Original Article

Microbial Biosystems 10(3) (2025) 2025.357317
10.21608/MB.2025.357317.1247

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Antifungal activity of *Senna alexandrina* leaves against fungal strain *Trichosporon inkin* in vitro

Alaa El-deen Ebrahim Eldaly[1], Sabha El-Sabagh[1], Anwer S. El-Badry[2*], Amany A. M. Ahmed[1]

[1]*Botany and Microbiology Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt.*
[2]*Botany and Microbiology Department, Faculty of Science, Tanta University, Tanta 31527, Egypt*

ARTICLE INFO

Article history
Received 02 February 2025
Received revised 17 February 2025
Accepted 24 April 2025
Available online 01 September 2025

Corresponding Editors
Farag, B.
Abdel-Azeem, A. M.
Mekky, A. E.

Keywords
Drug resistance prevention,
natural therapeutics for
immunocompromised patients,
plant-based antifungal agents,
side-effect-free antifungal
alternatives,
Trichosporon infections

ABSTRACT

Fungal skin infections have become a significant problem for people of all genders. Therefore, accurate diagnosis and treatment of the active disease, as well as the reduction of re-infection by continuing screening, following up with relatives, treating symptomatic carriers, and disinfecting their environment, are mandatory. Patients with health problems especially intensive care patients with weak immune systems need antifungal therapy without any side effects and without systemic treatment which may cause gastrointestinal symptoms, Chapped lips, nausea and vomiting, headache, Hypokalemia (low potassium levels in the blood), depression, cardiac toxicity, cardiac arrest, hepatotoxicity by elevation of liver enzymes and inflammation of liver and reduction of bile flow from the liver So this type of patients in pretty need of antifungal agents without all side effects that may arise by using commercial antifungal agents as Polyene macrolides , various Azloles, Allyamines , Griseofulvin, flucytosine. Studies have shown that the best alternative compounds for antifungal therapy are derived from plants and are effective with no side effects. Which don't add any physiological pressure on the pathogens to develop drug resistance and are easily degradable. This article evaluates the antifungal activity of *Senna alexandrina* leaf extract using different solvents against the fungal strain *Trichosporon inkin* in vitro.

Published by Arab Society for Fungal Conservation

Introduction

Patients with health problems especially intensive care patients with weak immune systems need antifungal therapy without any side effects and without systemic treatment which may cause gastrointestinal symptoms, photophobia(sensitivity to bright light), Chapped lips, nausea and vomiting, headache, Hypokalemia (low potassium levels in the blood), depression, cardiac toxicity, cardiac arrest, hepatotoxicity by elevation of liver enzymes and inflammation of liver and reduction of bile flow from the liver So this type of patients in pretty need of antifungal agents without all side effects that may arise by using commercial antifungal agents as Polyene macrolides, various Azloles, Allyamines, Griseofulvin, flucytosine.

This study was considered to find out the antifungal activity of plant extracts using various solvent extracts to inhibit human skin pathogenic fungi and show significant inhibition as well as or more than inhibition of commercial antifungal also the mode of

***Corresponding author Email address*: anwer.elbadry@science.tanta.edu.eg. (**Anwer S. El-Badry**)

action of plant extracts that cause the inhibition to the pathogenic fungi was examined.

Trichosporon inkin fungi cause a disease called white Piedra. Which is an asymptomatic fungal infection of the hair shaft, resulting in the formation of nodules of different hardness on the infected hair. The conditions caused are considered superficial mycoses (Zapata-Zapata *et al*. 2023). White Piedra, caused by other *Trichosporon* species, occurs in semitropical and temperate countries. The *Trichosporon* genus is subdivided into six distinct human pathogenic species of which *Trichosporon ovoides*, *Trichosporon inkin*, *Trichosporon mucoides*, and *Trichosporon asahii* are linked to White Piedra**.**

Trichosporon species in particular has also been considered urophilic due to its ability to occupy strongly acidic locations when colonizing pubic hair and its capacity to utilize urea and uric acid. The fact that they select hair as a substratum may be due to their keratinophillic affinity (De Hoog *et al*.1998). Usual treatments for white Piedra using shampooing with Ketoconazole (Singh *et al* 2019).

After increasing resistance of fungi towards these Azoles besides patients with weak immune systems not in need of any additional therapies (Bauer *et al*. 1996) all scientific engines were directed towards native plants and essential oils to adapt suitable antifungals with fewer side effects and effective antifungal activity equal to alternative azoles.

In this study, *Trichosporon inkin* was tested to find out the antifungal activity against *Senna alexandrina* leaves using various solvents (methanol, ethanol, ethyl acetate, and cold water) extracts to inhibit human skin *Trichosporon inkin* infection. *Senna alexandrina* leaves in methanol extract with a concentration of 20% (W/V) showed the largest inhibition zone against *Trichosporon inkin* with inhibition zone equal to (30± 0.57) mm the largest inhibition between other solvents.

Materials and Methods

Source of the Trichosporon inkin strain

The *Trichosporon inkin* strain (RCMB 040002.1) was obtained from the Regional Center for Mycology and Biotechnology (RCMB), Culture Collection Unit (CCU), Al-Azhar University, Cairo, Egypt.

