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Research Article

Biological Activities and GC-MS Analysis of *Aloe vera* and *Opuntia ficus-indica* Extracts

Afaf Alghamdi,[1] Wafa Alshehri ,[1] Bayan Sajer,[2] Mada Ashkan,[3] Ruba Ashy,[1] Rukaia Gashgari,[1] and Haifa Hakmi[1]

[1]*Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia*
[2]*Department of Biological Sciences, College of Science, King Abdulaziz University, Jeddah 80200, Saudi Arabia*
[3]*Biological Sciences Department, College of Science & Art, King Abdulaziz University, Rabigh 21911, Saudi Arabia*

Correspondence should be addressed to Wafa Alshehri; waalshehri@uj.edu.sa

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To evaluate the potential antimicrobial activity, *Aloe vera* and *Opuntia ficus-indica* plants were collected from the Jeddah, Al Baha, and Taif areas of the Kingdom of Saudi Arabia (SA), and their ethanolic extracts were screened by gas chromatography mass spectrometry (GC–MS/MS). The di(2-propylpentyl) ester and hexadecenoic acid ethyl ester of phthalic acid were the most abundant compounds in the *A. vera* extract, and 1-(benzyloxy)-3,5-dinitrobenzene and phenol, 5-ethenyl-2-methoxy were the most abundant compounds in the *O. ficus-indica* extract. The antimicrobial activity of aqueous and ethanolic extracts of these plants against seven fungi and five pathogenic bacteria was also tested. Among all the tested fungi, *A. chevalieri* showed the largest inhibition zone when treated with the *A. vera* gel ethanolic extract, followed by *P. funiculosum* and *P. minioluteum*, which were more sensitive to and showed larger inhibition zones upon treatment with aqueous extract. For the *O. ficus-indica* ethanolic extract, T. funiculosus showed the largest inhibition zone. The aqueous extract of the *O. ficus-indica* showed low antimicrobial activity against all tested fungi. By contrast, both the *A. vera* and *O. ficus-indica* extracts showed antibacterial activity against *S. aureus*, *Shigella* sp., *E. coli*, and *MRSA* except *S. typhimurium*, which was the most resistant bacterium to both the aqueous and ethanol extracts of *A. vera* and *O. ficus-indica.*

1. Introduction

Cacti and cacti-like plants are important food sources for wild animals besides their use in medicine, chemical, spinning, and cosmetic industry. These plants are a low-cost source of readily available raw materials [1, 2] for various uses. As medicinal plants, they are rich sources of novel antimicrobial agents [3]. Throughout human history, various infectious diseases have been treated with traditional herbal medicines. A wide range of medicinal plant parts are extracted as raw drugs that possess various medicinal properties [3]. The antimicrobial agents of *A. vera* gel effectively kill or greatly reduce the growth of a wide range of pathogens [4–7]. Both *Opuntia* and *Aloe* species have wide applications, as bactericidal, antibiotic, fungicidal, and anti-inflammatory agents; in tissues as moisturizing creams; and as pain relievers for joint and muscle pain [6, 8–11]. Several studies have reported that *A. vera* gel is effective against both Gram-positive and -negative bacteria [12]. Irshad and Butt [13] found that *A. vera* gel has a good antibacterial effect toward some bacteria, such as *Escherichia coli*, *Salmonilla typhimurium*, *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Pseudomonas.* Jain et al. [12] reported that bioactive components in *A. vera* crude extracts have powerful antibacterial activity. Hence, higher concentrations of *A. vera* extracts can be utilized as secondary antibacterial agents for the treatment of some diseases. Another study reported the antibacterial properties of *A. vera* gel ethanolic extracts towards selected pathogens [6]. Arbab et al. [14] stated that *A. vera* is effective against infections caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*.

Pourmajed et al. [15] showed that the ethanolic extracts of the *O. ficus-indica* possess antimicrobial activity against *E. coli* isolated from patients with urinary tract infection (UTI). The ethyl acetate extract of the cactus showed antibacterial activity against five food-borne bacteria, namely, *B. subtilis*, *Staphylococcus aureus* subsp. *aureus*, *E. coli*, *S. typhimurium*, and *Pseudomonas fluorescens*. No study exists exploring the chemical composition and antimicrobial activities of *O. ficus-indica* and *A. vera* plants. Hence, current study was designed to evaluate these parameters of both plants collected from Jeddah, Taif, and Al Baha, in SA, by screening their ethanolic extracts using GC–MS/MS.

2. Materials and Methods

2.1. Collection of Plant Samples. The fresh naturally grown stems, leaves, and roots of *A. vera* and *O. ficus-indica* were harvested from the Jeddah, Taif, and Al Baha areas, in SA, in September 2019 and January 2020. The selected plants are listed in Table 1.

