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Assessment Of The Antifungal Bioactivity Of Aqueous Extract From Ziziphus Spina-Christi Leaves Against Candida SPP. Responsible For Vulvovaginal Candidiasis

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Abstract. *The present study was aimed at gaining knowledge about the type of pathogen responsible for Vulvovaginal Candidiasis (VVC) infections and evaluating the antimicrobial activity of plant extracts against Candida spp. antibiotic resistance. 112 samples were taken from infected women with VVC of different ages from September to December 2024. All specimens were collected from Al Hussein Teaching Hospital/ Al- Thiqar-governorate. The results showed that 67 (59.82%) of all cases had positive results for Candida spp., while 45 (40.18%) had negative results. Candida spp. Isolated from VVC were C. albicans, the most prevalent 33 (49.26%), C. glabrata, 12 (17.91%), C. krusi and C. kefyr, 9 (13.43%), and C. guilliermondii, 4 (6.90%). The antifungal sensitivity of the pathogenic Candida spp. was tested against five types of antifungals, and the results showed the highest resistance to ketoconazole was 66 (98.51%) of Candida spp. Because of the presence of these physiologically active chemicals, Zizyphus spina-christi L. exhibits antimicrobial capabilities. In this study, the main aim is to study the antifungal activity of Ziziphus spina-christi leaf extract on some resistant candida and conduct analysis to confirm that the Ziziphus spina-christi leaves have phytochemical contents. Gas chromatography-mass spectrometry (GC-MS) was used to find the bioactive components present in extracts of Z. spina-christi leaves. Finally, we decided the inhibitory activity of Ziziphus spina-christi leaves extract. The extract of Ziziphus spina-christi had alkaloids, saponins, flavonoids, phenols, tannic acid, and terpenoids, according to a preliminary phytochemical screen. In addition, GC-MS analysis of the extract confirmed the presence of many beneficial chemicals. The extract showed promising antimicrobial activity for Candida spp. (C. albicans, C. glabrata,C. krusi , C. kefyr, and C. guilliermondii) . These results show that the Ziziphus spina-christi leaves extract is a valuable resource for bioactive chemicals with potential uses in a variety of biological contexts.*

Keywords: *Vulvovaginal Candidiasis (VVC),Candida spp.,Antifungal,Ziziphus spina-christi leaves extract, Antimicrobial Activity.*

Abstrak. Penelitian ini bertujuan untuk mengetahui jenis patogen yang bertanggung jawab atas infeksi Vulvovaginal Candidiasis (VVC) dan mengevaluasi aktivitas antimikroba ekstrak tanaman terhadap resistensi antibiotik Candida spp. Sebanyak 112 sampel diambil dari wanita yang terinfeksi VVC dari berbagai usia pada periode September hingga Desember 2024. Semua sampel dikumpulkan dari Rumah Sakit Pendidikan Al Hussein / Provinsi Al-Thiqar. Hasil penelitian menunjukkan bahwa 67 (59,82%) dari seluruh kasus menunjukkan hasil positif untuk Candida spp., sementara 45 (40,18%) menunjukkan hasil negatif. Candida spp. yang diisolasi dari VVC adalah C. albicans, yang paling prevalen yaitu 33 (49,26%), C. glabrata 12 (17,91%), C. krusi dan C. kefyr 9 (13,43%), dan C. guilliermondii 4 (6,90%). Uji kepekaan antifungal terhadap Candida spp. patogen dilakukan terhadap lima jenis obat antijamur, dan hasilnya menunjukkan resistensi tertinggi terhadap ketokonazol yaitu 66 (98,51%) dari Candida spp. Karena adanya senyawa aktif fisiologis, Zizyphus spina-christi L. menunjukkan kemampuan antimikroba. Dalam penelitian ini, tujuan utamanya adalah untuk mengkaji aktivitas antifungal ekstrak daun Ziziphus spina-christi terhadap beberapa Candida yang resisten dan melakukan analisis untuk memastikan bahwa daun Ziziphus spina-christi mengandung zat fitokimia. Gas kromatografi-spektrometri massa (GC-MS) digunakan untuk menemukan komponen bioaktif yang terkandung dalam ekstrak daun Z. spina-christi. Akhirnya, kami memutuskan aktivitas penghambatan dari ekstrak daun Ziziphus spina-christi. Ekstrak daun Ziziphus spina-christi mengandung alkaloid, saponin, flavonoid, fenol, asam tanat, dan terpenoid, menurut skrining fitokimia awal. Selain itu, analisis GC-MS dari ekstrak mengonfirmasi adanya banyak senyawa

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bermanfaat. Ekstrak ini menunjukkan aktivitas antimikroba yang menjanjikan terhadap Candida spp. (C. albicans, C. glabrata, C. krusi, C. kefyr, dan C. guilliermondii). Hasil ini menunjukkan bahwa ekstrak daun Ziziphus spina-christi merupakan sumber yang berharga untuk senyawa bioaktif dengan potensi penggunaan dalam berbagai konteks biologis.