Preparation of plant extracts

Four types of solvents (cold water, methanol, ethanol and ethyl acetate solvents) were used in this study according to their polarity. The temperature during preparation was kept below 70°C (Altemimi *et al*. 2017)**.**

Leaves of *Senna alexandrina* were collected from the local market of Egypt, examined, and identified by the International Herbarium in Tanta University under supervision of Dr. Kamal H. Shaltout, who confirmed the classification. The collected plants were washed, disinfected, rinsed with distilled water, and shade-dried. The dried material was ground into fine powder to pass a 100 mm sieve.

Fifty grams of powder were soaked in 200 ml of solvents (methanol, ethanol, ethyl acetate, and cold water) with stirring for 48 h, filtered through muslin, centrifuged at 3000 rpm for 10 min, and finally filtered again through Whatman No. 41 filter paper to obtain a clear filtrate. The filtrates were evaporated and dried at 40 °C under reduced pressure using a rotary evaporator. After complete solvent evaporation, 2 g of solid extracts were dissolved in 10 ml of 10% dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml and stored at 5 °C in labeled sterile screw-capped bottles for further use (Mohamed et al., 2020).

Antifungal potency of methanol extract of Senna alexandrina against Trichosporon inkin strain.

The antifungal activity of plant extract was evaluated using the well agar diffusion method, and minimal inhibitory concentration (MIC) was examined. The well diffusion assay was conducted according to Perez et al. (1990). A suspension of *Trichosporon inkin* (5×10^6 spores/ml) was pipetted into the center of sterile Petri dishes. Molten cooled Sabouraud's dextrose agar (SDA) was then poured into the dishes containing the inoculum and mixed well. After solidification, wells (8 mm in diameter) were made using a sterile cork borer. Then, 100 µl of *Senna alexandrina* methanol extract (20% w/v) was added to the wells. Plates were kept in the refrigerator for 30 min to allow diffusion, then incubated at 30 °C for 3 days. Antifungal activity was determined by measuring the inhibition zone (including well diameter) after incubation.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined using the agar well diffusion assay (Eloff et al. 1998). Different concentrations (20, 10, 5, and 2.5% w/v) were prepared by two-fold serial dilution. A suspension of *Trichosporon inkin* (5×10^6 spores/ml) was pipetted into sterile Petri dishes, followed by addition of Sabouraud's dextrose agar and mixed at a temperature that allowed inoculum survival and spread. Four wells were made in each plate, and 100 µl of each concentration was added to respective wells. Plates were

refrigerated for 30 min to allow extract settlement, then incubated at 30 °C for three days.

MIC was defined as the lowest concentration inhibiting fungal growth. All assays were performed in triplicate. Zones of inhibition were measured after incubation and compared to standard interpretive criteria to determine susceptibility of *Trichosporon inkin*. Graphs were drawn with fungal growth percentage versus antifungal concentrations. The measured MIC of *Senna alexandrina* methanol extract was 5 mg/ml.

Mode of action of Senna alexandrina methanol extract against Trichosporon inkin

Antifungal mechanism was investigated through imaging with TEM (Transmission electron microscope), measuring total cell protein ad total soluble ions of *Trichosporon inkin* after addition of *Senna alexandrina* methanol extract.

Antifungal mechanism of the effective plant extracts using transmission electron microscope.

Transmission electron microscopy (TEM) was used to investigate the antifungal activity of plant extracts on fungal strains. For sample preparation, 2.5% buffered glutaraldehyde in 0.1 M phosphate buffer saline (PBS) was used as a fixative. Samples were first fixed in this solution (pH 7.4) at 4 °C for 2 h, then washed three times with PBS (10 min each). Post-fixation was carried out in 1% osmium acid for 30 min, followed by three additional PBS washes (10 min each).

Samples were dehydrated through a graded ethanol series (30, 50, 70, 90%, and absolute ethanol, 30 min each), then infiltrated with acetone for 1 h. After dehydration, they were embedded in Araldite 502 resin. Plastic molds were sectioned using a LEICA Ultracut UCT ultramicrotome and stained with 1% toluidine blue for examination of semi-thin sections. Ultra-thin sections were then cut, stained with uranyl acetate, counterstained with lead citrate, and examined with a JEOL-JEM-100 SX transmission electron microscope (Japan) at the Electron Microscope Unit, Tanta University (Burghardt et al., 2006).

Measuring total cell protein and total soluble ions after treating fungal strain Trichosporon inkin with Senna alexandrina methanol extract

Measuring total cell protein and total soluble ions in fungal cells was used to confirm inhibition, following the same principle as amphotericin B action, which damages the fungal cell membrane, causes leakage of soluble ions, and prevents formation of essential cell proteins (Ghannoum & Rice, 1999). *Trichosporon inkin* was cultured in Sabouraud's dextrose broth to obtain a suspension of 5×10^6 cells/ml. The fungal suspension was mixed with the tested antimicrobial agent (*Senna alexandrina* methanol extract) at the previously determined MIC. The mixture was incubated overnight in a shaking incubator at 60 rpm under appropriate temperature conditions.

After incubation, the treated mixture was centrifuged at 3000 rpm for 20 min, washed with sterile distilled water, and centrifuged again to collect the cell pellet in a clean Eppendorf tube. Cells were disrupted for intracellular content extraction by grinding with glass beads in 1 ml distilled water. The suspension was filtered, supernatants collected, and stored at 4 °C for further analysis (Bradford, 1976).

