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Research Article

Effects of Natural Products and Essential Oils on Pathogenic Fungi

Sara Al-Ali[1] and Awatif Al-Judaibi[1,2]*

[1]*King Abdulaziz University, Jeddah, Saudi Arabia*

[2]*Jeddah University, Jeddah, Saudi Arabia*

***Corresponding Author:** Awatif Al-Judaibi, King Abdulaziz University and Jeddah University, Jeddah, Saudi Arabia.

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Abstract

Background: Despite the harsh conditions and limited water resources in the Arabian Peninsula, certain plants thrive in this environment. Some of these plants contain bioactive compounds and have been used for thousands of years in traditional medicines. This study would circumvent the problem of the growing drug-resistance amongst pathogens.

Methods: We investigated the ethanol extracts and essential oils of two plants, *Azadirachta indica* and *Olea europaea*, and the ethanol extracts of *Phoenix dactylifera* (Ajwa) and *Ziziphus spina-christi*, to determine their efficacy against *Candida albicans* (ATCC CA 10231), *Candida tropicalis* (ATCC CT 2697), *Cryptococcus neoformans* (ATCC CT 2697), *Trichophyton mentagrophytes* (ATCC 9533), and *Microsporum canis* (isolated from patients) in terms of minimum inhibitory concentrations, minimum fungicidal concentrations and kill-time.

Results: The results were compared to treatment with the antifungal itraconazole. We also observed a decrease in *C. albicans* dry weight and an increase in glucose uptake compared to the untreated cells.

Conclusion: We conclude that the bioactive compounds in the ethanol extracts of *P. dactylifera* may offer a less expensive and natural alternative to pharmaceutical drugs.

Keywords: *Microsporum canis; Trichophyton mentagrophytes; Candida albicans; Candida tropicalis; Cryptococcus neoformans*

Introduction

Fungi are present in soil and on plants, as well as on many indoor surfaces and on human skin. Of the approximately 1.5 million different species of fungi on earth, only approximately 300 are known to cause human illness [1,2]; however, these fungi are generally common in the environment. A number of fungal pathogens are completely dependent on their host for survival, while others can prosper in a broader range of environments. Fungal host restrictions also vary considerably, from species that require a particular host to pathogens that can cause disease in many hosts. An extreme example is the genus Fusarium, with species that cause diseases in thousands of plant species, as well as in animals (including humans). The number of fungi reported to cause disease in humans is increasing rapidly, especially in individuals with compromised immune systems [3]. Some fungal infections, such as systemic mycoses, can be life-threatening and are also difficult to treat.

Fungal infections of the skin, hair, and nails are primarily caused by a group of keratinophilic fungi known as dermatophytes, which include species belonging to *Trichophyton, Microsporum*, and *Epidermophyton*. *Trichophyton* species cause skin, hair, and nail infections, while *Microsporum* species infect skin and hair and Epidermophyton species infect skin and nails. These species cause ringworm, or tinea, which can occur on the feet (tinea pedis and tinea cruris), trunk and limbs (tinea corpora), face (tinea faciei), hair (tinea capitis), hand and feet (tinea manuum), and nails (tinea unguium) [4,5]. Infection rates increase if the skin has an injury, such as burns or scars, or when temperature and humidity are high [6,7]. Infections caused by dermatophytes are particularly common in hot and humid climates of tropical and subtropical countries [8]. These fungi can survive in the environment for up to 15 months depending on species.

Dermatophyte transmission can occur by direct contact with an infected host, such as a human or an animal, or by direct or indi-

rect contact with exfoliated skin or hair on clothing, towels, hair brushes or combs, caps, shoes, and other objects. Dermatophytes are also found in temporary residences, such as hotels, and in furniture, saunas, and swimming pools. Reusing unsterilized equipment, for example in barber shops, can also lead to fungal infections [7,8].

