than *Z. album*, although Khelil et al. [45] found a negative effect of the methanol extract on this yeast. It was also noted that the antibacterial effect is noted in *Candida albicans* better than *Aspergillus fumigatus*, according to a dose-response relationship. Also, the polyphenolic extracts have a more antifungal power than the oils. This high activity of the phenolic derivatives would be linked to a good solubilization of these in the aqueous medium due to the free hydroxyl group [46], but also to a toxic action of these molecules towards the cell membrane of the microorganism [47, 48] studied the antifungal activity of primary alcohols, phenolic compounds, aromatic aldehydes, and organic acids. They have realized that this increases with the hydrophobicity of these compounds, suggesting hydrophobic interactions between these compounds and the fungal cells tested [49] also studied the question by studying the action of eugenol and vanillin, which are phenolic compounds. According to these authors, the targets of phenols are the cell wall, the cytoplasmic membrane, and the cytoplasm. Their effects on these three sites depend on the concentration used: at low concentrations, they produce reversible effects, whereas at high concentrations they produce general coagulation followed by cell death.

Table 4. Disc diffusion test.

	M. communis		***Z. album***		**MIC (10µg)**	**Gris (10µg)**
	Aq.E	**EO**	**Met.E**	**EO**		
Candida albicans	14±0,02	10,5±0,01	8±0,02	7,5±0,2	24 ±0,6	0,8 ±0,3
Aspergillus fumigatus	10±0,5	8,5±0,3	7,2±0,5	7,5±0,01	26 ±0,15	-

The values represent the mean ± standard deviation (n = 3), -: no inhibition zone.