Estimation of total soluble cell proteins concentration:

Coomassie brilliant blue G-250 dye of 100 mg was dissolved in 50 ml of 95% ethanol + 100 ml of 85% phosphoric acid, diluted to 1 L. dist. Water, then filtered and 0.1 ml of previously treated cell extract was mixed with 5 ml of Comassie dye, and was shaken well for 5 min., The light absorbance of the mixture was recorded at λ=595 nm. Records of absorbance indicate the conc. Of total soluble cell proteins in tested supernatants using a previously drawn standard curve of light absorbance of known conc. Of bovine serum albumin as a standard protein stained and measured in the same way as the unknown sample (Bradford 1976).

Estimation of total cell soluble ions content:

All minerals, and ionic components of the treated cells were released in the previously treated, and stored extract of broken cells. Each tested suspension was diluted to 10 ml with dist. Water, then the electrode of the EC-meter (Sensorex CS200TC lab conductivity sensor, USA) was immersed in the suspension, and the potential of electric current was recorded. The concentration of total ions present in the tested suspension was indicated by the records of electric current potential, which can be read from a previously prepared standard curve of electric conductivity of known concentration of known electrolyte **(**Kissinger 1996).

Gas chromatography–mass spectrometry (GC-MS) analysis for Senna alexandrina methanol extract inhibiting fungal strain Trichosporon inkin

Samples were introduced to the scientific research center and measurement at Tanta University for analyzing the contents of plant extract. The instrument

was Clarus 580/560S PerkinElmer, Inc., Waltham, MA, USA) gas chromatograph/mass spectrometer (GC/MS) was employed to determine the chemical constitution of plant extract *Senna alexandrina* leaves. The column used in this investigation was an Elite-5MS (30 m × 0.25 mm × 0.25 µm film thickness) column, and the oven temperature was first held at 80 °C for 7 min before being increased by 10 °C min−1 to 140 °C withhold for 1 min, followed by an increase to 200 °C withhold for 1 min by a rate of 10 °C min−1, and finally increased to 280 °C withhold for 10 min by a rate of 5 °C min−1. Temperatures were maintained in the transfer and input lines at 250 °C. Helium was the carrier gas, and a column flow rate of 1.21 ml/min was used. Using the auto sampler AS3000 and GC in split mode (1:20), a sample of 1 µl was automatically injected after a solvent delay of 5 min. At an ionization energy of 70 eV, EI mass spectra were obtained over the range of m/z 40–650 in full scan mode. The temperature of the ionization chamber was fixed at 200 °C. By comparing their retention times and mass spectra to those of the WILEY 09, replib, and NIST 11 mass spectral databases, all acquired components of the examined extract were recognized (Adams, 2007).

Statistical analysis

All data results were obtained from three replicates. Information entered and investigated utilizing Microsoft Excel programming. The resulting data was arranged and analyzed by a statistical software package (SPSS). Results were introduced as mean ± standard deviation (Chia *et al*., 2013).

Results

Antifungal susceptibility tests of Senna alexandrina leaves methanol extract against Trichosporon inkin

Senna alexandrina leaves extract was prepared with four solvents (methanol, Ethanol, ethyl acetate, and cold water) the best inhibition for *Trichosporon inkin* was by methanol extract as shown in the table 1.

Table 1 Inhibition of *Trichosporon inkin* by *Senna alexandrina* leaf extracts using different solvents

Solvent	Inhibition zone (mm)
Methanol	(30 ± 0.57)
Ethanol	N
Ethyl acetat	(14 ± 0.57)
Cold water	N

values are means of triplicate determination (n=3)± standard deviation. N, no zone of inhibition was found.

The methanol extract of *Senna alexandrina* leaves showed the strongest antifungal activity against *Trichosporon inkin*, with an inhibition zone of 30 ± 0.57 mm. The ethyl acetate extract had a moderate effect, producing a 14 ± 0.57 mm inhibition zone, while extracts using ethanol and cold water showed no activity.

The antifungal potential of the methanolic extract was tested using the agar well diffusion method on Mueller Hinton (MH) agar. *T. inkin* strain RCMB 040002.1 was exposed to a 20% (w/v) methanol extract. Methanol was evaporated to remove the solvent, and the extract was dried. Then, 2 g of the dried extract was dissolved in 10 mL of 10% (w/v) dimethyl sulfoxide (DMSO) to prepare the final 20% (w/v) solution.

The methanol extract consistently produced a clear inhibition zone of 30 ± 0.57 mm. Each test was repeated three times, and the mean values are presented in Figure 1.

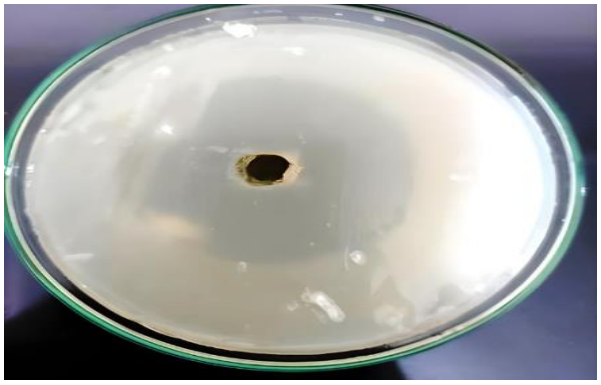

Fig 1. Antifungal activity of *Senna alexandrina* methanolic extract (20% w/v) against *Trichosporon inkin* strain**.**

Determination of the MIC of plant extract inhibiting Trichosporon inkin

To confirm the effective antifungal concentration of plant extracts, the minimum inhibitory concentration (MIC) test was carried out with the well-agar diffusion method. The minimum inhibitory concentration that can inhibit fungal strain was 5 mg/ml (figure 2).