Kata kunci: Vulvovaginal Candidiasis (VVC), Candida spp., Antifungal, Ekstrak daun Ziziphus spina-christi, Aktivitas Antimikroba.

1. INTRODUCTION

Infections caused by commensal yeasts like *Candida* spp. are increasing due to their flexibility and resistance to antifungal medications, prompting the development of effective techniques for fungal survival. (Ciurea et al., 2020). *Candida* species, a normal part of the microbiota in healthy individuals, can become opportunistic when the host is weak (Brillowska-Dabrowska et al., 2010). The efficiency of Candida spp. in invading and infecting hosts is attributed to the expression of virulence factors like hydrolytic enzyme production, surface adhesion, biofilm development, and morphological transition. (Deorukhkar et al., 2014) Candidal vulvovaginitis, a multifactorial infectious disease-causing pathologic inflammation in the lower female reproductive tract, is primarily caused by Candida albicans and related Candida species. (Willems et al., 2020). Around 75% of all females are expected to experience VVC at some point in their lives (Sobel & Chaim, 1997). Vaginal candidiasis, a non-lethal disease affecting immunocompetent, healthy women, has a significant global incidence and impact on quality-of-life, needing further study of host and fungal mechanisms. (Willems et al., 2020). Multidrug-resistant fungal infections cause global morbidity and mortality. Standard antifungal drugs have low efficacy and potential side effects. Limited systemic antimycotic medications make antifungal resistance a significant clinical issue (Perfect & Ghannoum, 2020). Fluconazole, an azole antifungal, is the most effective treatment for most cases of *C.albicans* infection (Pfaller et al., 2010). Imidazoles, including ketoconazole, clotrimazole, and miconazole, are major class of azole antifungals, while triazoles, including Itraconazole, Fluconazole, Voriconazole, and Posaconazole, are minor classes (Rós Ásmundsdóttir et al., 2008). Researchers have combined fluconazole and amphotericin B to create new antifungals, enhancing yeast cell permeability and inducing biofilm formation defects in *C. albicans* cells (do Nascimento Dias et al., 2020).

Ziziphus is a plant genus belonging to the family Rhamnaceae, consisting of approximately 100 species of both deciduous and evergreen trees and shrubs. These plants are primarily found in tropical and subtropical regions (10). Research has proven the antimicrobial properties of preparations derived from Zizyphus spina-christi L. leaves and fruits against various microorganisms such as Staphylococcus aureus, Candida albicans, Bacillus subtilis,

and Escherichia coli (11). However, despite the potential of Z. spina-christi as a source of antimicrobial compounds and other functional groups, there is currently no available literature on the antifungal efficacy of methanol extract obtained from the leaves and fruits of Z. spina-christi against Alternaria sp. (11). The Ziziphus genus has been found to contain useful compounds such as vitamin C, amino acids, triterpene acids, polysaccharides, polyphenols, flavonoids, saponins, alkaloids, indole derivatives, and fatty acids (11). While these compounds have been more commonly studied for their medical applications, their potential in crop protection has not been as extensively explored (12).

Methanol extract is often preferred due to its higher extraction efficiency for a wide range of bioactive chemicals. Studies have shown that the methanolic extract of Z. spina-christi roots and fruits shows antifungal efficacy against dermatophytes, including Trichophyton rubrum, T. mentagaphytes, Microsporum canis, Aspergillus fumigatus, and Candida albicans (13). However, further research is needed to investigate its effectiveness against Alternaria sp. In summary, the Ziziphus genus has various bioactive compounds with potential antimicrobial and antifungal properties. While studies have proven the effectiveness of Z. spina-christi extracts against certain microorganisms, further research is necessary to evaluate its specific impact on Alternaria sp. Additionally, the potential applications of these compounds in crop protection call for further exploration. Natural and plant-based products are on the rise since they pose no threat to human health and have no impact on the environment. In addition to being more cost-effective than chemicals, these materials also do negligible harm to their hosts. There are many issues with chemical fungicides, including their high acute and chronic toxicity, slow breakdown times, buildup in the food chain, and the fact that they can now kill out beneficial as well as harmful pests. These drawbacks are mitigated by plant extracts (14). Because of this, researchers are starting to pay more attention to biological control of plant diseases and biological control programs in order to learn more about the long-term effects of the application (15). the plant leaves are commonly known by their antibacterial efficacy.