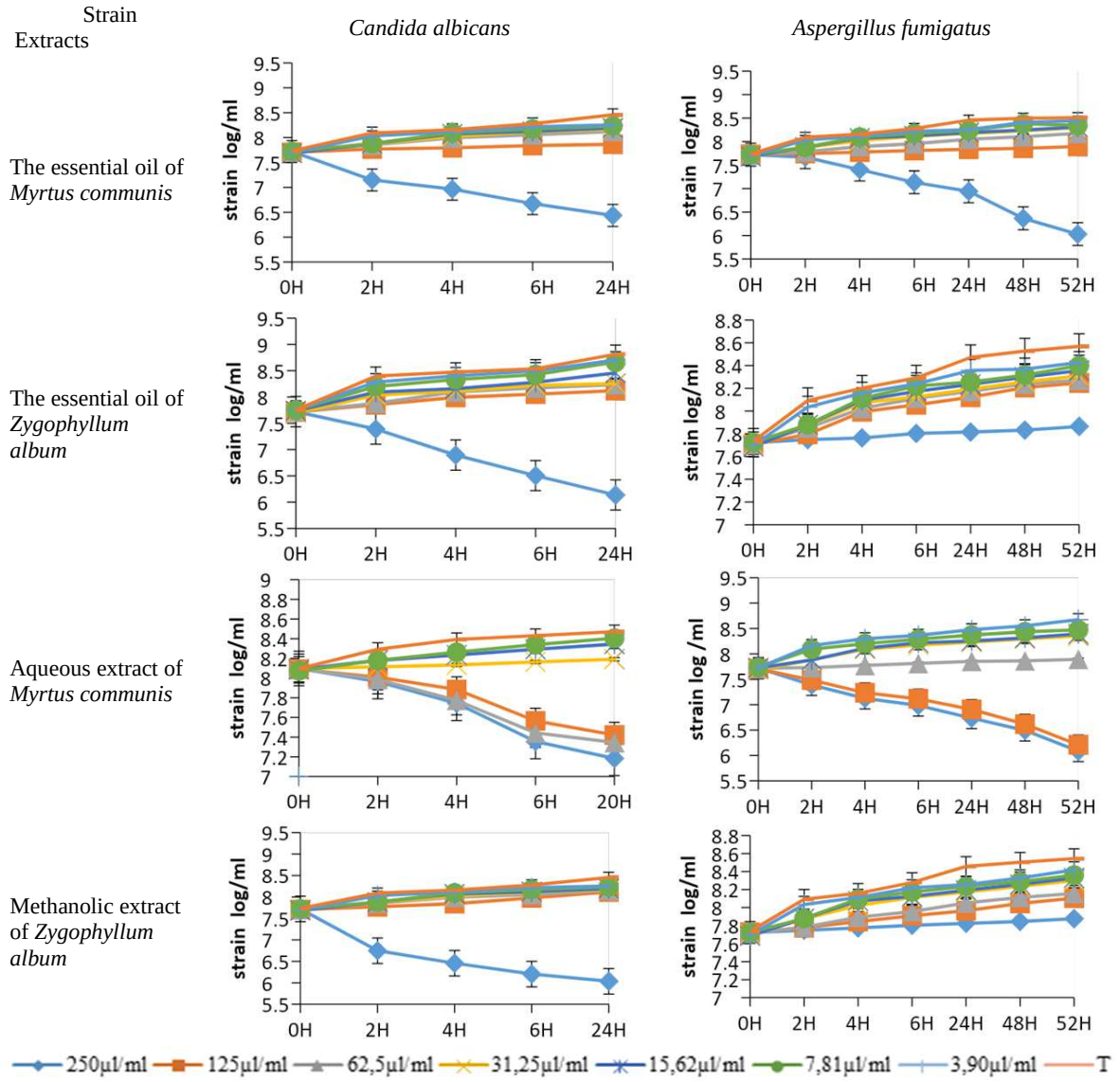

Figure 3. Kinetics of growth of strains in the presence of plant extracts.

Table 4 and Figure 3 of the MFC clearly show that there is great variability in the results obtained. Significant antifungal activity of the two extracts studied (MCA and ZAM) was observed on *C. albicans.* Of these two extracts, MCA was found to be most active with a MFC value of 25 mg/ml versus 100 mg/ml for ZAM. Nevertheless, *Z. album* does not exert any fungicidal activity vis-à-vis *A. fumigatus.*

On the basis of MFC, the efficacy ratios determined by Ben Arfa [46], namely MFC_{MCA}/MFC_{ZAM} (for *A. fumigatus*) and MFC_{MCA}/MFC_{ZAM} (for *Candida albicans*) are respectively 100 and 25. This means that MCA Is 100 times more active on *A. fumigatus* and 25 times more active on *C. albicans* than ZAM. Similarly for essential oils, MCO is 40 times more active for *C. albicans* and 250 times more active for *A. fumigatus* than ZAO.

The antifungal potency of the essential oil of essential oils could be attributed to the presence of antifungal components listed in the list of constituents with antifungal activity [50, 51] reported that antifungal oil Essential of *Myrtus communis* is bound to β-pinene, p-cimene, 1,8-cineole, and α-pinene. The majority or minor compounds may increase the antifungal activity. For the *Z. album*, the action of

caryophyllene, which one of the components of its essential oil is known to have inhibitory effects against some fungal strains including *C. albicans* [52].

Table 5. The minimum inhibitory concentrations (MICs) and the minimum fungal concentrations (MFCs) of plant extracts and the antifungal used.

	Phenolic extracts (mg/ml)				**Essential oils (µl/ml)**			
	M. communis		***Z. album***		***M. communis***		***Z. album***	
	CMI	**MFC**	**CMI**	**MFC**	**CMI**	**MFC**	**CMI**	**MFC**
Candida albicans	31.25	250	250	250	62.5	250	250	250
Aspergillus fumigatus	62.5	125	250	-	125	250	250	-

4. CONCLUSION

In conclusion, the findings of the present study indicate that the phenolic extracts and essential oils of *Myrtus communis* and *Zygophyllum album* have potential as a topical antifungal agent against fungi that are pathogenic to humans. These extracts are broad-spectrum agents that inhibit *Aspergillus fumigatus* and *Candida albicans* species which are intrinsically resistant to fluconazole or whose resistance is easily inducible. Given the results described above, particularly the possible mechanisms of action, which might induce side-effects in humans, these antifungals require further investigation. The results presented should stimulate studies on toxicity, improved formulations and the determination of optimal concentrations for clinical applications, as well as comparative studies alongside currently used drugs of the therapeutic efficacy of plant extracts to control many infections.

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Conflict of Interest: The authors declare no conflict of interest.

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