2.2. Preparation of Plant Extracts

2.2.1. Aqueous Extract. The *Opuntia ficus-indica* (leaves and stems) were soaked in a solution of sodium hypochlorite (0.1%; w/v) for disinfection and removal of any adherent soil material [16]. The samples were washed with distilled water to remove the sodium hypochlorite. The leaves and stems samples were taken by cutting 1/2 inch from the plant base to remove the yellow sap material and 1/2 inch from the apex. These were then cut into two or more parts for easy handling. The spiny leaf margins were removed using a clean knife. The inner gel was carefully scraped out using a clean spoon to avoid the green areas. The gel was mixed using a grinder to obtain a juice gel. The juice gel was heated in a hot water bath at 70°C for 20 min. The juice was then filtrate to obtain a clear juice gel.

2.2.2. Ethanol Extract. The fresh stems of cacti and fresh leaves of cacti-like plants were rinsed several times with both tap water and distilled water [17]. 95 g of the samples were then grounded in an electrical blender to obtain a fine paste by adding a few drops of 99% ethanol. The gel was equally distributed in conical flasks, after which 150 mL of 99% alcohol was added to all conical flasks. Subsequently, the flasks were kept in a rotary shaker for 3 days. The gel was filtered using Whatman filter paper no.1 (Filter Papers Grade 1, Turkey), evaporated in a heating mantle yielding 14.8 g powder, which was stored in a screw cap test tube at 4°C. 10 mg/ml of the powder, and was used later on in the antimicrobial activity testing.

2.3. Pathogenic Microorganisms. Seven pathogenic fungi (Aspergillus chevalieri (MT487830.1), *Aspergillus terreus* (MT558939.1), *Penicillium funiculosum* (JX500735.1), *Talaromyces funiculosus* (KX262973.1), *Penicillium minioluteum* (JN620402.1), *Aspergillus niger* (MT628904.1), *Curvularia khuzestanica* (MH688044.1)), and five human pathogenic bacteria (*Escherichia coli* (11775), *Staphylococcus aureus* (12600), *Shigella* sp., methicillin-resistant *Staphylococcus aureus* (33591), and *Salmonella typhimurium* (14028)) were obtained from the King Fahad Researcher Centre in Jeddah and used during the experiment.

2.3.1. Determination of Antimicrobial Activity. PDA medium was used for subcultivation of the fungal and bacterial strains. Each microbe was evenly inoculated on the PDA media in plates using the streak method. Filter papers (5 mm diameter) were completely immersed in the aqueous and ethanol extracts of *A. vera* and *O. ficus-indica* extract and then placed on the agar surface. The plates were incubated for 2–5 days at 37°C and 28°C for fungal and bacterial growth, respectively. All experiments were performed thrice [18]. The antimicrobial activity was determined by observing the inhibition zone.

2.3.2. Antimicrobial Sensitivity Test on Microbes (Positive Control). For the positive control samples for microbe inhibition, the antibiotic itraconazole was used for fungi and ciprofloxacin for bacteria.

2.3.3. Antimicrobial Effect of Solvents (Negative Control). Ethanol and distilled water were used as negative control.

2.4. Gas Chromatography-Mass Spectrometry Conditions. About 500 μL ethanol and 500 μL samples were added. The mixture was vortexed for 30 seconds and then injected for GC–MS analysis.

2.4.1. Gas Chromatography–Mass Spectrometry (GC–MS/MS) for Aloe vera. The *A. vera* extracts were analyzed by gas chromatography (Agilent 7890A GC System). A Hp-5 ms fused silica capillary column was used (5% phenyl/95% dimethylsiloxane 30 M × 0.25 mm film thickness 0.32 Lm). The oven temperature was 110°C (isothermal for 2 minutes), with an increase to 200°C (10°C/min) and then 280°C (5°C/min), with final temperature (isothermal for 9 minutes).

The carrier gas used was helium (flow rate of 1 mL/min). GC–MS analyses were performed using an Agilent Technologies 7000 GC/MS Triple Quad coupled to an Agilent Technologies 7693 Autosampler. The capillary column and GC conditions were calculated as described above. MS spectra were recorded at 70 eV, and the scanning rate was 1 scan/s with a run time of 90 minutes [19].

2.4.2. Gas Chromatography–-Mass Spectrometry (GC–MS/MS) for Opuntia ficus-Indica. Gas chromatographic analysis was performed using an Agilent Technologies 7890A GC System. The separation was achieved using an HP-5 ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness). The GC oven temperature was programmed for an increase from 40°C (5 min) to 250°C @ 2°C/min, held for 15 minutes and eventually increased to 270°C@ 10°C/min. Helium was used as a carrier gas, with a 1.0 mL/min flow

Table 1: List of Cacti and cacti-like plants utilized in this study.