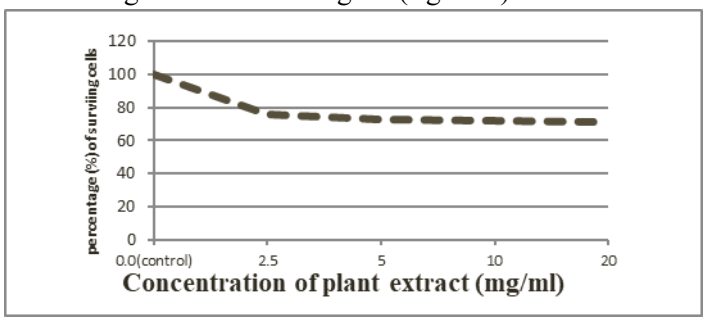

Fig 2. Determination of the minimum inhibitory concentration (MIC) of *Senna alexandrina* methanol extract against *Trichosporon inkin.*

Antifungal mechanism of S. alexandrina methanol extract using TEM and physiological analyses

Another approach to investigate the antifungal effect was using transmission electron microscopy (TEM) to observe morphological changes in viable fungal cells exposed to the active plant extract and its impact on the cell wall and internal organelles. The effect of Senna *alexandrina* methanol extract at 5 mg/mL on *Trichosporon inkin* was examined under TEM.

Untreated control cells displayed normal morphology (Fig. 3-A). After treatment with the methanol extract, substantial alterations were observed: deterioration of organelles, accumulation of solutes and crystals within the cells due to membrane lysis (Fig. 3-B1, C3, H3, F2), complete discharge of cellular contents (Fig. 3-B2, C2, D4, E3, G2), rupture of the cell wall and membrane with leakage of organelles (Fig. 3-C1, D2, E2, F1, F2), and cytoplasmic shrinkage from loss of internal components (Fig. 3-G1). Additionally, in some cells, the contents of two adjacent cells merged following wall rupture, accompanied by coagulation of cytoplasmic proteins (Fig. 3-F1).

Measurement of total cellular protein inside and outside Trichosporon inkin cells"

Investigation of antimicrobial action of *senna* leaves on *Trichosporon inkin* by calculating cell protein in and outside cell was total cell protein in cell breakage suspension (18 mg/ml) and total cell protein in culture filtrate was(67 mg/ml*)*. This can confirm the antimicrobial action of *senna* leaves on *Trichosporon inkin* in protein synthesis and disrupting cell membranes which is found in larger percent in cell filtrate than inside cell breakage suspension as represented in figure 4.

Calculation of total soluble ions inside and outside cells of Trichosporon inkin

Analysis of total soluble ions revealed 0.035 mol inside the cells and 0.082 mol in the culture filtrate, indicating disruption of the fungal cell structure and leakage of ions from the cells (Fig. 5).

Identification of individual components in Senna alexandrina leaf methanol extract by GC-MS

The results obtained in table (2) and figure (6) showed that the methanol extract of *Senna alexandrina* leaves contains numerous bioactive compounds that are considered with antimicrobial and antifungal impacts for example, (Glycerin) which act as strong antifungal, (1-Butanol, 2-methyl-acetate) which working as an antifungal anti-inflammatory, (2,4-Dihydroxy -2,5 dimethyl 3(2H)-furan-one) which has strong antimicrobial activity, (3-O-methyl-d-glucose) which has

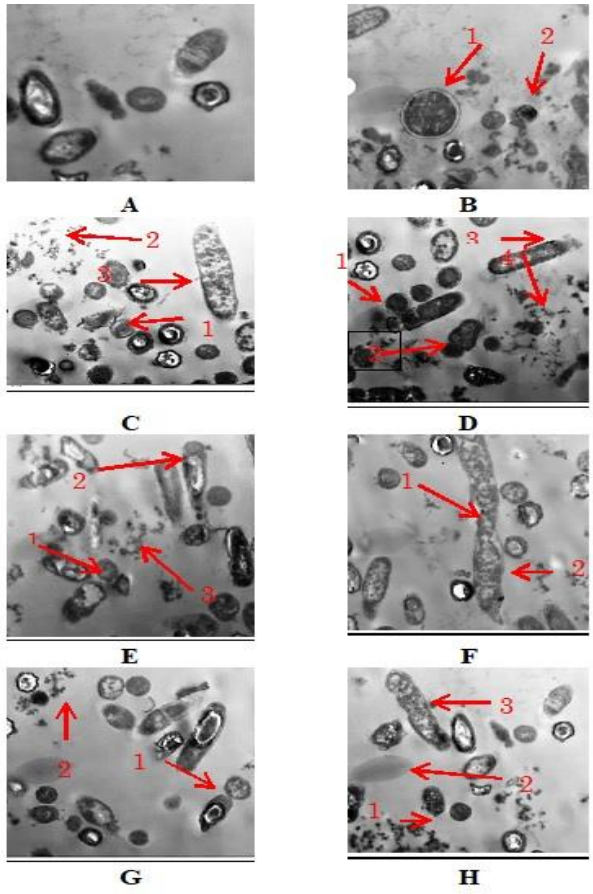

Fig 3. Transmission electron microscope (TEM) micrographs showing the effect of *Senna alexandrina* methanol extract on *Trichosporon inkin* cells. (A) Control; (B–H) Deterioration of cell organelles following treatment with the methanol extract."