2. MATERIALS AND METHODS

Samples Collection

In this study, 112 samples were collected from infected women with (VVC) from period September to December 2024. All specimens were collected from Al Hussein Teaching Hospital/ Al- Thiqar governorate. Clinical presentations were done by specialized doctors, and Informational on each patient was collected using a designed questionnaire. The specimens were taken by sterilized cotton swabs and were divided in to two smears: one smear was

examined at once under microscope for direct examination; the other usually was cultured on Sabouraud Dextrose agar (SDA).

Sample Culture and Identification

The samples were streaked directly onto SDA enriched with chloramphenicol (250 mg/liter) and HiChrome candida differential media (HiMedia Laboratories, India). and incubated aerobically at 35±2°C for 48 hours (16) (Golla Eshwara Chandra, Goteti Venkata Padmaja, 2023).

To ensure reproducibility, three replicates of each sample were used. The isolates were phenotypically found based on gram reaction and direct wet preparation (10% KOH). The species status of each isolate compared to control strains was confirmed using the Vitek Compact 2 system (Laouini & Ouahrani, 2017) (BioMerieux Inc., Durham, NC 27712, USA).

Antifungals Agents' Susceptibility Test

The modified Kirby-Bauer method (Vandeputte et al., 2012) (60). The MICs for ketoconazole, itraconazole, clutraimazole, amphoterecin-B, and fluconazole were interpreted based on the Clinical and Laboratory Standard Institute (CLSI, 2011).

Plant Collection and Aqueous Extraction

Fresh leaves of Ziziphus Spina-Christi from a single population were collected from Al Hussein Teaching Hospital /Al- Thiqar governorate. The leaves were dried, crushed, and blended into powder. A cold extraction process was used, followed by homogenization, filtering, and concentration. The extracts were stored in sterile bottles and stored at 2-4°C. according to Ingle *et al*. (18).

Quantitative Determination of Phytochemical Constituents of Ziziphus spina-christi Leaves

The sample was dried in the shade at room temperature for 24h, then ground to a fine powder by an electric mixer, 5g of it was taken and placed in a Soxhlet and extracted with 300ml of ethanol at 50-55°C for 3-4 hours. The extract was filtered through a filter paper (Whatman no. 1), and the extract was concentrated using a rotary evaporator at low pressure and at a temperature of 40°C, then the extract was weighed at 2.6g and kept in a bottle until use at 4°C. The total phenols were revealed according to the larva that was brought by the scientist Follen using Gallic acid (Sigma, Aldrich, Germany) by taking 150ml of Folin reagent

(Folin-Ciocalteu, Merck, Germany) with 500µl of alcoholic extract, then adding 1.5ml of 20% sodium carbonate and then mixing well. The final volume was completed to a volume of 10ml, and the absorbance values were recorded after two hours of reaction at a wavelength of 765nm. The total concentration of phenols relative to the titration curve of gallic acid was calculated in units (mg/g dry weight) (19).

Phenol: The study involved drying a sample, grinding it into a powder, extracting it with ethanol, filtering it, and concentrating it. The total phenols were decided using Gallic acid, a reagent, and sodium carbonate. The absorbance values were recorded after two hours of reaction at a wavelength of 765nm. The concentration of phenols was calculated in units (mg/g dry weight) according to the titration curve of gallic acid (19).

Alkaloids: For detection of alkaloids, 20g of the sample was ground and then extracted with methanol for 24h in an extractor Soxholet The extract was filtered, and the methanol was evaporated by a rotary evaporator at a temperature of 45°C until it dried. The method that the scientist (20) came to detect was followed by using the Dragendroff reagent, where it was extracted in the Soxholet apparatus using alcohol, and then 1ml of the extract was taken and 50ml of HCL acid was added to it at a concentration of 10% and a few drops of Dragon drops were added. 10ml of chloroform were added and the pH was adjusted to 7 using sodium hydroxide at a concentration of 1N. A titration curve of atropine was prepared by preparing several concentrations and it was measured at a wavelength of 470 nm.

The study used Harborne's method to quantitatively decide alkaloid in wood powder samples. The extract was concentrated, precipitated, washed, and filtered. The residue was dried in an oven, and the percentage of alkaloid was calculated using an electronic weighing balance. The process involved several steps (20).

Flavonoid: The determination of the total flavonoid content in the crude extract was carried out using the aluminum chloride measurement method. The absorbance of the extract was recorded at a wavelength of 510 nm, and this measurement served as a reference. The quantification of the flavonoid concentration was then performed by comparing the titration curve obtained for rutin and expressed in mg/g of dry weight (21). Thus, the concentration of flavonoids in the crude extract was evaluated using aluminum chloride and a standard titration curve with rutin, and the results were reported in units of mg/g dry weight (21).