No	Scientific name	Family	Common name	Part of plant used	Collection site
1	*Aloe vera*	*Asphodalaceae*	*Aloe vera*	Roots and leaves	Jeddah
2	*Opuntia ficus-indica*	Cactaceae	*Opuntia* Teen Shouki Barshoumi	Roots and stems	Albaha and Taif

rate, and injector and detector temperature of 250°C, and 280°C, respectively. A fused silica HP-Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., 0.20 mm film thickness) was used. A mixture of aliphatic hydrocarbons (C8–C30) in hexane was directly injected into the GC injector under the abovementioned temperature program to calculate the retention index (as the Kovats index) of each compound. GC–MS analysis was performed on an Agilent Technologies 7000 GC/MS Triple Quad coupled to an Agilent Technologies7693 Autosampler. The separation was performed using a fused silica HP-5 capillary column (30 m × 0.25 mm i.d., 0.33 mm film thickness). The GC oven temperature was programmed similarly to in the gas chromatographic analyses. The MS scan conditions were source temperature, 25°C; interface temperature, 290°C; electron energy, 70 eV; and mass scan range, 40–450 AMU [20].

2.5. Statistical Analysis. Using the Windows Microsoft Excel 2013 software, version 15.0, analyzing the results was carried out using a one-way analysis of variance (ANOVA) test to determine any significant differences between the antimicrobial effect in both cacti and cacti-like plants. All columns versus control and the P value <0.05 are considered as significant.

3. Results

3.1. Determination of Antimicrobial Activity of Aloe vera Extract. The antimicrobial activity of *A. vera* extracted using two solvents, aqueous and ethanol, showed different response against the selected fungi and bacteria. Among all tested fungi, the largest inhibition zone for ethanol extracts was observed with *A. chevalieri* (1.00 ± 0.50 mm), while *P. funiculosum* and *P. minioluteum* were the most sensitive for aqueous extracts, with inhibition zones of 0.57 ± 0.40 and 0.43 ± 0.23 mm), respectively. *A. terreus* and *A. niger* were the most resistant fungi, with no activity revealed for both the aqueous and ethanol extracts. The aqueous extract of *A. vera* showed antibacterial activity against *S. aureus, Shigella* sp., and *E. coli*, with inhibition zones of 1.10 ± 0.10, 0.47 ± 0.25, and 0.40 ± 0.10 mm, respectively. MRSA showed the largest inhibition zone with ethanol extract (0.70 ± 0.26 mm). *S. typhimurium* was the most resistant bacteria, with no activity being shown for either the aqueous or ethanol extracts of *A. vera* (Tables 2 and 3).

ANOVA showed that the *A. vera* aqueous extract has the highest antibacterial activity with an average value of 0.4592 while the ethanolic extract has the highest antifungal activity with an average value of 0.2331. There is a statistically significant difference between aqueous and ethanolic extracts ($P < 0.05$) (Table 4).

3.2. Determination of Antimicrobial Activity of Opuntia ficus-Indica Extract. The antimicrobial activity of *O. ficus-indica* aqueous and ethanolic extracts, showed different response against the selected pathogens. Among tested fungi, T. funiculosus showed the largest inhibition zone against ethanolic extract (0.40 ± 0.10 mm), while P. funiculosum showed a smaller zone of inhibition against ethanolic extract (0.07 ± 0.06 mm). The aqueous extract showed low antimicrobial activity with all tested fungi, ranging from 0.13 to 0.17. The *O. ficus-indica* aqueous extract showed significant antibacterial activity against MRSA, *Shigella* sp., and *S. aureus.* MRSA showed the largest inhibition zone of 1.43 ± 0.40 mm, followed by *Shigella* sp. and *S. aureus*, with inhibition zones of 0.73 ± 0.06 and 0.66 ± 0.20 mm, respectively, against aqueous extracts. *S. typhimurium* was the most resistant bacteria, with no activity for either the aqueous or ethanolic extracts (0.00) (Tables 5 and 6).

The ANOVA showed that the *Opuntia ficus-indica* aqueous and ethanolic extracts have the highest antibacterial activity with an average of (0.59, 0.198), respectively. There is a statistically significant difference between aqueous and ethanolic extracts ($p < 0.05$) (Table 7).