Fungal infections can be controlled through the use of antifungal agents; however, fungal and human cells are similar at the molecular level and it is difficult to identify drugs that target fungi without affecting human cells. The side effects of antifungal drugs include allergic reactions, liver damage, and altered estrogen levels [7]. In addition, the indiscriminate use of commercial antimicrobial drugs has caused the development of multiple-drug resistant forms of human pathogenic microorganisms, which further increases the need for new antimicrobial agents that are safe and effective.

Plants constitute an important source of new drugs and may provide potential antifungal agents [9]. The World Health Organization (WHO) defines medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or plants that synthesize metabolites that can be used to produce drugs (WHO 2008) [10].

The effect of plant extracts on microorganisms has been studied by many researchers worldwide; however, of the estimated 250,000-500,000 plant species on the planet, only a small percentage have been investigated phytochemically and an even smaller fraction have been subjected to biological or pharmacological screening [11-21].

The aim of this study was to investigate the antifungal effects of ethanol extracts of *Azadirachta indica*, *Ziziphus spina-christi*, and *Olea europaea* and two essential oils, neem oil; *A. indica* and olive oil; *O. europaea*, on *Trichophyton mentagrophytes* and *Microsporum canis* and on three yeasts, *Candida albicans, Candida tropicalis*, and *Cryptococcus neoformans*. Minimum inhibitory concentrations (MICs), minimum fungicidal concentrations (MFCs), and kill times were determined. In addition, the effects of crude extracts and essential oils on the growth and dry weight of *Candida albicans* were also studied. The results of this study could lead to the development of novel anti-fungal agents for clinical use.

Materials and Methods

Test organisms

Microsporum canis was obtained from patients at King Fahd Armed Forces Hospital, Jeddah. In addition, the following antibiotic-resistant strains were obtained from the American Type Culture Collection (ATCC): the fungus *Trichophyton mentagrophytes* (ATCC 9533) and three yeasts, *Candida albicans* (ATCC CA 10231), Candida tropicalis (ATCC CT 2697), and *Cryptococcus neoformans* (ATCC CT 2697). All isolates and strains were grown under aerobic conditions at 27 ± 2°C for 7 d. Yeasts were grown at 27 ± 2°C for 48 h in an aerobic incubator. Fungi and yeasts were cultured on Sabouraud dextrose agar (SDA) and Mueller Hinton agar (MHA) and in Sabouraud dextrose broth and Mueller Hinton broth (MHB). The fast-growing fungi strains were maintained in culture under aerobic conditions at 27°C for 7 to 21 d, while yeast species were maintained for 48 h at 27°C.

Study specimens

Specimens of *Azadirachta indica*, *Ziziphus spina-christi*, and *Olea europaea* plants were collected from the Jeddah region in Saudi Arabia during autumn 2013. Ajwa date; *Phoenix dactylifera* seeds were collected from Almadina Almonawara city, Saudi Arabia. Plants were identified by the Department of Biological Sciences, Botany Section at King Abdulaziz University. Neem oil and olive oil were obtained from a Saudi market.

Alcohol extraction and essential oils

Plant leaves and Ajwa seeds were thoroughly washed and then dried in the shade at 32-35°C; the dried plants were then ground into powder. Plant extracts were obtained by placing the powdered samples in 100% ethanol (1:10 w/v) in a conical flask, which was shaken at 120 rpm at 30°C for 3 d. Flask contents were filtered through Whatman No. 1 filter paper and the filtrates were dried under reduced pressure at 40°C. The extracts were weighed and the percentage of yield was calculated relative to the initial material used for extraction. The crude extracts were then dissolved in a 1:1 volume of dimethylsulfoxide (DMSO) and stored in closed vials at 4°C.

Neem oil and olive oil were also used in the study. Itraconazole was used as the positive control in the antifungal assays (Sporanox, 100 mg per capsule, Janssen, 04100 Borgo San Michele, Latina, Italy).