Saponins: The determination of saponin content in the sample was conducted using an extraction method (22). Initially, butanol was employed for the extraction of saponins, and the resulting extract was after washed with a 5% NaCl solution. The extract was then evaporated to dryness in an evaporating dish that had been pre-weighed and dehydrated at 60°C in an

oven. After cooling in a desiccator, the dish was re-weighed to obtain the weight of the extract. This process was repeated two more times, and the mean value was calculated to decide the saponin content in the sample.(22) In another extraction method specified by the same reference (22), a mixture of powdered leaves was heated over a hot water bath for a period of 4 hours. Subsequently, another round of extraction with ethanol was performed, followed by evaporation. The resulting concentrate was mixed with diethyl ether, and the aqueous layer was separated. N-butanol was added to the aqueous layer and subjected to two extractions with sodium chloride. The remaining solution was then heated, dried, and the saponin content was calculated. It should be noted that the saponin content in the original sample may vary based on the percentage of the original sample used in the extraction process.

Percentage of saponins = (W2–W1/Wt. of sample) × 100

W1= weight of evaporating dish; W2 = weight of evaporating dish + sample.

tannic acid: The quantitative determination of tannin was performed following the analytical method described by Amadi et al. (23) and Ejikeme et al. (24). To prepare the Folin-Denis reagent, sodium tungstate was dissolved in distilled water, followed by the addition of phosphomolybdic acid and orthophosphoric acid. The resulting solution was then diluted .For the analysis, leaf powder samples were added to distilled water and subjected to boiling. The mixture was after filtered, and the filtrate was combined with distilled water and a diluted extract to ease color development. The optical density of the solution was measured at a wavelength of 700 nm using a Spectrum Lab 23A spectrophotometer .To construct the tannic standard curve, 0.20g of tannic acid was dissolved in distilled water and diluted. The optical density was measured for varying concentrations of tannic acid, and these values were plotted against the corresponding tannic acid concentrations. The later computation used the formula provided in the original reference.

Tannic acid(mg/100mg) =C × extract volume × 100/ aliquot volume ×weight of sample.

C: is concentration of tannic acid read in the graph.

Terpenoids: This study introduces a modified procedure for analyzing total terpenoids concentrations in plant material described by (Ghorai et al., 2012) homogenizing it with tungsten carbide beads and methanol, incubating it at room temperature for 48 hours, centrifuging the samples, adding chloroform and sample supernatant, vortexing the mixture, adding concentrated sulfuric acid, incubating the assay tube for 1.5-2 hours in the dark, and forming a reddish-brown precipitation. The precipitate is then decanted and dissolved in methanol. The sample is then transferred to a colorimetric cuvette to read absorbance at 538 nm. A standard curve is calculated from the blank corrected at a wavelength of 538 nm of the

linalool standard, and the total terpenoid concentration of the unknown plant sample as linalool equivalents is calculated using the regression equation of the linalool standard curve (23).

Gas Chromatography-Mass Spectrometry Analysis

The leaves powder (20 gm) is extracted using a n-hexane (350 ml) apparatus, which takes 10 hours, and then filtered and concentrated using a rotary vacuum evaporator. The Shimadzu GC-2010 plus chromatograph was used in a study, kept at 280oC. The DB-5 column was used, with a nonpolar, cross-linked, and cross-linked structure. The sample was carried from the injector to a quadrupole-kind detector using a 70-eV potential for electron impact.

Inhibitory Activity of Extract

The agar diffusion method described by (Yang et al., 2020) (25) was employed in this study. Mueller-Hinton agar (MHA) media was poured into plates and allowed to dry. Bacterial cultures from stock cultures were spread on the media using a cotton swab. Subsequently, a fungal tablet was placed on the surface of the inoculated medium using a Cork borer, which created a circular hole with an 8 mm diameter. The plates were then incubated at 37°C for 48 hours to allow for the growth of microorganisms. The inhibition zone, showed by clear zones surrounding the fungal tablet, was measured. Each microorganism with the corresponding antibiotic was combined three times for analysis.(25) Statistical analysis was performed by expressing the results as mean ± standard deviation (SD). A significance level of $p>0.05$ and $p>0.01$ was considered statistically non-significant, while a significance level of $p<0.05$ was considered significantly different. The statistical analysis was conducted using SPSS (version 20).

3. RESULTS

Distribution of *Candida* spp. from VVC

Sixty-seven (59.82%) of all cases (112) gave positive results for *Candida* spp., while 45 (40.18%) gave negative results (Fig 1).