3.3. GC–MS Analysis of Aloe vera Ethanolic Extract. The GC–MS/MS analysis for A. vera was performed for 18 identified compounds. The well-known phthalic acid, di(2-propylpentyl) ester (92.4%) was identified as the major compound, followed by hexadecenoic acid, ethyl ester (91.8%), lidocaine (89%), trib utyl acetyl citrate (88.1%), ethyl oleate (86.0%), 1,4-benzenedi carboxylic acid, bis(2-ethylhexyl)ester (80.8%), pyrolo[3,2-d]py rimidin-2,4(1H,3H)-dione (77.3%), phenol,4-[(5,6,7,8-tetrahy dro-1,3-dioxolo[4,5-g]isoquinolin-5yl)methyl],(R)- (76.4%), 3-methyl-1,2,3,4 tetrahydro-gamma-carboline (73.4%), 2,2 dim ethyl-5-phenyl-3(2H)furanone (71.7%), 1,2 benzenedi-carbox ylic acid,bis(2-methylpropyl)ester (71.2%), 2-fluoro-6-trifluoro methylbenzoic acid, 4-nitrophenyl ester (71.2%), 4-(benzyl-eth yl-amino)-butyric acid, methyl ester (70.7%), (2-aziridinyl ethyl)amine (69.8%), 3-methyl-4-nitrophenyl pentafluoro benzyl ether (67.7%), methane, difluoroiodo- (66.5%), pyrro le,2-methyl-5-phenyl- (66.0%), and benzamide,2-methoxy-N-b enzyl-N-phenethyl- (65.6%) (Figures 1 and 2).

3.4. GC–MS of O. ficus-Indica Ethanolic Extracts. The GC–MS analysis of *O. ficus-indica* led to 34 compounds, namely, 1-(benzyloxy)-3,5-dinitrobenzene (81.5%), phenol, 5-ethenyl-2-methoxy- (80.7%), hexadecanoic acid, ethyl ester (80.6%), 1,3-pentadiyne,1,5,5,5-nitropyrimidine (77.0%), benzene,1fluoro-4-methyl(75.6%), trifluoromethylthiocyanate (74.4%),1-(benzyloxy)-3,5dinitro-benzene (74.2%), furan,2-methoxy-(74.1%), phthalic acid, di(oct-3-yl)ester (72.1%), acetic acid,(4-chloro-2-methylphenoxy)-heptadecyl ester (71.6%), pyridine,3-propyl-(71.1%), benzphetamine (71.0%), l-alanine,

Table 2: Antifungal activities of aqueous and ethanolic extract of *A. vera*.

No	Fungi	Inhibition zone (mm)			
		A. vera aqueous extract (30 μ/disc)	*A.vera* ethanolic extract (30 μ/disc)	Itraconazole (positive control) (30 μ/disc)	Distilled water (negative control) (30 μ/disc)
1	*A. chevalieri*	0.33 ± 0.12	1.00 ± 0.50	0.27 ± 0.12	0.00
2	*T. funiculosus*	0.27 ± 0.06	0.13 ± 0.12	0.13 ± 0.06	0.00
3	*A. terreus*	0.00	0.00	1.7 ± 0.06	0.00
4	*P. funiculosum*	0.57 ± 0.40	0.20 ± 0.10	0.23 ± 0.15	0.00
5	*P.minioluteum*	0.43 ± 0.23	0.23 ± 0.23	1.33 ± 0.47	0.00
6	*A. niger*	0.00	0.00	1.20 ± 0.36	0.00
7	*C. khuzestanica*	0.07 ± 0.06	0.07 ± 0.06	0.20 ± 0.10	0.00

All values are expressed as mean ± SD.

Table 3: Antibacterial activities of aqueous and ethanolic extract of *A. vera*.

No	Bacteria	Inhibition zone (mm)			
		A. vera aqueous extract (30 μ/disc)	*A. vera* ethanolic extract (30 μ/disc)	Ciprofoxacin (positive control) (30 μ/disc)	Distilled water (negative control) (30 μ/disc)
1	*Staph. aureus*	1.10 ± 0.10	0.00	2.00 ± 0.00	0.00
2	*Shigella sp.*	0.47 ± 0.25	0.17 ± 0.06	0.23 ± 0.06	0.00
3	*S.thypimurium*	0.00	0.00	1.33 ± 0.3	0.00
4	MRSA	0.33 ± 0.06	0.70 ± 0.26	1.83 ± 0.3	0.00
5	*E.coli*	0.40 ± 0.10	0.07 ± 0.06	1.97 ± 0.06	0.00

All values are expressed as mean ± SD.

Table 4: One-way ANOVA for antifungal and antibacterial activity of *A.vera* extracts.

No	Bacteria	Inhibition zone (mm)			
		A. vera aqueous extract (30 μ/disc)	*A. vera* ethanolic extract (30 μ/disc)	Ciprofoxacin (positive control) (30 μ/disc)	Distilled water (negative control) (30 μ/disc)
1	*Staph. aureus*	1.10 ± 0.10	0.00	2.00 ± 0.00	0.00
2	*Shigella sp.*	0.47 ± 0.25	0.17 ± 0.06	0.23 ± 0.06	0.00
3	*S.thypimurium*	0.00	0.00	1.33 ± 0.3	0.00
4	MRSA	0.33 ± 0.06	0.70 ± 0.26	1.83 ± 0.3	0.00
5	*E.coli*	0.40 ± 0.10	0.07 ± 0.06	1.97 ± 0.06	0.00

Table 5: Antifungal activities of aqueous and ethanolic extract of *O. ficus-indica*.

