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RESEARCH ARTICLE

Biochemical profile, antioxidant effect and antifungal activity of Saudi *Ziziphus spina-christi* L. for vaginal lotion formulation

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Abstract

Ziziphus spina-christi L. extract from the northern region of Saudi Arabia, was investigated to determine its chemical composition and to evaluate its antioxidant and antifungal properties. Fresh leaves were extracted using Soxhlet apparatus and the yield was 8% w/w. Results of the qualitative study showed that this extract is rich in chemical compounds belonging to several classes (saponins, phenols, tannins). GC-MS analysis detected 38 chemical compounds with different concentrations representing 99.71 % of the total extract. However, Z. *spina-christi* leaves extract is mainly composed of Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester (18.80%). The extract has free radical scavenging activity at different concentrations and the best result was obtained with IC_{50} of 148.33 µg/ml. *C. albicans* and other *Candida* species caused vulvovaginal candidiasis, which is a high-risk occurrence in hospitalized patients. *In vitro* antifungal activity was investigated by the agar well diffusion test to measure and compare diameter of zones of inhibition (in mm) against *Candida albicans*, *Candida glabrata* and *Candida tropicalis.* Ethanolic extract of *Z. spina-christi* demonstrated a substantial inhibitory impact on several *Candida* species, especially against *C. glabrata* which has the highest inhibitory effect (90%). Therefore, Saudi *Z. spina-christi* leaves extract is a source of natural antioxidants and it can be used as well antifungal pharmaceutical product.

Keywords

Antioxidant activity, *Candida albicans*, Pharmaceutical preservative, Fresh leaves extract, GC-MS analysis

Introduction

In hospitalized patients, vulvovaginal candidiasis is a high-risk occurrence. *Candida albicans* and other *Candida* species were known as responsible for the great majority of vulvovaginal candidiasis cases (1). Fungal infection is more likely in those with several predisposing conditions, such as diabetes, cellular urine catheters, antibiotics and corticosteroid users (2). Due to a lack of acceptable treatment choices and pathogen cross-resistance to earlier medications (fluconazole and itraconazole), researchers are looking for novel antifungal agents from a variety of sources, including medicinal plants (3, 4).

Candidiasis can be caused by a variety of yeast species in the genus *Candida* (5) They are part of the skin's, mucous membranes and gastrointestinal tract's regular biota. All humans' mucosal surfaces are colonized by *Candida* species during or shortly after birth, whereas the possibil-

ity of endogenous infection is always present. Subsequently, the most common systemic mycosis is candidiasis. Difficulties that arise during *C. albicans* chemotherapy need the development of innovative treatment techniques (6).

Plants have been used from ancient times in folk medicine. They are important sources to develop therapeutic products for health care (7). Medicinal plants are rich in therapeutically bioactive molecules such as flavonoids, alkaloids, coumarins, tannins and terpenes. These compounds are of great interest as sources of natural antioxidants and are recognized by their diverse biological activities (8).

Z. spina-christi tree (family Rhamnaceae) grows in warm-dry areas, largely cultivated in South and West Asia, North and East Africa and the Middle East (9). It is of great concern for the reason that fruits, seeds, bark, roots and leaves have been used in traditional medicine for the treatment of several diseases (10). This plant is rich in biologically active components like flavonoids, terpenoids, vitamins, polysaccharides, polyphenols and tannins and is commonly used in medicine as antimicrobial, antioxidant, anti-inflammatory, antifungal, analgesic, sedative, anticancer, hypoglycemic and reducing cholesterol agent (11, 12). Fruits of *Z. spina-christi* are consumed as a source of energy due to their richness in carbohydrates (13). Moreover, leaves of *Z. spina-christi* had biological applications and can be used for their antipyretic, anti-diarrhoeal, immunomodulator and anti-fertility activities (10). In Arabic countries, *Z. spina-christi* tree has historical, religious and medicinal interests. In Saudi Arabia, *Z. spina-christi* tree is still growing and is distributed in different regions of the Kingdom (14). In traditional Arabic medicine, it is used to treat diarrhea, ulcers and fevers (15). Oxidative stress causes an alteration of the constituents of human cells and is responsible for premature cellular aging. In general, medicinal plants can be used to protect and fight against oxidative damage in the biological system, also to maintain the anti-oxidant system balance to prevent chronic degenerative diseases (16).