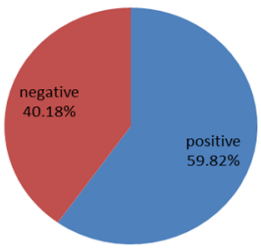

Figure 1: Distribution of Candida spp.

Distribution of *Candida* spp. According to Age groups

According to age group, the VVC candidiasis was distributed to five groups. Table 1, the highest incidence of infected women was noticed in the second (21–30) group 24(35.82%) and the third group (31–40) 18(26.86%), while the first and fourth groups had lower percent 7(8.96%) and 5(7.46%) respectively.

Table 1: Distribution of vulvovaginitis candidiasis according to age groups.

Age group	No. (%) of Infected woman
14-20	7(8.96%)
21-30	24(35.82%)
31-40	18(26.86%)
41-50	13(19.40%)
51-60	5(7.46%)
Total	67(100%)

Candida Species Identification Result

Candida spp. was found depending on the direct examination of morphological features in the culture medium and Vitek2 compact system. Table 2 shows the prevalence of *Candida* spp. in VVC. The results showed that *C. albicans* was the most prevalent 33(49.26%) species than others. *C. glabrata* was 12 *(17.91%),* while C. *guilliermondii* was lower percent 4(5.97%).

Table 2: Distribution of *Candida* spp. in Vaginal.

Pecies	No. (%)
C.albicans	33(49.26%)
C.glabrata	12(17.91%)
C. krusi	9(13.43%)
C.kefyr	9(13.43%)
C. guilliermondii	4(5.97%)
Total	67(100%)

Resistance of candida spp. to antifungal agents

The obtained results were recorded according to the appearance of a transparent zone around the antifungal drug disc. The current results showed *Candida* spp. were resistant 66(98.51%) of ketoconazole, 64 (95.52%) of Fluconazole, 60(89.55%) of traconazole, 53(79.1) of Amphotericin-B. and 49(73.13%) to Clotrimazole table 3.

Table 3: Resistance of candida spp. to antifungal agents isolated from vaginal infections.

No.	Yeast strains	No.	Amphotericin-B	Fluconazole	Itraconazole	Clutraimazole	Ketoconazole
1	*C.albicans*	33	21(63.63%)	30(90.90%)	27(81.81%)	19(57.57%)	32(96.97%)
2	*C.glabrata*	12	11(91.66)	12(100%)	12(100%)	9(75%)	12(58.33%)
3	*C. krusi*	9	8(88.88%)	9(100%)	9(100%)	9(100%)	9(100%)
4	*C.kefyr*	9	9(100%)	9(100%)	8(88.88%)	8(88.88%)	9(100%)
5	*C. guilliermondii*	4	4(100%)	4(100%)	4(100%)	4(100%)	4(100%)
	Total	67	53(79.1)	64(95.52%)	60(89.55%)	49(73.13%)	66(98.50%)

Phytochemical Screening of *Z. spina-christi* Extract

It's well knowledge that endophytic fungi generate a wealth of new antibacterial and antioxidant chemicals (27). Based on the data presented in Table 4, revealed that total flavonoid content was 148.11 mg rutin/100mg, total phenolic content (mg Gallic/100mg) was 282.37 mg gallic/100mg, total terpenoid content was 25%, Total alkaloid content was 10.4%, total saponin content was 10.0%, and total tannic acid was 930 mg/100g. It can be concluded that extract has the alkaloids, phenol, flavonoid, saponins, tannic acid, and terpenoids.

Table 4: Phytochemical screening of *Z. spina-christi* leaves extract.

Extract	Total flavonoid content (mg rutin/100mg)	Total phenolic content (mg Gallic/100mg)	Total terpenoid content (%)	Total alkaloid content (%)	Tannin (mg/100g)	Total saponin content (%)
Z. spina-christi leaves	148.11	282.37	25.0	10.4	930	10.0

Phytochemical Analysis by GC-MS Spectroscopy

Table 3 and Fig 2 list the phytochemical components discovered by GC-MS analysis from the extract of *Z. spina-christi* leaves. Table 2 display the bioactive components along with data on their retention period, peak, area, area %, height, and height%. Table 5 lists the GC-MS data for the 18 components found in the leaf extract. The GC-MS analysis results have shown the presence of 18 different compounds, where major compounds were 9-Octadecenoic acid, 12-hydroxy-, ethyl ester, [R-(Z)], 10-Undecenoic acid, ethyl ester, and Ricinoleic acid ratios 41.55, 37.70 and 11.59%, respectively. These compounds have different biological

activities such as antimicrobial, antioxidant, anti-inflammatory, anti-eczemic, and antistaphylococcal activities.