No	Fungi	Inhibition zone (mm)			
		O. ficus-indica aqueous extract (30 μ/disc)	*O. ficus-indica* ethanol extract (30 μ/disc)	Antibiotic itraconazole (positive control) (30 μ/disc)	Distilled water (negative control) (30 μ/disc)
1	*A. chevalieri*	0.10 ± 0.10	0.13 ± 0.06	0.27 ± 0.12	0.00
2	*T. funiculosus*	0.10 ± 0.10	0.40 ± 0.10	0.13 ± 0.06	0.00
3	*A. terreus*	0.00	0.20 ± 0.10	1.66 ± 0.06	0.00
4	*P. funiculosum*	0.07 ± 0.06	0.07 ± 0.06	0.23 ± 0.15	0.00
5	*P. minioluteum*	0.00	0.13 ± 0.06	1.33 ± 0.47	0.00
6	*A. niger*	0.17 ± 0.06	0.13 ± 0.06	1.20 ± 0.36	0.00
7	*C. khuzestanica*	0.13 ± 0.06	0.17 ± 0.57	0.20 ± 0.10	0.00

All values are expressed as mean ± SD.

n-propargyloxycarbonyl-, ethyl ester (70.4%), 2-aminohydra tropic acid (69.8%), thiophene,3-methyl- (69.6%), cyanogen-chloride (69.2%), alanine, N-methyl-n-propoxycarbonyl-, nonyl ester (69.1%), 3-amino2,4dimethylpentane (68.1%), 2,2′-bioxirane (68.2%), hydrazine-carbo-thioamide, N-methyl-(67.7%), 4-methyl-2,4-bis(p-hydroxy-phenyl)pent-1-ene,2TM Sderivative (67.6%), phthalic acid,bis(2-pentyl) ester (67.5%), phthalic acid, butyl hexyl ester (67.4%), 2-butynedinitrile (67.2%), bis(2-ethylhexyl)phthalate (67.1%), silane, dimethyl(4methoxyphenoxy)heptadecyloxy- (67.0%), 4-hexen-2-one(66.4%), furfural (66.4%), 1-penten-3-one (65.7%) and 2propanol,1,3-dibromo (65.1%) (Figures 3 and 4).

Table 6: Antibacterial activities of aqueous and ethanolic extract of *O. ficus-indica*.

No	Bacteria	Inhibition zone (mm)			
		O. ficus-indica aqueous extract (30 μ/disc)	*O. ficus-indica* ethanolic extract (30 μ/disc)	Ciprofoxacin (positive control) (30 μ/disc)	Distilled water (negative control) (30 μ/disc)
1	*Staph .aureus*	0.66 ± 0.20	0.00	2.00 ± 0.00	0.00
2	*Shigella sp.*	0.73 ± 0.06	0.10 ± 0.10	0.23 ± 0.06	0.00
3	*S. thypimurium*	0.00	0.00	1.33 ± 0.28	0.00
4	MRSA	1.43 ± 0.40	0.76 ± 0.25	1.83 ± 0.28	0.00
5	*E.coli*	0.13 ± 0.06	0.13 ± 0.06	1.97 ± 0.06	0.00

All values are expressed as mean ± SD.

Table 7: One-way ANOVA for antifungal and antibacterial activity of *O. ficus-indica* extracts.

	Average	Variance	*P* value
Antifungal activity of *O. ficus-indica* aqueous extract	0.0814	0.0040	0.0019
Antifungal activity of *O. ficus-indica* ethanolic extract	0.1757	0.0113	
Antibacterial activity of *O. ficus-indica* aqueous extract	0.59	0.3224	0.0011
Antibacterial activity of *O. ficus-indica* ethanolic extract	0.198	0.1021	

Coppounds in Aloe vera			
Classes of compounds	Bioactive Compounds	MW	Molecular
long-chain fatty acid ethyl ester	Phthalic acid, di (2-propylpentyl) ester	402.5	$C_{26}H_{26}O_4$
long-chain fatty acid ethyl ester	Hexadecanoic acid, ethyl ester	284.4	C18H36O2
Monocarboxylic acid amide	Lidocaine	234.34	$C_{14}H_{22}N_2O$
plasticizers	Tributyl acetylcitrate	402.5	C20H34O8
polyphenols	Pyrolo[3,2-d] pyrimidin-2,4 (1H,3H)-dione	151.12	$C_6H_5N_3O_2$
Ester	2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester	329.2	$C_{14}H_7F_4NO_4$
plasticizers	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	278.3	$C_{16}H_{22}O_4$
Pyrroles	Pyrrole, 2-methyl-5-phenyl-	157.21	$C_{11}H_{11}N$
polyphenols	Benzamide, 2-methoxy-N-benzyl-N-phenethyl	257.29	$C_{15}H_{15}NO_3$

Figure 1: The various compounds found in *A. vera*.