In this context, the purpose of this research is to provide a comprehensive study about Saudi *Z. spina-christi* leaves grown on Al-Jouf region, to determine the chemical profile, to evaluate the antioxidant and the *in vitro* antifungal activities of *Z. spina-christi* extract for the treatment of a variety of vaginal infections and inflammatory illnesses using natural substances.

Materials and Methods

Plant material

Fresh leaves of *Z. spina-christi* L. were collected in March 2021 from Sakaka city a region in Aljouf located the north of KSA (latitude: 29.953894, longitude: 40.197044, 29° 57' 14.0184'' N and 40° 11' 49.3584'' E) and were stored in plastic bags in the dark for further use. Then, the plant identified by Doctor Ben Amor botanist in the faculty of sciences, Gafsa, Tunisia.

Leaves extraction

Extract was prepared from fresh leaves of *Z. spina-christi* using Kimax ® Soxhlet extractor apparatus. Fifteen gms of fresh leaves were placed in the Soxhlet extractor and then 250 ml of ethanol were added to the distillation flask. After refluxing for 8 hr, the solvent was evaporated under reduced pressure and the concentrated extract was stored at 4 °C in obscurity until the beginning of the analysis. Extraction was done in triplicate and the yield was 8% w/w (17).

Qualitative phytochemical determination

Qualitative analysis was carried out to detect the presence or absence of alkaloids, glycosides, saponins, phenols, tannins, steroids, terpenoids, anthraquinone and flavonoids according to the common methods described in the literature (18).

Quantitative chemical determination by GC-MS

Fresh leaves extract of *Z. spina-christi* was analyzed using Shimadzu GC-MS- QP2010SE single quadrupole apparatus. GC was equipped with SLB-5MS capillary column (30 m x 0.25 mm; thickness= 0.25 µm). Injector temperature was set at 270 °C and the oven temperature increase from 40 °C to 220 °C (4 °C/min), kept for 10 min then up to 280 °C (5 °C/min). Detector temperature was 270 °C and Helium (carrier gas of 99.99% purity) was used at a flow rate 1 ml/min. A mass spectrum was recorded at energy of ionization of 70 eV. The total analysis time was 120 min and components were identified based on the comparison of their retention time and mass fragmentations patterns with those of standards data of WILEY and NIST libraries (19).

Evaluation of in vitro antioxidant activity

The anti-free radical activity of DPPH· of leaves extract of *Z. spina-christi* was determined based on the standard assays with some modifications (20). Thus, at different concentrations, 1 ml of each tested extract was added to 2 ml of DPPH · solution (0.1 mM). After vigorous stirring, the mixture is incubated for 30 min in the dark and at room temperature and then the absorbance was measured at 515 nm by a UV visible spectrophotometer (JASCO-V530).

The estimated anti-free radical activity was expressed by the value of the percentage inhibition (% I) calculated using the following formula:

$$\% I = [(A_0 \text{ blank} - A_1 \text{ sample}) / A_0 \text{ blank}] * 100$$

A_0 blank is the absorbance of the control reaction containing all reagents except the tested extract (1 ml of ethanol and 2 ml of DPPH) and A_1 sample is the absorbance of the tested sample. Trolox was used as a positive control.

Antioxidant activity was expressed as IC50 (µg/ml) which represent the extract concentration providing 50% inhibition, calculated from the graph plotting inhibition % against sample concentration. A Low IC50 value means a high antioxidant activity of the extract. Tests were carried out in triplicate.

Evaluation of in vitro antifungal activity

Collection of samples

A sterile swab was used to collect vaginal fluids from females with different ages from (25 to 50) years old. Under sterile conditions, 3 specimens were taken simultaneously, one for light microscopic investigation and the other for fungal culture. Materials were inoculated with Sabouraud's Dextrose Agar and incubated at 35 °C until colonial appearance. Fungal cultures were maintained in Sabouraud's Dextrose Agar at 5 °C followed by serial subculturing every 3 months. All fungi were kept at the Biology Department, College of Science, Jouf University with a number of *C. albicans* (JU 01032), *C. parasilopses* (JU 01033) and *C. tropicalis* (JU 10134).