Table 5: Phytochemical components in leaves extract of *Z. spina-christi* found by GC-MS spectroscopy.

Peak	Compound	R.Time	Area	Area%
1	**Benzene, (1-ethoxyethyl)-**	3.452	43388	0.11
2	**Silane, triethoxymethyl-**	3.637	42419	0.11
3	**Glycerin**	4.315	165753	0.42
4	**Ethanethiol, 2-(diethylboryloxy)-**	4.481	28739	0.7
5	**Heptanoic acid, ethyl ester**	5.915	30000	0.8
6	**Octanoic acid, ethyl ester**	6.991	102151	0.26
7	**Decanoic acid, ethyl ester**	8.976	32847	0.08
8	**Tetradecanoic acid, ethyl ester**	9.296	217355	0.55
9	**n-Hexadecanoic acid**	9.499	221450	0.56
10	**Z-12-Tetradecenal**	9.630	74727	0.19
11	**Nonanoic acid, 9-oxo-, ethyl ester**	9.780	196555	0.49
12	**Methyl 19-methyl-eicosanoate**	10.062	1008074	2.53
13	**Eicosanoic acid, ethyl ester**	10.175	1238914	3.11
14	**Ricinoleic acid**	10.573	4618210	11.59
15	**9-Octadecenoic acid, 12-hydroxy-, ethyl ester, [R-(Z)]-**	10.814	16554510	41.55
16	**10-Undecenoic acid, ethyl ester**	10.920	15017984	37.70
17	**(E)-9-Octadecenoic acid ethyl ester**	**11.391**	182665	0.46
18	**Glycerin octanoate**	11.532	62714	0.16
			39838455	100.00

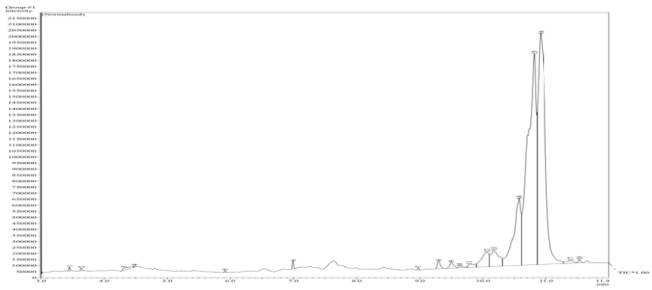

Figure 2: GC-MS spectral chromatogram of *Z. spina-christi* leaves extract.

Inhibitory Activity of Z. *Spina-Christi* Leaves Extract

The antimicrobial activity of Z. spina-christi leaf extracts against pathogenic microorganisms were assessed using the agar well diffusion method. The results presented in Table 6 proved varying degrees of inhibition by the extracts. Strikingly, the extract showed strong inhibitory activity against Candida spp. Specifically, the extract showed the highest inhibitory activity against C. glabrata, with an inhibition zone of 27.7±5.29 mm. This was followed by C. albicans with an inhibition zone of 23.1±12.49 mm, C. guilliermondii with an inhibition zone of 23±2.40 mm, C. kefyr with an inhibition zone of 15.3±13.39 mm, and C. krusi with an inhibition zone of 13.7±13.37 mm. These results proved that extract presented

high antimicrobial activity. That's because extract is packed with bioactive chemicals, the highest concentration of which influences the inhibitory zone.

Table 6. Effect of *Z. spina-christi* leaves extract on growth inhibition of *candida* spp.

Yeast strains	Inhibition zone (mm)				P value at 0.01
	100%	150%	200%	Control	
C.albicans	14.7±10.24	17.8±11.31	23.1±12.49	0.0	0.668
C.glabrata	13.9±9.61	20.9± 3.19	27.7± 5.29	0.0	
C. krusi	9.6±12.19	10.8±13.60	13.7±13.37	0.0	
C.kefyr	11.9±10.41	12.8±11.17	15.3±13.39	0.0	
C. guilliermondii	19.8±0.28	21.5±4.24	23±2.40	0.0	
P value at 0.01	0.00				
The results show that there are no moral differences between innate insulations as the P-value value was greater than 0.01 and 0.668. The results also show that there are moral differences between the concentrations used at a level of 0.01 and that the probability was less than 0.00.					

4. DISCUSSION

This study focuses on patients diagnosed with vulvovaginal candidiasis (VVC). The vagina normally harbors various Candida species, and an increase in colonization can lead to the development of VVC. Statistical research shows that approximately 77.5% of women have experienced at least one episode of VVC in their lifetime (Yano et al., 2018) (28). Among Iraqi women with VVC, around 46.6% showed symptomatic characteristics, while 53.4% were asymptomatic (Midhat et al, 2020) (29). VVC is commonly categorized into symptomatic and asymptomatic infections (Fournier & Mendling, 2015)**.(30)** In our study, the diagnosis of VVC was more frequent in patients aged 20 to 30 years and 31 to 40 years. This finding aligns with a study on college-aged women with VVC, which also saw a higher prevalence in the 20-30 age group compared to those below 20 or above 50 years (Emeribe et al., 2015) (31).