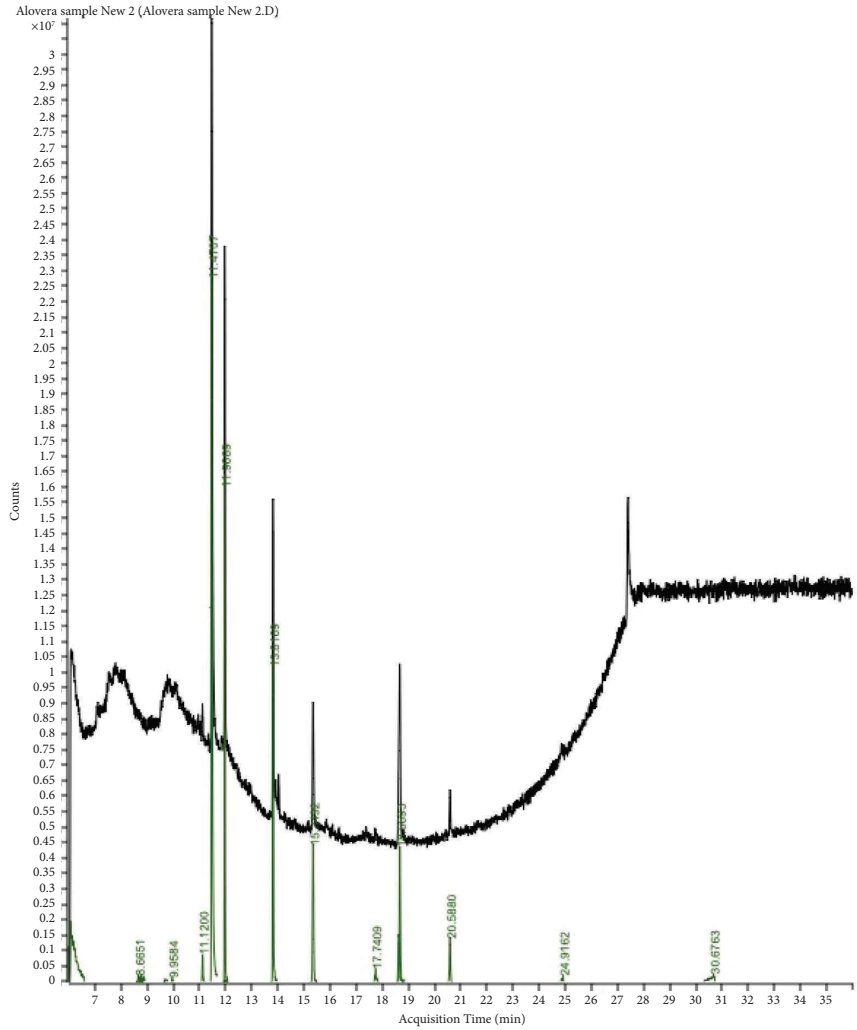


Figure 2: Continued.

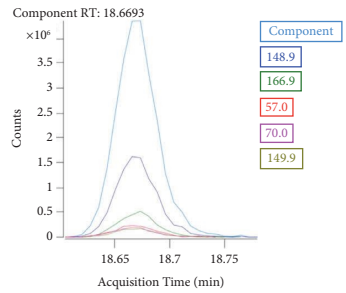
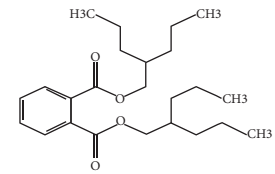
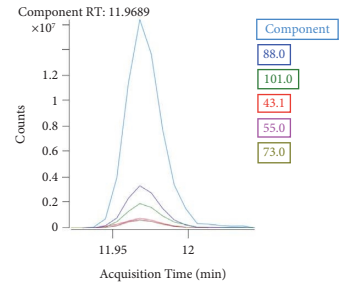
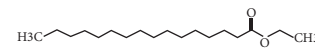
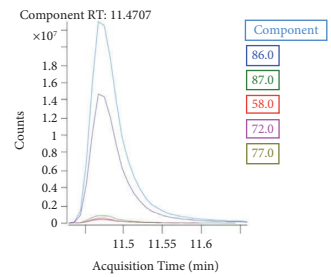
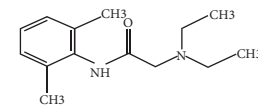
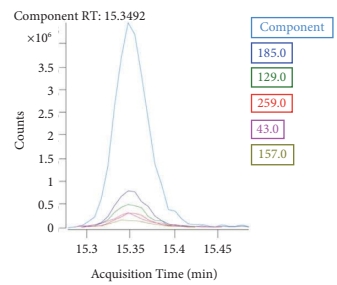
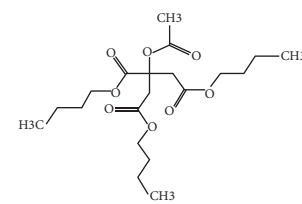
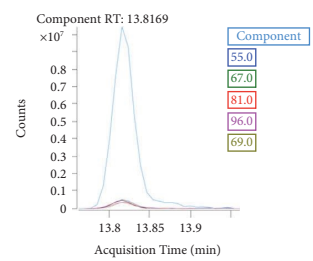
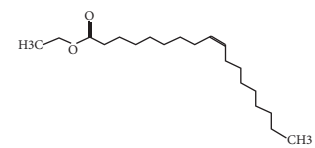
Compound name	GCMS Analyses	Structure
Phthalic acid, di (2-propylpentyl) ester		
Hexadecanoic acid, ethyl ester		
Lidocaine		
Tributyl acetylcirate		
Ethyl Oleate		