Antifungal assays

The antimicrobial activity of each crude extract against the tested fungi and yeast was determined *in vitro*. Yeast inoculums were prepared by seeding SDA (HiMedia, Mumbai, India) with 100 µl of 1–5 × 10^6 colony-forming units (CFU) of each yeast culture. Dermatophytes were prepared by inoculating SDA (HiMedia) with 1-mm

discs from the radial growth of fungi. The antifungal assays were conducted in 90-mm-diameter petri dishes containing SDA thoroughly mixed with different concentrations (1–5%) of the extracts. The rate of mycelial growth inhibition was measured by placing an active mycelial disc of fungi on petri dishes and incubating them at 27°C; radial growth was recorded after 3 and 7 d. All tests were performed in triplicate. The antimicrobial activity of each crude plant extract and oil against *Candida* species was also determined using the agar diffusion method, as previously described by the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards) [22-24]. Each extract was dissolved in DMSO at 50 µg/ml and filtered through a 0.22-µm pore filter (Millipore, Billerica, MA, USA). Zones of inhibition were recorded after 48 h. All tests were performed in triplicate.

Determination of MICs

MIC is defined as the lowest concentration of an antimicrobial that prevents the growth of a microorganism following a specific incubation period. MICs were determined using a broth-micro dilution method, as described by Wiegand., *et al*. [25]. Yeast strains were cultured at 27°C on MHA and then resuspended in 1 ml MHB to obtain a final concentration of 100 CFU/ml. Each extract was serially diluted with MHB. Following incubation, the MIC was determined as the lowest concentration of each specimen for which there was no visible growth compared with the control [26].

Determination of MFCs

MFC is defined as the lowest concentration of an antifungal agent needed to kill 99.9% of the initial inoculum. MFCs were determined by inoculating 0.1 ml from wells showing no growth in the MIC assay onto sterile SDA [27] and the plates were incubated at 27°C for 48 h. The concentration with no growth of the tested fungi was considered as the MFC; the negative control was a plate containing only medium. Values were recorded as mg/ml [28-30]. Each treatment was performed in triplicate.

Growth curve

Ten milliliters of SDB were inoculated with 1 ml of 5×10^6 *C. albicans* cells/ml and incubated at 27°C under aerobic conditions. The optical density (OD) at 750 nm of 1 ml of culture was determined using a spectrophotometer at 0, 1, 2, 4, 8, 12, and 48 h and a line graph was then plotted to analyze differences in growth [29].

Kill-time determination

Liquid cultures (1 ml) were diluted to an initial C. albicans inoculum of $2–5 \times 10^5$ CFU/ml in MHB containing the MIC concentrations of the *P. dactylifera* and *Z. spina christi* extracts and the cultures were incubated for 0, 2, 4, 8, 12, and 48 h at 27°C. At each time point, 50-µl aliquots were plated on SDA and incubated at 27°C for 48 h. Visible colonies were identified using an Interscience scan 500 colony counter. Yeast colonies were counted as CFU/ml [31-35]. Each treatment was performed in triplicate.

Effect of *P. dactylifera* and *Z. spina-christi* extract on yeast dry weight and glucose uptake

The dry weight and glucose uptake of *C. albicans* were measured to determine the antimicrobial effects of *P. dactylifera* and *Z. spina-christi* extracts. *C. albicans* (5×10^5 CFU/ml) was inoculated into 1 ml of MHB in tubes of known weight, to which 150 µl of *P. dactylifera* or *Z. spina-christi* extract were added. The tubes were incubated with shaking (180 rpm) at 27°C for 48 h. The samples were then collected and centrifuged at 10,000 rpm for 10 min. Suspended solutions were transferred to sterilized quartz tubes for measurement of glucose uptake using the COBAS INTEGRA 400 plus system, Roche Diagnostics Ltd. Switzerland. Yeast growth was estimated by washing the centrifuged samples with triple-distilled water (in 27-30°C) and centrifuging them again at 9,800 x g, after which they were dried at 80°C [29].

Statistical analysis

Microbial zone of inhibition and cell count (CFU/ml) data were collected, summarized, and tabulated. Statistical analyses were performed using the Package for Social Science, IBM SPSS 20 (SPSS Inc., Chicago, IL, USA). The results are expressed as mean ± standard deviation (mean ± SD). Differences between samples and the homogeneity between groups were determined using the ANOVA test. Results were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

Results

This study, conducted in Saudi Arabia, was designed to investigate the antifungal activity of plants from the Jeddah region (*A. indica*, *Z. spina-christi*, and *O. europaea*), *P. dactylifera* seeds, and neem oil and olive oil. The tested organisms included the fungi *M. canis* and *T. mentagrophytes* and the yeasts *C. albicans*, *C. tropicalis*, and *C. neoformans*.