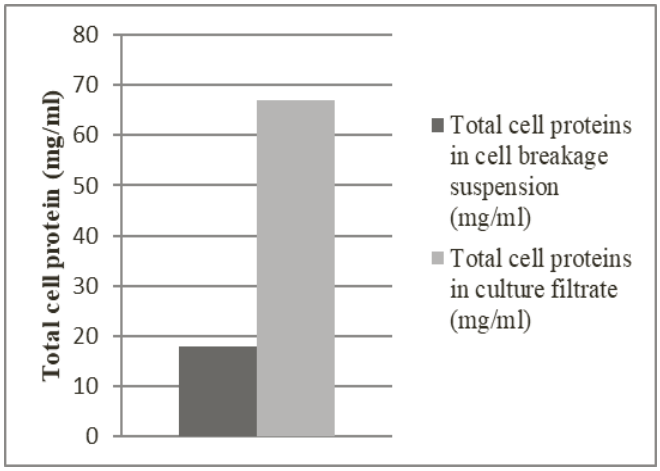

Fig 4. Effect of methanolic extract of *Senna alexandrina* leaves on total cell protein of *Trichosporon inkin*

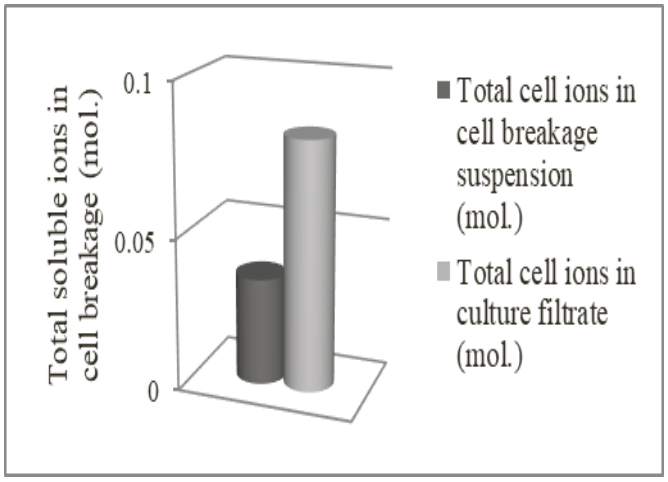

Fig 5. Effect of *Senna alexandrina* leaves on total soluble ions of *Trichosporon inkin.*

antimicrobial activity, (2-myristynoyl pantetheine)which exhibit anti-inflammatory activity, (Decanoic acid-10 bromo methyl ester) which acts as an antimicrobial anti-inflammatory, Myristic acid which exhibits strong antifungal activity, (Palmetic acid) which showed stronger antifungal than the unsaturated fatty acids, Linoleic acid) exhibit antifungal activity, (11.14.17-Eicosatrienoic acid methyl ester) which is an anti-inflammatory anti-oxidant, and (Cis-vaccenic acid) which is the most component in large concentration acting as anti hypolipedimic effect antimicrobial and strong antibacterial Other compounds were found in medium concentrations as (n-butyric acid 2-ethylhexyl ester) which has antimicrobial activity,(Benzoic acid) which increases the antimicrobial activity and increases the immune response,(Thymol) which has antifungal and chemosensitizing activities,(2,3,4,4 Tetramethyl-pentane 1,3 diol)which has antimicrobial antifungal activities. (Benzoic acid,3-hydroxy, methyl ester) which has antimicrobial activities, (2-myristynoyl pantetheine) which exhibits anti-inflammatory activity, (Ledene-oxide-II) which has antifungal properties, (phytol) has a potential antimicrobial agent. Compounds with small concentrations inside the chemical analysis (Arachidonic acid) which has antifungal and antimicrobial activities, (Ethylphosphonic acid, dicyclopentyl ester) Which possess the highest fungicidal activity against powdery mildew, (Benzoic acid,3-methyl-2-trimethylsilyloxy-trimethylsilyl-ester) which has antifungal activities, (11-hexadecanoic acid,15 methyl methyl ester) which has the highest antimicrobial effect against clinical pathogenic bacteria,(Stearic acid) has anti-microbial activity,(Sulfurous acid,2-ethylhexyl nonyl ester) which has anti-microbial activity, (Ledene-oxide-(ll)) exhibit anti-fungal activity. (cycloheptadecanone) Has broad spectrum anti-microbial properties, (2-dimethyl silyoxypentadecane) which possess high antimicrobial and high antitumor activities. The outcome fraction of mass spectra mentioned compounds (Fig 6, Table 2).