Figure 2: Continued.

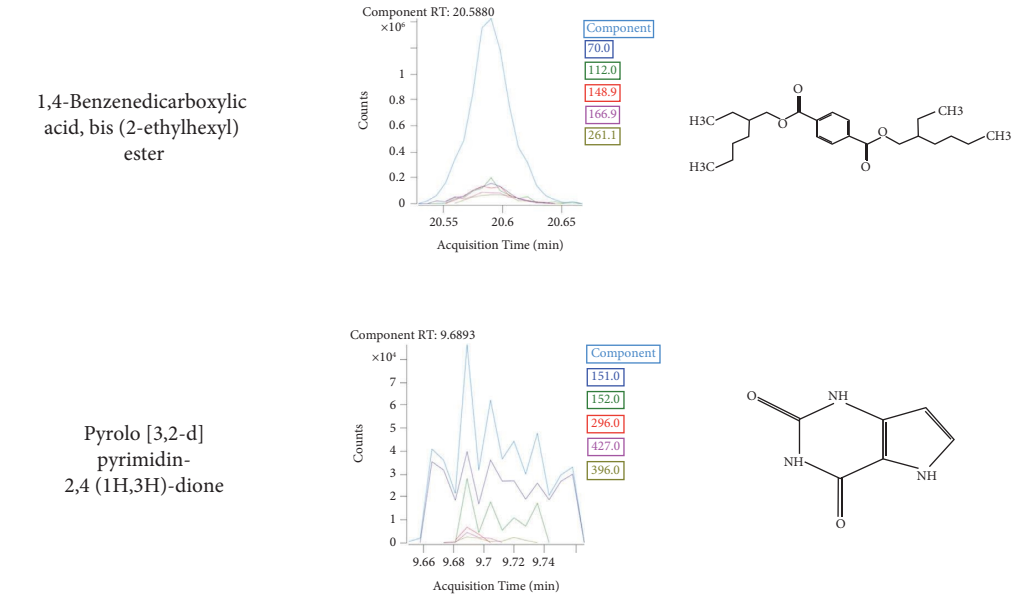


Figure 2: Active metabolites of *Aloe vera* ethanolic extract using GC–MS/MS. Base peak chromatogram of *Aloe vera* ethanolic extract (A) and identified secondary metabolites. A: The chromatogram obtained from the GCMS of O. *ficus-indica* ethanolic extract.

3.5. The Biological Activities of A. vera and O. ficus-Indica Compounds. GC–MS was conducted using the National Institute Standard and Technology (NIST) database. In total, 18 and 34 compounds were studied from *A. vera* and *O. ficus-indica*, respectively. Hexadecenoic acid, ethyl ester, found in extracts of both plants, has antioxidant, nematicide, hypocholesterolemic, pesticide, lubricant, antiandrogenic, flavor, and hemolytic activity [21–23]. Javahershenas and Khalafy [24] showed that pyrolo[3,2-d] pyrimidin-2,4(1H,3H)-dione has antimicrobial, antibacterial, antifungal, anti-inflammatory, antitumor, antioxidant, antiviral, anti-HIV, anti-asthmatic, and anticoagulant activity. Benzamide,2-methoxy-*N*-benzyl-*N*-phenethyl has antibacterial and antifungal activity [25, 26]. Phthalic acid, di(2-propylpentyl) ester, tributyl acetyl citrate, phthalic acid, di(oct-3-yl)ester, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester, and pyrrole,2-methyl-5-phenyl-, all have antimicrobial activity [27–31]. However, 2-fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester is an acidifier and an arachidonic acid inhibitor, which increases aromatic amino acid decarboxylase activity, according to [32] (Tables 8 and 9).

4. Discussion

The present study aimed to evaluate the potential antimicrobial activity of *O. ficus-indica* and *Aloe vera* plants collected from Saudi Arabia. To our best knowledge, this is the first report of being tested on the selected microbial species from Saudi Arabia.