Determination of antifungal activities of the essential oils and extracts.

This experiment aimed to determine the concentrations of *A. indica*, *Z. spina-christi*, *O. europaea*, and *P. dactylifera* extracts exhibiting the highest antifungal activity. The antifungal activity of neem

oil and olive oil was also tested. The results in Table 1 show that neither of the essential oils had an effect on *C. albicans, C. tropicalis, or C. neoformans*; all tested yeast strains demonstrated visible growth. However, all yeasts were sensitive to *O. europaea*, *A. indica, Z. spina-christi*, and *P. dactylifera* extracts and itraconazole. *C. tropicalis* and *C. neoformans* growth was inhibited 15.16% and 19.83%, respectively, following treatment with 150 mg/ml of *O. europaea* and 14.50% and 17.50%, respectively, following treatment with same concentration of *A. indica*. A concentration of 150 mg/ml Z. spina-christi and *P. dactylifera* was effective against *C. albicans*, *C. tropicalis*, and *C. neoformans*. The highest inhibition percentages, 21.16% and 15.83%, were observed for *C. neoformans* following treatment with *Z. spina-christi* and *P. dactylifera*, respectively. *A. indica* and *O. europaea* extracts did not affect *C. albicans*; however, they significantly inhibited *C. tropicalis* growth, reducing it by 15.16% and 14.50%, respectively (P ≤ 0.01).

	mg/ml ml/ml	*C. albicans*	*C. tropicalis*	*C. neoformans*	*M. Canis*	*T. mentagrophytes*
O. europaea oil	50	0.00	0.00	0.00	0.00	27.50 ± 1.75**
	100	0.00	0.00	0.00	0.00	25.00 ± 1.52**
	150	0.00	0.00	0.00	0.00	18.50 ± 0.76**
A. indica oil	50	0.00	0.00	0.00	26.66 ± 0.57**	28.83 ± 0.57**
	100	0.00	0.00	0.00	23.33 ± 0.57**	23.00 ± 0.57
	150	0.00	0.00	0.00	20.66 ± 1.32**	19.66 ± 1.00**
O. europaea	50	0.00	13.16 ± 0.76**	0.00	0.00	0.00
	100	0.00	14.00 ± 0.50**	16.33 ± 0.28**	0.00	12.00 ± 1.60**
	150	0.00	15.16 ± 0.28**	19.83 ± 0.57**	0.00	17.00 ± 1.04**
A. indica	50	0.00	0.00	0.00	0.00	27.33 ± 0.76**
	100	0.00	13.00 ± 0.86**	15.00 ± 0.86**	19.50 ± 0.76**	24.83 ± 1.52**
	150	0.00	14.50 ± 0.28**	17.50 ± 0.86**	24.66 ± 1.00**	22.83 ± 1.52**
Z. spina christi	50	0.00	0.00	0.00	0.00	0.00
	100	14.33 ± 1.15**	15.33 ± 0.28**	18.50 ± 0.28**	0.00	0.00
	150	16.00 ± 0.28**	19.50 ± 0.50**	21.16 ± 0.88**	0.00	16.33 ± 2.02**
P. dactylifera	50	12.00 ± 0.86**	11.33 ± 0.76**	12.00 ± 0.50**	0.00	14.83 ± 1.60**
	100	13.50 ± 0.28**	12.00 ± 0.50**	14.50 ± 1.00**	0.00	12.00 ± 1.75**
	150	15.16 ± 0.33**	15.83 ± 1.04**	15.83 ± 0.57**	0.00	11.33 ± 0.76**
Itraconazole	50	23.83 ± 0.76**	23.00 ± 0.50**	19.33 ± 0.76**	0.00	23.16 ± 1.00**
	100	26.66 ± 0.76**	26.33 ± 1.04**	25.00 ± 0.50**	16.00 ± 0.28**	22.33 ± 1.04**
	150	30.16 ± 0.76**	28.00 ± 0.86**	26.66 ± 0.76**	18.33 ± 0.57**	21.16 ± 1.80**

**: P ≤ .01

*: P ≤ .05

Table 1: Inhibition of yeast/epidermophyton growth (mm) following incubation (2–7 d) with 100 µl of different concentrations of plant ethanol extracts (mg/ml)/ and essential oils (ml/ml).