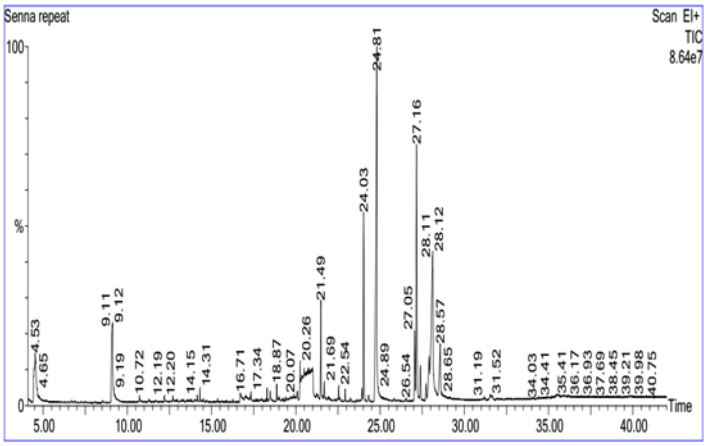

Fig 6. GC-MS analysis profile of *Senna alexandrina* leaf methanol Extract

Discussion

In recent years, there has been growing interest in discovering antifungal compounds from natural sources. Many studies have explored plant extracts as potential antifungal agents (Saral et al., 1991). This interest is driven by the increasing resistance of some fungal strains to standard antifungal drugs, which are often toxic (Saral et al., 1991). Hospital-acquired infections caused by drug-resistant fungi, coupled with the limitations and high costs of conventional treatments, have emphasized the need for safer and more effective natural alternatives. Plants are a rich source of bioactive compounds, which benefit from chemical diversity, evolutionary pressures to produce active molecules, and similarities in protein targets across species (Larsson et al., 2007).

Our study showed that the antifungal activity of *Senna alexandrina* leaf extracts varied depending on the solvent used (Table 1). Methanol extracts were the most effective against *Trichosporon inkin*, producing a clear inhibition zone of 30 ± 0.57 mm. Ethyl acetate and ethanol extracts were less effective, likely due to differences in the bioactive compounds they contain.

We determined the minimum inhibitory concentration (MIC) of the methanol extract using an agar well diffusion assay with concentrations of 2.5, 5, 10, and 20 mg/mL. The MIC for *T. inkin* was 5 mg/mL. This aligns with previous findings by Manandhar et al. (2019), who evaluated methanolic extracts of various medicinal plants against human pathogens using similar methods.

Table 2 Composition of methanol extract of *Senna alexandrina* leaves as evaluated by GC-MS chromatography

Peak no.	RT (min.)	Area %	Compound name
1	4.53	4.713	Glycerin
2	4.76	0.361	2-propyl-tetrahydropyran-3-ol
3	9.120	5.109	1-Butanol,2-methyl-acetate
4	9.406	0.283	n-butyric acid 2-ethylhexyl ester
5	10.721	0.250	2,4-Dihydroxy-2,5 dimethyl-3(2H)-furan-3-one
6	11.281	0.129	Benzoic acid
7	12.202	0.188	2-dimethyl silyoxypentadecane
8	12.712	0.178	phenylacetaldehyde
9	14.313	0.260	Thymol
10	16.709	0.566	2,3,4,4-Tetramethyl-pentane-1,3 diol
11	17.034	0.233	Lactose
12	17.339	0.204	Benzoic acid,3-hydroxy, methyl ester
13	18.869	0.354	Dodecanoic acid
14	19.890	0.173	2,5-octadecanoic acid ,methyl ester
15	20.495	3.691	3-O-methyl-d-glucose
16	20.715	3.494	2-myristynoyl pantetheine
17	20.790	1.302	2--myristynoyl pantetheine
18	21.005	3.701	Decanoic acid,10 bromo, methyl ester
19	21.230	0.228	13-oxadispiro(5.0.5.1)tridecane
20	21.310	0.188	17-octadecynoic acid
21	21.486	2.109	Myristic acid
22	21.691	0.421	Ledene-oxide-(ll)
23	22.541	0.333	3,7,11,15-tetramethyl-hexadecen-1-ol
24	22.626	0.129	cycloheptadecanone
25	22.926	0.305	Myristic acid
26	23.246	0.132	1-octadecyne
27	23.922	0.301	Cyclopropaneoctanoic acid,2-hexyl-methyl ester
23	22.541	0.333	3,7,11,15-tetramethyl-hexadecen-1-ol
24	22.626	0.129	cycloheptadecanone
25	22.926	0.305	Myristic acid
26	23.246	0.132	1-octadecyne
27	23.922	0.301	Cyclopropaneoctanoic acid,2-hexyl-methyl ester
28	24.027	4.540	Palmetic acid
29	24.322	0.290	Linoleic acid
31	25.192	0.333	Palmetic acid
32	25.372	0.289	5,8,11-Heptadecatrien-1-ol
33	26.543	0.153	Sulfurous acid ,2-ethylhexyl nonyl ester
34	27.053	1.751	Myristic acid
35	27.163	7.723	Linoleic acid
36	27.398	1.066	Phytol
37	27.723	0.519	Stearic acid
38	27.913	1.845	11,14-Eicosatrienoic acid,methyl ester
39	28.123	11.669	Cis-vaccenic acid
40	28.569	2.652	Octadecanoic acid
41	31.540	0.350	Arachidonic acid
42	31.650	0.267	Ethylphosphonic acid, dicyclopentyl ester
43	35.877	0.319	Benzoic acid,3-methyl-2-trimethylsilyloxy-trimethylsilyl-ester
44	35.879	0.259	11-hexadecanoic acid,15 methyl methyl ester

To understand how the methanol extract acts on fungal cells, we measured total cellular protein and soluble ions inside and outside *T. inkin* cells and compared the results with the antifungal Amphotericin B. Previous studies have shown that Amphotericin B disrupts fungal cell membranes, altering permeability and causing leakage of proteins and ions (Clancy & Nguyen, 1998; Ghannoum & Rice, 1999). Using the Bradford method (1976), we found that treated cells had 18 mg/mL protein inside the cells and 67 mg/mL in the culture filtrate, indicating significant leakage due to membrane disruption. Similarly, total soluble ions measured 0.035 mol inside the cells and 0.082 mol in the filtrate, further confirming cell membrane damage.