Identification of isolated fungi

Candida spp. were identified using morphological and physiological methods such as growth characteristics and carbon source assimilation or fermentation, as well as the appearance on differential isolation media. According to the manufacturer's recommendations, the HiCandida identification kit was used to accurately identify *Candida* species. A plastic strip had twelve wells containing sterile media for several biochemical assays as follows: well 1, medium for urease detection, and wells 2-12, medium for carbohydrate utilization (with 11 different sugars in respective wells, including, melibiose, lactose, maltose, sucrose, galactose, cellobiose, inositol, xylose, dulcitol, raffinose and trehalose) (21, 22).

The homogeneous yeast suspension (10^6cells/ml) was produced and injected into kit wells, then incubated for 24-28 hrs at 22.5 2.5 °C. The color of the kit changed after the incubation period: well 1 containing urease was considered positive if the yellow color changed to pink. If the color of wells 2-12 changed from orange to yellow after 72 hrs, the result was considered positive; if the color remained orange, the result was regarded as negative. Findings were interpreted according to the manufacturer's guidelines.

Well Diffusion Assay

Inhibitory zones of *Z. spina-christi* leaves extract were tested using the well assay technique against *C. albicans*, *C. glabrata* and *C. tropicalis* strains to determine the effective concentration. Overnight inoculum of *Candida* spp. were disseminated over Sabouraud's dextrose agar media and 1 ml of different concentrations of the extracts (5, 10, 15 and 20 mg/ml) were added to each well (10 mm diam.) and incubated at 26 °C for 48 hrs (23, 24). As a positive control, miconazole was utilized (25).

Statistical Analysis

Analysis of variance as One-way (ANOVA) and Statistical Package for Social Sciences (SPSS) software version 12.0 were conducted for statistical analysis of the obtained results. Antioxidant and antifungal activities were carried out in 3 experiments. Differences among the mean values of the various treatments were determined by the least significant difference test. A probability level of $P < 0.05$ was used in testing the statistical significance of all experimental data (26).

Results and Discussion

Qualitative phytochemical determination

The qualitative analysis showed that the leaves extract of *Z. spina-christi* was rich in phytochemicals belonging to different classes as illustrated in Table 1.

Table 1. Phytochemical screening of *Z. spina-christi* leaves extract.

Phytochemicals	Extract
Alkaloids	+
Glycosides	+
Saponins	++
Flavonoids	-
Phenols	++
Tannins	++
Steroids	+
Terpenoids	+
Anthraquinone	-

++ phytochemical detected at appreciable amount; + phytochemical detected at trace amount; - phytochemical not detected

This analysis was based on the study of the presence or absence of several phytochemicals. Alkaloids, glycosides, saponins, phenols, tannins, steroids and terpenoids were detected with different amounts, while anthraquinone and flavonoids were absent. To obtain a more detailed result on the phytochemical composition and their %, this study will be completed by an analysis using the GC-MS technique.

Quantitative chemical determination by GC-MS

The chemical composition of green leaves extract obtained from *Z. spina-christi* was identified and quantified by GC-MS technique. Analysis of the obtained result shows that the extract is rich in chemical compounds. Indeed, it contains 38 phytochemical constituents with different diverse chemical groups representing 99.71 % of the total extract (Table 2).

Table 2. Identified volatile compounds of *Z. spina-christi* leaves extract.