Most studies have reported a positive correlation between age and VVC prevalence, although some studies did not find such a correlation (Emeribe et al., 2015; Hedayati et al., 2015) (32-33) (34).

VVC is most observed in women of reproductive age (Ramírez-Lozada et al., 2019; Rati et al., 2015) and is known to increase the risk in women between 18 and 45 years old (Kamya Ramesh Swaminathan et al., 2017) (35).

In this study, *C. albicans* caused more infections than non-albicans. This result is compared with the earlier finding by Ghajar *et al.* (2018) (36). They reported the prevalence of *Candida albicans* (67.7%), *Candida glabrata* (25.8%), and *Candida kefyr* (3.2%), respectively. The sensitivity of the pathogenic *Candida* spp. caused VVC. According to *Candida* spp., guilliermondii were 100% resistant to the five antifungals, and each of *C.glabrata , C. krusi, C.kefyr,* and *C. guilliermondii* were 100 % resistant to Ketoconazole.

While *C. albicans* was resistant to Ketoconazole, Fluconazole, Itraconazole, Amphotericin-B, and Clotrimazole (32 (48.48%), 30 (90.90%), 27 (81.81%), 21 (63.63%), 19 (57.57%). Membrane proteins in pathogenic yeast, including cell, vacuole, and mitochondrial membranes, play various physiological functions such as environmental sensing, nutrient transport, signal transduction, drug efflux, modification, and detoxification (Khandelwal et al., 2019) (**37**). Polyene medicines like amphotericin B are resistant to fungi due to alterations in ergosterol ERG 3 and ERG6, as proved in C. albicans. (17) (Vandeputte et al., 2012). Candida isolates in this study showed susceptibility to antifungal agents, showing their suitability for VVC treatment. Available in topical and orally available forms (38) (Gonçalves et al., 2016). Antibiotics, despite not affecting fungus promotion, create a conducive environment for fungal growth by altering the number of fungi and intestinal bacteria (39) (Feglo & Narkwa, 2012). The rise of antifungal-resistant organisms is causing challenges in treatment, with around 10% of candida vaginitis patients not responding to conventional treatments. (40) (De Pádua et al., 2003).

Our study showed that total flavonoid, phenolic, terpenoid, alkaloid, and tannic acid saponin contents were 148.11 mg/100g, 285.37 mg /100g, 25.0%, 10.4%, 930mg/100g and 10%. The disruption of the plasma membrane, induction of mitochondrial malfunction, and inhibition of cell wall construction, cell division, RNA and protein synthesis, and the efflux-mediated pumping system are just some of the mechanisms by which flavonoids impede fungal development (30). (41) The hydroxyl groups on phenolic compounds, a class of secondary metabolites, confer scavenging ability (31). This makes phenolic compounds crucial bioactive molecules. Terpenoids can inhibit tumor growth, reduce inflammation, kill germs and viruses, treat and prevent heart disease, lower blood sugar levels, and even treat diabetes (32). Phenol compound has been shown to inhibit the growth of microorganisms (33). Antioxidant, anticancer, anti-inflammatory, antibacterial, and antiviral properties were also found (34). These physiologically active mixtures of components in *Z. spina-christi* leaf methanol extracts have been shown to have antibacterial, antifungal, and antioxidant effects. According to (Bukvicki et al., 2013). (35), the presence of 2-methyldecane has an antibacterial effect. Our results agreed with those of (Mukherjee et al., 2013). (36)

: It was found that methanolic extracts of *Zizyphus* species leaves have antifungal activity against (*Aspergillus flavus, A. niger,* and *A. alternata*) in a study assessing antioxidant and antifungal properties. These results were at odds with those found in (Abalaka et al., 2010) . (38), which looked into the antifungal activity of ethanolic extracts of leaves from two *Ziziphus* species and found that they had no effect on the fungus A. niger. This may be because

the phytochemical components in plant extract have varying degrees of antifungal activity depending on the solvents employed to extract them.