Transmission electron microscopy (TEM) allowed us to visualize these effects directly. Untreated cells appeared normal, while methanol extract-treated cells showed severe changes: organelle deterioration, accumulation of solutes and crystals, membrane lysis, cytoplasmic shrinkage, leakage of cellular contents, rupture of the cell wall, merging of cellular contents, and coagulation of cytoplasmic proteins (Jothy et al. 2012).

GC-MS analysis of the methanol extract identified numerous bioactive compounds with antifungal potential. These included glycerin (strong antifungal) (Ralet et al., 2022), 1-butanol, 2-methyl-acetate (antifungal, anti-inflammatory) (Lawal et al., 2018), 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-one (antimicrobial) (Wein, 2002), 3-O-methyl-D-glucose (antimicrobial) (Jumina et al., 2019), myristic acid (strong antifungal) (Vijayarohini et al. 2020), palmitic acid (strong antifungal) (Prasath et al. 2020), linoleic acid (antifungal) (Walters et al. 2004), cis-vaccenic acid (antimicrobial, antihyperlipidemic) (Yazıcı, 2024), Ledene-oxide-(II) (antifungal) (Taha et al. 2023), cycloheptadecanone (broad-spectrum antimicrobial) (Rajakrishnan et al. 2022), and 2-dimethylsilyoxypentadecane (antimicrobial, antitumor) (Wanda 2023). These compounds likely work together synergistically to inhibit *T. inkin*.

Conclusion

The skin, as the body's outermost barrier, is constantly exposed to environmental stressors, making it vulnerable to infections, including those caused by fungi. Plants produce a remarkable variety of bioactive compounds that serve as natural defense mechanisms. Many of these compounds, or phytochemicals, can effectively inhibit the growth of microorganisms, including pathogenic fungi, by targeting multiple cellular mechanisms.

Among the extracts tested, the methanol extract of *Senna alexandrina* leaves demonstrated potent antifungal activity against *Trichosporon inkin*, largely due to its diverse bioactive components. This study highlights the potential of *S. alexandrina* as a natural antifungal agent and supports further pharmacological research to explore its safety and efficacy in human applications.

Conflict of interest statement:

The authors declare that they have no conflict of interest.

Funding

No funding sources for this study.

References

Adams R P .(2007).Identification of Essential Oil Components by Gas Chromatography/mass Spectrometry. Carol Stream, IL, USA: Allured Publishing Corporation

Altemimi A, Lakhssassi N, Baharlouei A ,Watson DG.(2017). Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. plants6040042.

Bauer AW, Kirby W M, Sherris J C, Turck M. (1996). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol. 44, 493-496.

Bradford M. (1976). A rapid and sensitive method for the quantitative of microgram quantities of protein utilizing principle of protein-dye binding. In: Biochem., 72:248-52

Burghardt RC, Droleskey R. (2006). Transmission electron microscopy. Curr Protoc Microbiol.2006,Dec;Chapter2:Unit2B.1.

Chamlagain M, Hu J, Sionov RV, Steinberg D. (2024). Anti-bacterial and anti-biofilm activities of arachidonic acid against the cariogenic bacterium Streptococcusmutans.Front Microbiol. 26;15:1333274.

Chia M A, Lombardi AT, Melao M D G G. (2013). Growth and biochemical composition of *Chloralla vulgaris* in different growth media. Academia Brasileira de Ciências, 85(4): 1427-1438.

Clancy C J, Nguyen M H. (1998). The combination of amphotericin B and as a potential new therapeutic approach to fusariosis. *J. Antimicrob. Chemother.* 41 127–130. 10.1093/jac/41.1.127

De Hoog G S, Gueho E. (1998). Agents of White Piedra, Black Piedra and Tinea nigra. In: Collier L Ballows A and Sussman M.(1998). Topiey & Wilson's Microbiology and microbial infections. Vol. 4: Medical mycology. Ajello L, Hay RJ(Eds.) London, Publ Arnold, 191-197.

Eloff A N. (1998). Sensitive and quick microplate method to determine the minimal Inhibitory concentration of plant extracts for bacteria Planta

Med., 64 (08) (1998), pp. 711 713.

Ghannoum MA, Rice LB. (1999). Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev. 1999 Oct;12(4):501-17.

Jumina J, Mutmainah M, Purwono B, Kurniawan YS , Syah YM. (2019). Antibacterial and Antifungal Activity of Three Monosaccharide Monomyristate Derivatives. Molecules. 2019 Oct 14;24(20):3692.