Peak	RT (min)	Chemical Compound	Peak Area (%)
1	11.684	N-[(E)- 3- methyl-2-butenylidene] methanamine	0.56
2	13.314	Isooctanol	0.73
3	16.428	2-tridecanol	0.64
4	16.559	Diethyl phthalate	0.45
5	19.845	11-Dodecenol	1.53
6	20.160	1-Ethynylcyclopentanol	0.52
7	20.396	Dodeca-1,6-dien-12-ol	0.62
8	21.402	n-hexadecanoic acid	0.60
9	21.465	Dibutyl phthalate	0.75
10	23.211	Phytol	2.74
11	23.520	1,2,3,4-tetrahydrostyrene	0.74
12	24.008	Hexadecanoic acid butylester	1.45

13	26.146	Octadecanoic acid butyl ester	1.32
14	27.085	2,4-Difluorophenol	0.39
15	27.252	1-Chloroheptacosane	1.31
16	27.431	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester	3.20
17	27.789	1-(4-bromobutyl)-2-piperidinone	0.53
18	28.217	Pentatriacontane	0.82
19	29.014	7-hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	1.12
20	29.145	Tritetracontane	2.10
21	29.286	3-chloropropionic acid octadecyl ester	1.61
22	29.428	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester	18.80
23	30.046	14B-Pregnane	3.85
24	30.366	Squalene	5.94
25	30.943	Carbonic acid,isobutyl octadecyl ester	4.53
26	31.173	Diethylmethylborane	1.60
27	31.278	8-nitro-12-tridecanolide	1.88
28	31.750	Sulfurous acid, octadecyl 2-propyl ester	6.64
29	31.912	(cis)/trans-2-(2,2-dimethylpropanoyl)-5-methylcyclopentanone	1.40
30	32.300	Bacchotricuneatin c	4.37
31	32.463	1-bromo-11-iodoundecane	2.81
32	32.636	17-pentatriacontene	5.80
33	33.029	Vitamin E	9.70
34	33.228	(8R, 12R)-8,12-Epoxy-13,14-dihydroxy-labdane	3.31
35	34.051	HAHNFETT	1.27
36	34.392	Oxalic acid, allyl pentadecyl ester	0.67
37	35.052	Beta- sitosterol	2.35
38	36.127	1,7-dimethyl-4-(1-methylethyl)cyclodecane	1.06

Detailed chemical analysis shows that the extract was mainly composed by Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester (18.80%), followed by Vitamin E (9.70%), Sulfurous acid, octadecyl 2-propyl ester (6.64%), Squalene (5.94%), 17-pentatriacontene (5.80%), Carbonic acid, isobutyl octadecyl ester (4.53%), Bacchotricuneatin c (4.37%), 14B-Pregnane (3.85%), (8R, 12R)-8,12-Epoxy-13,14 -dihydroxy-labdane (3.31%), Hexadecanoic acid, 2-hydroxy -1-(hydroxymethyl) ethylester (3.2%), 1-bromo-11-iodoundecane (2.81%), Phytol (2.74%), Beta- sitosterol (2.35%), Tritetracontane (2.10%), 8-nitro-12-tridecanolide (1.88%), 3-chloropropionic acid octadecyl ester (1.61%), Diethylmethylborane (1.60%), 11-Dodecenol (1.53%), Hexadecanoic acid butylester (1.45%), (cis)/trans-2-(2,2-dimethylpropanoyl)-5-methylcyclopentanone (1.40%), Octadecanoic acid butyl ester (1.32%), 1-Chloro heptacosane (1.31%), Hahnfett (1.27%), 7-hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin (1.12%) and 1,7-dimethyl-4-(1-methylethyl) cyclodecane (1.06%). The other compounds were detected in traces (less than 1%).

The chemical analysis of leaves extract of *Z. spina-christi* showed that fatty ester derivatives (32.78%), hydrocarbons (28.27%) and alcohols (19.22%) were the dominant compounds and represent 80% of the total bioactive constituents (Table 3).

Table 3. Classification of the volatile compounds of *Z. spina-christi* leaves extract according to the chemical classes.