Several studies have proven the efficacy of Ziziphus sp. leaves and fruits, along with other plant extracts, against a broad range of pathogenic fungi. For instance, leaf tannins and flavonoid compounds from Z. spina-christi L. have been found to show physiological activity against Drechslera biseptata and Fusarium solani in vitro (38), supporting our own findings. In relation to tomato damping-off diseases caused by Fusarium oxysporum, Pythium aphanidermatum, and Rhizoctonia solani, another study (39) discovered highly active methanolic extracts from the leaves of Thymus vulgaris and Zingiber officinale, which exhibited fungistatic and fungicidal effects against these pathogens. The fungicide carbendazim, at 8 ppm, proved more effective in restraining the mycelial growth of all phytopathogenic fungi compared to the two methanolic plant extracts, which is consistent with our results. Furthermore, (Yahia et al., 2020). (40) reported that extracts from the leaves and fruits of the Ziziphus genus have abundant bioactive chemicals with potential applications in both industrial and culinary contexts. Contrasting the antibacterial activity and the total phenolic, flavonoid, and tannin content of the fruit extract, the leaf extract showed significantly higher levels. The bioactive components in plant extracts synergistically work to combat fungi by inhibiting enzyme production, reducing ergosterol levels in filamentous fungi, or both (41).

Earlier research has shown that the leaf extract of Ziziphus sp. possessed the highest concentration of total polyphenols compared to other extraction solvents (42) and across various plant species (43). Additionally, studies have proven that the lipophilic flavonoids present in the leaf epidermis and/or cuticle are highly effective phenolic compounds that contribute to the plant's resistance against a wide range of phytopathogenic fungi (44). Gas chromatography coupled with mass spectrometry is a commonly used technique for analyzing phytochemical substances derived from natural sources due to its stability, sensitivity, and efficiency (45). Traditional medicinal plants have a rich historical background, and their endophytic fungi are increasingly recognized for their ability to produce similar bioactive secondary metabolites with pharmacological properties as their host plants (46, 47).

The GC-MS analysis of the leaf extract revealed the presence of five different chemicals with varying retention times. Polyphenolic substances, chemically characterized by having a benzene ring with one or more hydroxyl groups, are produced from phenylalanine through the shikimic acid and phenylpropanoid pathways. It is worth noting that increased hydroxylation of polyphenols correlates with increased toxicity (48, 49). Like other plant extracts, where phenolic components prove active antifungal properties (50), polyphenols also

play a crucial role in protecting against microbes, herbivores, and insects. Our analysis leads us to hypothesize that the high gallic acid content in the leaf extract may contribute to its potential as a defensive phytochemical with antifungal properties. Earlier reports have highlighted the pharmacological properties of tannins, such as gallic acid, coffeic acid, ellagic acid, and catechin, as well as flavonoids, present in the leaves, bark, inner bark, fruits, and seeds of Z. joazeiro extract sample (51).

Our study findings align with an earlier study (52) that investigated the potential of gallic acid and its derivatives to mitigate the negative effects on tomato plants. We saw that the extract showed potent inhibitory activity against both Gram-negative and Gram-positive bacteria, as well as fungi. The inhibition of protein synthesis, nucleic acid synthesis, and metabolic pathways are all contributing factors to the efficacy of secondary metabolites against bacteria (47, 53). Earlier research has highlighted the interrelation between the polarity and activity of components used in leaf and fruit extracts (54, 55). Moreover, multiple studies have shown that extracts with varying polarities show diverse pharmacological and toxicological effects (56, 57).

Earlier studies have reported varying inhibitory concentration values depending on the specific fungus and plant species under investigation. For example, when exposed to methanolic extracts of Ziziphus mauritiana Lam leaves, two different strains of C. albicans displayed inhibitory concentrations of 125 μg/mL and 500 μg/mL, respectively (58). The antifungal activity of Z. spina-christi L. methanolic leaf extracts were examined against multiple fungal strains (59). At an inhibitory concentration of 400 mg/100 mL, the extract effectively inhibited the growth of Aspergillus niger ATCC 102, Aspergillus flavus ATCC 247, and Fusarium moniliform ATCC 206. Additionally, Alotibi (9) proved that the aqueous extract of Z. spina-christi at a concentration of 50 mg/mL significantly reduced the development of Helminthosporium rostratum, Alternaria brassicae, and Rhizoctonia solani. These findings support the notion that secondary metabolites in plants have great potential as a source of effective antifungal drugs (36), and earlier studies have proven the efficacy of various plant extracts against the pathogenicity of different fungi, consistent with our own findings.

5. CONCLUSION

In our study, we conducted GC-MS and phytochemical analyses to find the bioactive compounds present in the extract of Z. spina-christi leaves. The results revealed that the extract showed significant antibacterial and antifungal activity against both Gram-negative and Gram-

positive bacteria, as well as fungi. These findings highlight the potential of the endophytic leaf extract of Z. spina-christi as a source of bioactive substances with various biological applications.

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