Mean ± SD

Determination of MICs of the essential oils, extracts and itraconazole

Table 2 shows the MIC levels of the essential oils, plant extracts, and itraconazole. The lowest MICs were associated with *C. albicans* and *C. neoformans*. *M. canis* and *T. mentagrophytes* treated with olive oil and neem oil; *O. europaea*, *A. indica*, *P. dactylifera*, and *Z. spina-christi* extracts; and itraconazole had MICs of 32 µl/ml. The most sensitive yeast was C. neoformans with MICs of 0.25 µl/ml, > 0.06 µl/ml, and 0.25 µl/ml following treatment with *O. europaea*, *A. indica*, and *P. dactylifera* extracts, respectively.

	Oil		Extract				Antibiotic
	O. europaea	*A. indica*	*O. europaea*	*A. indica*	*P. dactylifera*	*Z. spina christi*	Itraconazole
M. canis	-	32	-	32	-	-	32
T. mentagrophytes	32	32	32	32	32	32	32
C. albicans	-	-	-	-	0.25	1	1
C. tropicalis	-	-	0.5	1	1	4	1
C. neoformans	-	-	0.25	0.06	0.25	1	1

Table 2: MICs (µl/ml) of fungal and yeast growth following 2–7 d growth in Mueller-Hinton broth.

Determination of MFCs of the essential oils, extracts and itraconazole

The MFCs shown in Table 3 confirm the MIC results. The highest efficiencies were against *M. canis*, *T. mentagrophytes*, *C. albicans*, *C. tropicalis*, and *C. neoformans*. The most effective extract in terms of MFC was that of *P. dactylifera* with values of 4 µl/ml, 8 µl/ml, 0.125 µl/ml, > 0.06 µl/ml, and > 0.06 µl/ml for *M. canis*, *T. mentagrophytes*, *C. albicans*, *C. tropicalis*, and *C. neoformans*, respectively. The most sensitive yeast was *C. neoformans* with an MFC value > 0.06 µl/ml, compared with 2 µl/ml for the antifungal itraconazole.

	Oil		Extract				Antibiotic
	O. europaea	*A. indica*	*O. europaea*	*A. indica*	*P. dactylifera*	*Z. spina-christi*	Itraconazole
M. canis	-	32	-	**> 32**	-	-	32
T. mentagrophytes	32	**> 32**	**> 32**	**> 32**	**> 32**	**> 32**	32
C. albicans	-	-	-	-	0.25	1	1
C. tropicalis	-	-	0.5	1	2	4	1
C. neoformans	-	-	0.25	-	0.25	1	2

Table 3: MFCs (µl/ml) of fungal and yeast growth following 7 d and 28 h incubation, respectively, on Sabouraud agar.

Determination of *C. albicans* kill-time by cell vitality (CFU) following treatment with *Z. spina-christi* and *P. dactylifera* extracts

Figures 1 and 2 show the growth curve and kill-times of *C. albicans* treated with 150 mg/ml of *Z. spina-christi* and *P. dactylifera*. Decreased cell vitality with 83.02% inhibition was observed after 2 h of incubation with the *Z. spina-christi* extract. Cells showed decreased vitality with 31.24% and 59.33% inhibition after 2 and 4 h of incubation with the *P. dactylifera* extract, respectively. The results were highly significant (P ≤ 0.01).