S.No	Chemical classes	Percentage (%)
1	Esters	32.78
2	Hydrocarbons	28.27
3	Alcohols	19.22
4	Sulfur containing compounds	6.64
5	Pyrans	4.37
6	Ketones	3.05
7	Nitrogen containing compounds	2.44
8	Others	2.34
9	Acids	0.6
	Total	**99.71**

Among the identified phytochemicals, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester was found to be the most dominant compound which is a fatty acid ethyl ester of glycerol derivative belonging to fatty acid esters secondary metabolites. A recent study has shown that Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester can be used for the treatment of C. Violaceum infections and can be evaluated for other pharmacological activities (27). Also, a theoretical predictive study showed that Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester has interesting biological activities such as All-trans -retinyl-palmitate hydrolase inhibitor, Lipid metabolism regulator, Eye irritation, inactive, Antieczematic, CYP2J substrate, Acylcarnitine hydrolase inhibitor, CYP2J2 substrate, Linoleate diol synthase inhibitor, Lipoprotein lipase inhibitor, GST A substrate, Macrophage colony-stimulating factor agonist, Alkenylglycerophosphocholine hydrolase inhibitor, Phosphatidylglycerophosphatase inhibitor (28).

Furthermore, some other identified phytochemicals have been reported to have interesting biological properties as antibacterial, antioxidant, analgesic, antiviral, hypoglycemic, anti-inflammatory, antifertility and more as shown in Table 4.

Table 4. Biological activities of some phytochemicals identified from *Z. spina-christi* leaves extract according to Dr. Duke's Phytochemical and Ethnobotanical databases.

S. No	Name of compounds	Biological Activity
1	Vitamin E	Analgesic, Antiaggregant, Antiagiant, Antialzheimeran, Antiatherosceloric, Antidecubitic, Antifibrostic, Antihereptic, Anti-inflammatory, antioxidant, Anti-tumor…
2	Squalene	Antibacterial, antitumor, cancer-preventive, Immunostimilant, lipoxygenase inhibitor, perfumery, pesticide, sunscreen

3	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester	Pesticide, hemolytic, flavor, anti-oxidant.
4	Phytol	Cancer-preventive
5	Beta- sitosterol	Anorexic, Antibacterial, anti-cancer, antiestrogenic, antifertility, anti-inflammatory, antioxidant, antiviral, hypoglycemic….
6	Hexadecanoic acid butylester	Antimicrobial, antioxidant
7	1,7-dimethyl-4-(1-methylethyl) cyclodecane	Cytotoxic
8	Pentatriacontane	Herbistat
9	n-Hexadecanoic acid	Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Hemolytic, Pesticide, Lubricant, 5 -Alpha reductase inhibitor, anti-psychotic.

The study of the bioactive components of leaves methanolic extract of *Z. spina-christi* from the campus of South Valley University, Qena, Egypt showed the presence of 13 components, while Phenol, 2,5-bis (1,1-dimethylethyl) (40.24%) and Decane, 2-methyl-(18.53%) were the most abundant components (29). Comparing to our results, the difference of chemical composition may be due to geographic conditions (humidity, temperature, altitude), the origin and the period of leaves harvest and the soil-growth conditions (30).

Evaluation of in vitro antioxidant activity

The antioxidant activity was evaluated by studying the reducing effect of different concentrations of *Z. spina-christi* leaves extract on DPPH radical compared to those of Trolox (positive control). The obtained results show that the extract has a great free radical scavenging activity with IC_{50} of 148.33 µg/ml (Fig. 1).