Jothy SL, Zakariah Z, Chen Y, Sasidharan S. (2012). In vitro, in situ and in vivo studies on the anticandidal activity of Cassia fistula seed extract. Molecules. 7;17(6):6997-7009.

Kissinger P. (1996). Laboratory Techniques in Electroanalytical Chemistry (2nd ed.), Harcourt Brace College Publishers.

Larsson J, Gottfries J, Muresan S , Backlund A. (2007). ChemGPS-NP: tuned for navigation in biologically relevant chemical space. J Nat Prod 70:789–794.

Lawal O, Knobel H, Weda H, Nijsen TME, Goodacre R, Fowler SJ, BreathDx consortium. (2018). TD/GC-MS analysis of volatile markers emitted from mono- and co-cultures of Enterobacter cloacae and Pseudomonas aeruginosa in artificial sputum. Metabolomics. 2018;14(5):66. doi: 10.1007/s11306-018-1357-5.

Manandhar S, Luitel S, Dahal RK.(2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J Trop Med. 2019 Apr 2;2019:1895340. doi: 10.1155/2019/1895340.

Mohamed EAA, Muddathir AM, Osman M A. (2020). Antimicrobial activity,phytochemical screening of crude extracts, and essential oils constituents of two Pulicari spp.growing in Sudan, Sci. Rep. 10 (1) 17148.

Perez C , Paul M , Bazerque P. (1990).An antibiotic assay by the agar well diffusion method. Acta Bio Med Exp 15: 113-5.

Prasath KG, Tharani H, Kumar MS, Pandian SK.(2020). Palmitic Acid Inhibits the Virulence Factors of *Candida tropicalis*: Biofilms, Cell Surface Hydrophobicity, Ergosterol Biosynthesis, and Enzymatic Activity. Front Microbiol. 2020 May 8;11:864.

Rajakrishnan R, Palaniselvam K, Rengasamy S, Ponnumuthu N, Asha D V B, Biji GD.(2022). Antifungal phytochemicals from the methanol and aqueous extract of Acacia concinna and Lantana camara and synergistic biological control of the Hibiscus mealybug (Maconellicoccus hirsutus). Physiological and Molecular Plant Pathology. 119. 101813. 10.1016/j.pmpp.2022.101813.

Rajeswari G, Murugan M. (2012) GC-MS analysis of bioactive components of *Hugonia mystax L.* (Linaceae), Research Journal of Pharmaceutical, Biological and Chemical Sciences, ISSN: 0975-8585,

Ralte L, Khiangte L, Thangjam NM, Kumar A, Singh YT. (2022). GC-MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkia timoriana revealed candidate anti-cancerous and anti-inflammatory agents. Sci Rep. 2022 Mar 1;12(1):3395

Saral R. (1991). Candida and Aspergillus infection in immune compromised patients: an overview.Review infectious disease 13:487-492.

Singh A, Nyati A, Mohta A, Kushwaha RK, Jain SK. (2019). A Case of Scalp White Piedra Caused by *Trichosporon ovoides*. Int J Trichology. 2019 May-Jun;11(3):134-137.

Taha AS, Ibrahim IHM, Abo-Elgat WAA, Abdel-Megeed A, Salem MZM, El-Kareem MSMA. (2023). GC-MS, quantum mechanics calculation and the antifungal activity of river Red gum essential oil when applied to four natural textiles. Sci Rep. 2023 Oct 25;13(1):18214.

Vijayarohini P , Kavitha G, Bangaru S, Sudarsan Alwar, Caroline M, Andrew S. (2020). Antimicrobial activity of selective transition metal co-ordination complexes of myristic acid,Materials Today: Proceedings, Volume 33, Part 7,2020,Pages 4198-4205,ISSN 2214-7853

Walters D, Raynor L, Mitchell A, Walker R , Walker K.(2004). Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. 2004 Jan;157(1):87-90.

Wanda M. (2023) Natural Phenolic Compound with Antimicrobial Properties, Department of Food Chemistry and Bio catalysis, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland.

Wiraswati H L, Fauziah N, Pradini G W, Kurnia D, Kodir R A, Berbudi A, Arimdayu A R , Laelalugina A, Supandi Ma'ruf I.F. , Breynia cernua. (2023). Chemical Profiling of Volatile Compounds in the Stem Extract and Its Antioxidant, Antibacterial, Antiplasmodial and Anticancer Activity In Vitro and In Silico. Metabolites 2023, 13, 281.

Wein M, Lavid N, Lunkenbein S, Lewinsohn E, Schwab W, Kaldenhoff R. (2002). Isolation,

cloning and expression of a multifunctional O-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2H)-furanone, one of the key aroma compounds in strawberry fruits. Plant J. 2002,31, 755–765.

Yazıcı A. (2024). The Strain-Dependent Antimicrobial and Antibiofilm effect of Cis and Trans-Vaccenic Acid against *Pseudomonas aeruginosa*. Cumhuriyet Science Journal. 45. 1 7. 10.17776/csj.1341700.

Zapata-Zapata C, Giraldo-Galeano AM, Rojo-Uribe C, Campo-Polanco L, Gómez-Velásquez JC, Mesa-Arango AC. (2023), Antifungal susceptibility profile of Trichosporon inkin: About three cases of White Piedra. Med Mycol Case Rep. 22;40:8-11. doi: 10.1016/j.mmcr.2023.02.007.