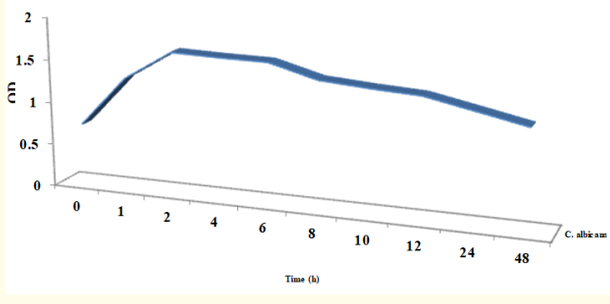

Figure 1: Growth curve of *C. albicans*

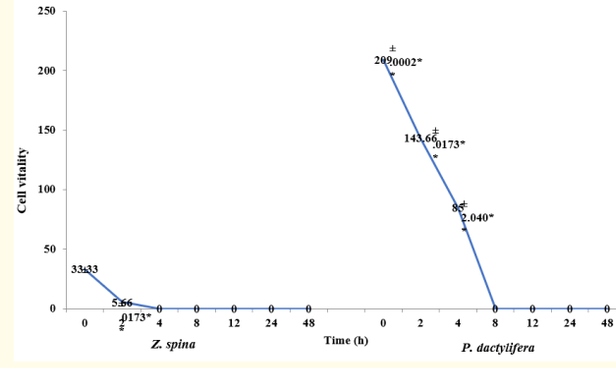

Figure 2: Kill-time of *C. albicans* following treatment with *Z. spina-christi* and *P. dactylifera* extracts

C. albicans dry weight and glucose uptake (mg/ml) following treatment with *Z. spina-christi* and *P. dactylifera* extracts

Glucose uptake in the fungal biomass was determined based on the dry weight of untreated cells. The biomass of *C. albicans* incubated with *Z. spina-christi* and *P. dactylifera* extracts was compared with that of untreated cells. Table 4 shows that the dry weight of *C. albicans* generally increased following incubation with increasing amounts of *Z. spina-christi* and *P. dactylifera* extracts, with the

exception of incubation with 150 µl *Z. spina-christi* extract, which showed a decrease in dry weight compared with the untreated cells. We hypothesize that the yeast was more stressed by treatment with high concentrations of the extract resulting in decreased dry weight. *Z. spina-christi* and *P. dactylifera* extracts increased the percentage of glucose uptake (reflecting fungal metabolism) compared to the control strain. *C. albicans* glucose uptake increased to 1149.41% when incubated with 150 µl of *Z. spina-christi*, while treatment with 150 µl *P. dactylifera* decreased glucose consumption to 1258.87%.

	Control (untreated cells)	Itraconazole (100)	Z. spina- christi			P. dactylifera		
			50	100	150	50	100	1
Dry weight	0.072	0.064±.0168	0.0055 ±0.00001**	0.01 ±0.0060	0.005 ±0.00011*	0.0079 ±0.0002**	0.017 ±0.0011**	0.017 ±0.0010**
Glucose uptake	16.59	1286.06±.0186**	1484.7 ± 29.18**	1242.55 ± 54.72**	1149.41 ± 18.86**	1478.83 ± 22.63**	1340.15 ± 61.21**	1258.87 ± 33.19**

**: P ≤ .01

*: P ≤ .05

Table 4: *C. albicans* dry weight and glucose uptake (mg/ml) following treatment with various concentrations (µl) of *Z. spina-christi* and *P. dactylifera* extracts (48 h incubation).

Discussion

The overuse of prescribed antibiotics can lead to resistance among pathogenic fungi and yeast. Thus, new antibiotics that are effective against resistant strains of microorganisms are needed; plants may offer such novel compounds. Secondary metabolism components have been found to act as bioactive compounds that can inhibit fungal and yeast growth [36]. In the current study, ethanol extracts of *A. indica*, *Z. spina christi*, and *O. europaea* leaves; *P. dactylifera* seeds; and the essential oil of two plants, *A. indica* and *O. europaea*, were tested against fungi strains *M. canis* and *T. mentagrophytes* and the yeasts *C. albicans*, *C. tropicalis*, and *C. neoformans*

Several studies have investigated the antifungal activity of different species of plants including *Cymbopogon citratus* DC. Stapf., *Lantana camara* L., *Nerium oleander* L., *Ocimum basilicum* L., *Lawsonia inermis* L., and *Amyris balsamifera* essential oil [37-39]. Our findings are consistent with the results of these studies; *A. indica*, *Z. spina-christi*, *O. europaea*, and *P. dactylifera* extracts were found to have antifungal activity against selected fungi and yeast.