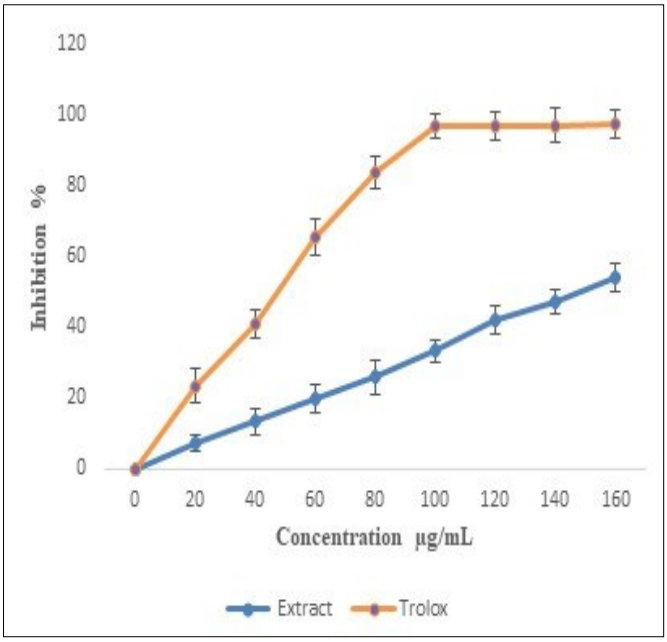

Fig. 1. Antioxidant activities of *Z. spina-christi* leaves extract at different concentrations. Trolox was used as a positive control.

A study of different *Z. spina-christi* leaves extract from five provenances in Saudi Arabia showed that our results were in consistence with those obtained from Mecca Road Provenance (0.15 mg/ml), while Al-Taif, Riyadh and Jeddah provenances have a lower value of IC50 (0.06, 0.08 and 0.07 respectively) (31).

The chemical analysis shows the richness of *Z. spina-christi* leaves extract by bio-actives compounds belonging to different classes such as esters, hydrocarbons, alcohols, ketones, nitrogen and sulfur compounds and fatty acids which might be the origin of a good antioxidant capacity as Vitamin E (32), Squalene (33), 14B-Pregnane (3.85%) (34, 35), Bacchotricuneatin c (36), Phytol (37), Beta-sitosterol (38), Hexadecanoic acid butylester, (39) and Hexadecanoic acid (40).

The extract can be applied as a natural supplement in the field of the food industry due to its antioxidant activity.

Evaluation of in vitro antifungal activity

From 40 vaginal swab samples that were collected from patients with vaginitis, 28 samples were positive and 12 samples were negative, as well as the frequency of isolates according to a woman's age was described in Table 5. Identification tests for all isolates reproach the following species: *C. albicans* 17 isolates, (42.5 %), *C. glabrata*, 9 isolates, (22.5%), and *C. tropicalis* 14 isolates (35%), respectively. Statistical analysis for age groups (25, 30, 35, 40 and 45) showed non-significant differences between means ($p \leq 0.05$) (Table 5). It was obvious that the majority of isolates belonged to the genus *C. albicans*, followed by *C. tropicalis* and *C. glabrata* respectively. The less frequent yeast species was *C. glabrata*.

Table 5. Statistical analysis of the relationship between age groups and Candida isolate.

***Candida* spp.**	**Age groups (8 samples/each group)**				
	21- 25	**26- 30**	**31- 35**	**36- 40**	**41- 45**
C. albicans	5±0.3	2±0.34	3±1.54	4±0.32	3±1.54
Candida glabrata	0	1	3±0.98	0	3±.05
Candida tropicalis	3±1.43	5±0.24	2±0.54	4±0.54	2

Values are given as mean ± SD of three replications.
Statistical significance of differences between the means of groups: No Significant at $p < 0.05$ according to ANOVA test.

Using an agar well diffusion test, antifungal effects of different concentrations of *Z. spina-christi* leaves extract on *Candida* spp. were evaluated in Table 6, indicating different concentration manifested inhibition zones with different diameters on Sabouraud's dextrose agar medium.

It has been shown that the concentration of 20 mg/ml of ethanolic *Z. spina-christi* leaves extract displayed the strongest antagonist effect against *C. glabrata, C. tropicalis and C. albicans* by 77, 75 and 70 mm respectively. Followed by a concentration of 15 mg/ml of extract appeared a moderate effect against *C. glabrata, C. albicans* and *C. tropicalis* (62, 60 and 58 mm respectively). Then, 5 mg/ml of extract gave the lowest effect, when compared with the control (Table 6 and Figs. 2-3).

Table 6. Mean zones of inhibition (mm) of *Z. spina-christi* leaves extract against *candida* spp**.**

Species	Mean zone of inhibition (mm)			
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml
Miconazol	60 ± 0.02	70 ± 0.18	80 ± 0.10	80 ± 0.32
C. albicans	50 ±0.20	56 ± 0.16	60 ± 0.13	70 ± 1.9
Candida glabrata	48 ± 0.12	54 ± 0.14	62 ± 0.24	77 ± 0.6
Candida tropicalis	48 ± 0.25	56 ± 0.20	58 ± 0.5	75 ± 0.31

Values are given as mean ± SD of three replications.
Statistical significance of differences between the means of groups: Highly Significant at p <0.05 according to ANOVA test

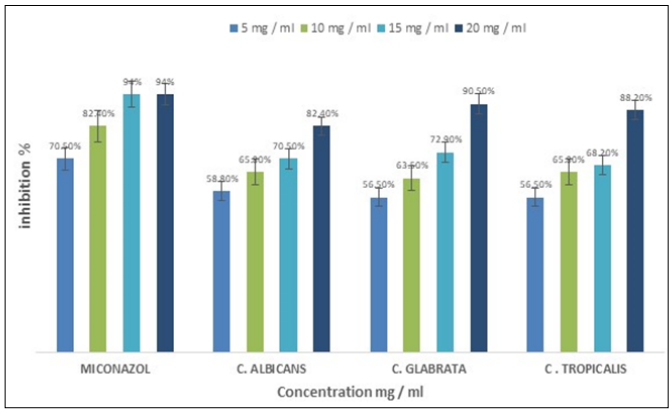

Fig. 2. Inhibition % of *Candida* spp. exposed to different concentrations of *Z. spina-christi* leaves extract on Sabouraud's dextrose agar at 35 °C for 48 hr in the dark.

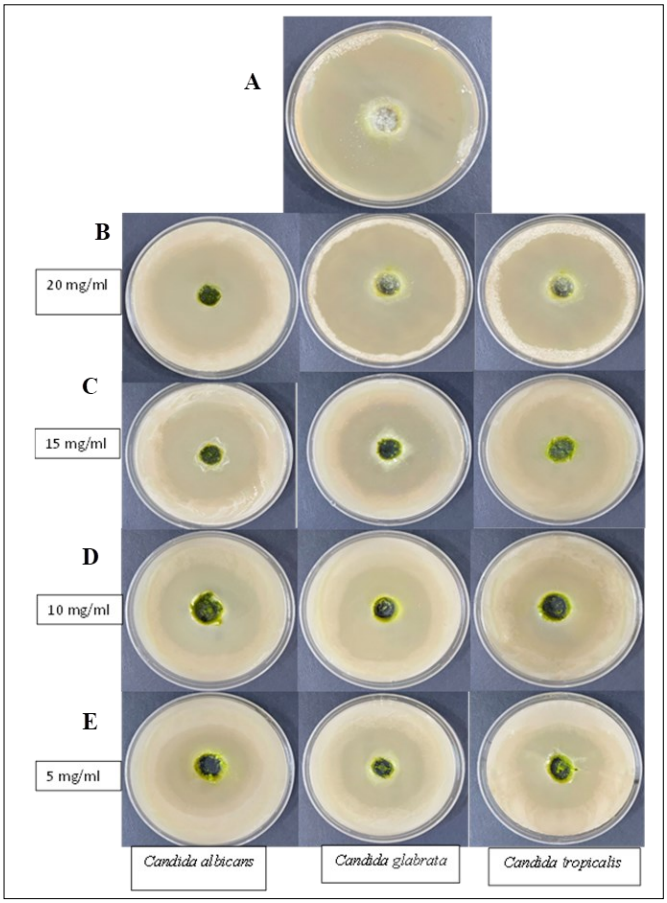

Fig. 3. Inhibition of *Candida* spp. **A**) Control containing miconazole, **B**) Treated containing *Z. spina-christi* leaves extract (20 mg/ml), **C**) Treated containing *Z. spina-christi* leaves extract (15 mg/ml), **D**) Treated containing *Z. spina-christi leaves extract* (10 mg/ml) and **E**) Treated containing *Z. spina-christi leaves extract* (5 mg/ml) on Sabouraud's dextrose agar media at 26 °C for 48 hr at the dark.