The bioactive compounds of plants differ according to species and habitat. Plant secondary metabolism is influenced by environmental conditions including temperature, sunlight, and dryness. Secondary metabolism is also affected by the presence of organic compounds such as flavonoids, sugar alcohols (sorbitol, ribitol, and inositol), soluble sugars (saccharose, raffinose, stachyose, and trehalose), and nitrogenous compounds (proline, glycine, and betaine) [40]. A previous study has demonstrated the effects of habitat and found that extracts of *Gnaphalium luteo-album* and *Adonis microcarpa* collected from the desert possess high inhibitory activities against pathogenic fungi [41]. Furthermore, extracts of *Rhaponticum acaule* L. and *Scorzonera undulata* L. collected from the desert strongly inhibited *C. albicans*, *C. neoformans*, *T. rubrum*, and *M. canis* growth [42].

Emerging drug resistance has necessitated the development of new therapeutic alternatives for the treatment of fungal infections. Essential oils possess mixed functional groups and complex molecular structures and are highly volatile at very low temperatures; which enables them to spread easily in the environment and the surrounding medium. Essential oils act more efficiently against drug-resistant microbes and show less residual effects in the body; they inhibit the growth and metabolism of a variety of infectious pathogens, mainly microbes [43,44].

Our results show that the ethanol extracts possess high activities against fungi and yeast, as reflected by the MICs and MFCs. These results are consistent with previously reported MICs and MFCs following treatment with several other types of extracts in organic solvents [45-48]. Several studies have demonstrated that plant extracts can inhibit the growth of some fungi with a low MIC [49,50]. However, another study has shown that a high MIC is required for effective anti-yeast activity [51].

Various concentrations of active crude extracts and oils were tested against susceptible fungi and yeast. Our results demonstrate significant variations in MIC values; the most sensitive fungi showed low MICs and the MICs of most extracts were between 0.06–32 µl/ml.

Medicinal plants containing phenolic compounds represent an important source of drugs. *A. indica* contains a variety of bioactive components with biological and pharmacological properties [52]; *Z. spina-christi* extracts have been phytochemically screened and the results identified the presence of alkaloids, sterols, triterpenes, and saponins [53]. Medicinal effects may also be due to the presence of various other phytochemical components, such as carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phlobatannins, saponins, steroids, tannins, and cardiac glycosides, in the chosen plant species [54-58].

The kill-time results for *C. albicans* treated with *Z. spina-christi* and *P. dactylifera* extracts are likely due to an increase in the bioactive compounds present in the extracts. Antifungal agents primarily target the cell wall; cytoplasmic membrane permeability; or enzyme, protein, and toxin function. The glucose uptake reflected on dry weight from *C. albicans* cells following treatment with *Z. spina-christi* and *P. dactylifera* ethanol extracts was likely caused by damage to cellular regulation and the effect of the extract on cytoplasmic membrane permeability [59]. Several studies have examined the effect of antimicrobial agents on glucose leakage and on cell energy and metabolism; our results are in agreement with these previous findings of decrease dry weight and an increase in glucose uptake, which could be attributed to a compensatory mechanism to counteract the loss of energy expended in preventing the effect of the extract [60-62].

Conclusion

The results obtained in this study reinforce the importance and necessity of examining the potential use of *P. dactylifera* and *Z. spina- christi* ethanol extracts and *O. europaea* and *A. indica* extracts and oils. Further research into the properties of these extracts, or the isolation of their active ingredients would enable the development of novel antifungal which would circumvent the problem of the growing drug-resistance amongst pathogens, and test the effects of the extracts on mammalian cells or higher eukaryotes to check their toxicity.

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