According to microscopy, *Z. spina-christi* leaves extract at 20 mg/ml caused significant changes in the shape and density of *Candida* spp. mycelia. When compared to the control, Fig. 4 demonstrates more morphological changes such as mycelium deformation, perforation, cell lysis and mycelium destruction.

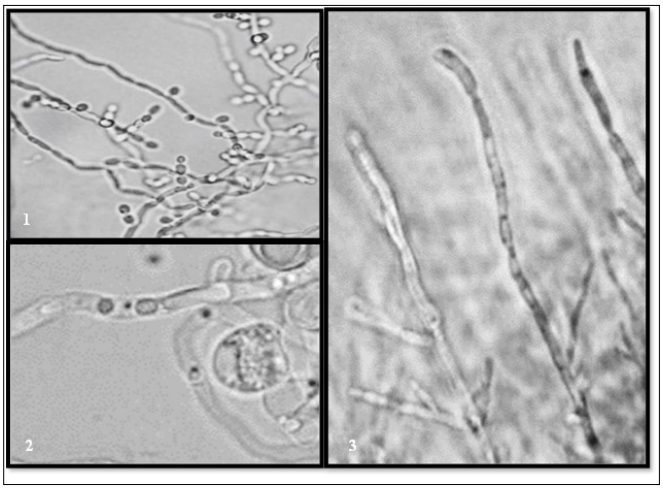

Fig. 4. Represented microscopic examination (magnification: X 10) of antagonistic effect *Z. spina-christi* leaves extract (20 mg/ml) against *C. albicans*. (**1**) Healthy mycelium. (**2** and **3**) Abnormal structure, lysis and coagulation of mycelium structure.

Several studies have highlighted the antifungal properties of the *Ziziphus* genus. It was demonstrated that the ethanolic extract of *Z. spina-christi* has antifungal efficacy against *Candida* spp. Confirmation was on the antifungal efficacy of *Z. spina-christi* extract against fungus strains in another study (41, 42). According to one report, methanolic and ethanolic extracts of *Z. Spina-christi* displayed antifungal efficacy against *Candida* spp. The microemulsion of ethanolic and methanolic extracts showed to be more fungicidal than ethanolic and methanolic extracts against *C. albicans* (43).

It was showed that aqueous extract of *Ziziphus* sp. can control the growth of *Alternaria brassicae* and *Fusarium oxysporum* (44).

Previous research has found a link between the antifungal properties of plant extracts and the solvents used; the polar extract contains saponins and glycosylated flavonoids, whereas the non-polar extract contains non-polar components such as terpenoids (45). Alkaloids, glycosides, saponins, phenols, tannins, steroids and terpenoids were detected with different amounts in *Z. spina-christi* leaves extract (Table 1) is thought to be responsible for its dominating action. Some of these chemicals, particularly terpenes were previously described to have antibacterial, fungicidal and insecticidal properties (46-49).

By entering between the fatty acyl chains, terpenes have been shown to alter the fungal cell permeability. Furthermore, terpenes impede Candida's respiratory chain, implying negative effects on mitochondria (50, 51). Extracts of *Z. spina-christi* were found to reduce *C. albicans* biomass by raising glucose levels and decreasing cell dry weight. This process might be the result of cell wall breakdown and subsequent sterilization (52). As a result, it is possible that the ethanolic extracts increased antifungal

activity and served as a catalyst for extract penetration through the fungal cell wall. These support the use of *Z. spina-christi* extract to treat yeasts that attack vagina.

Conclusion

This study demonstrated that the extract of *Z. spina- christi* L. grown in is rich in bioactive compounds and predominantly by Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester. *Z. spina-christi* leaves extract provide a good antioxidant activity, which makes it possible to use in food industry. Furthermore, this extract can be used to treat a variety of infections and inflammatory disorders caused by *C. albicans* and *C. tropicalis*. These results indicate that this raw material could be used in pharmaceutical formulation as a vaginal lotion.

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Authors contributions

SMNM performed the isolation, identification of fungal and writing the antifungal section. HB prepared the plant extract, phytochemical analysis, antioxidant activity and writing the chemical section. HMAA completed the statistical analysis and editing the final draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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