The results showed that *A. vera* extract has higher antifungal activity than the *O. ficus-indica* extract, as evidenced by the larger inhibition zone. The ethanolic extract of both plants had a significant effect on the microbes. The best results in terms of inhibition were observed against A. chevalieri and P. funiculosum were with *A. vera* ethanolic and aqueous extract, respectively. For *S. aureus* and *Shigella* sp., the best inhibition was observed with the aqueous extract, and for MRSA, this was with the ethanolic extract of *A. vera*.

The *O. ficus-indica* extract showed the best inhibition result with *T. funiculosus* using the ethanolic extract, while the aqueous extract showed insignificant effects against all fungi. The best inhibitory activity with both aqueous and ethanolic extract was observed for MRSA, followed by *Shigella* sp., and then *S. aureus* but only with the aqueous extract. *O. ficus-indica* has a stronger effect on bacteria than fungi, while *A. vera* has good effects on both fungi and bacteria. Both *S. aureus* and *B. subtilis* are significantly inhibited by *A. vera* gel extract [33]. Previous studies showed that the *A. vera* has a significant effect on *S. aureus* and *B. subtilis* and an insignificant effect on *A. ficuum* [34]. Ethanolic extract of *A. vera* had a greater inhibition zone than methanolic extract with *S. aureus*, *B. subtilis, E. coli*, and *S. typhimurium*. *A. vera* and *O. ficus-indica* ethanolic extracts had inhibitory effects on *E. coli*, *S. aureus*, *Acinetobacter*, and *S. epidermidis* with different concentrations [6]. Both plant extracts had a significant impact on the aforementioned bacteria [35]. Antimicrobial activity of the *O. ficus-indica* seeds oil against *C. albicans*, *E. coli*, *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *S. cerevisiae*, *and S. typhimurium* [36] showed that the oil extracts have high antimicrobial activity against Gram-positive and -negative bacteria.

The discovery of novel antimicrobial metabolites from medicinal plants such as *A. vera* and *O. ficus-indica* is an important alternative to overcome the increasing drug

Coppounds in *O. ficus-indica*			
Classes of compounds	Bioactive Compounds	MW	Molecular
Ketones	4-Hexen-2-one	98.14	$C_6H_{10}O$
Organosulfur	Thiophene, 3-methyl-	98.16	$CH_3C_4H_3S$
long-chain fatty acid ethyl ester	Phthalic acid, di (oct-3-yl) ester	418.6	$C_{24}H_{38}O_4$
Polyphenols	4-Methyl-2,4-bis (p-hydroxyphenyl)pent-1-ene, 2TMS derivative	268.3	$C_{18}H_{20}O_2$
long-chain fatty acid ethyl ester	Phthalic acid, bis (2-pentyl) ester	306.4	$C_{20}H_{30}O_4$
long-chain fatty acid ethyl ester	Hexadecanoic acid, ethyl ester	284.4	$C_{18}H_{36}O_2$
Anorectic	Benzphetamine	239.3	$C_{17}H_{21}N$
Plasticizers	Bis (2-ethylhexyl) phthalate	390.5	$C_{24}H_{38}O_4$
Fatty acid	2-Aminohydratropic acid	165.19	$C_9H_{11}NO_2$
Local anaesthetic	Tolycaine	278.35	$C_{15}H_{22}N_2O_3$
Hydrocarbons	1,3-Pentadiyne, 1,5,5,5-tetrafluoro-	136.05	C_5F_4
Amino acids derivative	l-Alanine, n-propargyloxycarbonyl-, ethylester	199.20	$C_9H_{13}NO_4$
long-chain fatty acid ethyl ester	Phthalic acid, butyl hexyl ester	306.3	$C_{18}H_{26}O_4$
polyhenols	1- (Benzyloxy)-3,5-dinitrobenzene	274.2	$C_{13}H_{10}N_2O_5$
Hydrocarbons	2-Butynedinitrile	76	C_4N_2
Aldehyde	2-Propanol, 1,3-dibromo	217.89	$C_3H_6Br_2O$
Aliphatic amine	3-Amino-2,4-dimethylpentane	115.22	$C_7H_{17}N$
Polyphenols	Phenol, 5-ethenyl-2-methoxy-	150.17	$C_9H_{10}O_2$
Ketone	1-Penten-3-one	84	C_5H_8O
Terpenes	Silane, dimethyl (4-methoxyphenoxy)heptadecyloxy-	436.7	$C_{26}H_{48}O_3Si$
Pyrimidines	5-Pyrimidinol, 2-methyl-	94	$C_5H_6N_2$
Amino acids derivative	Alanine, N-methyl-n-propoxycarbonyl-, nonyl ester	259.34	$C_{13}H_{25}NO_4$
polyphenols	Acetic acid, (4-chloro-2-methylphenoxy)-, heptadecylester	439	$C_{26}H_{43}ClO_3$
Pyridines	Pyridine, 3-propyl-	121	$C_8H_{11}N$
polyphenols	Phenol, 5-ethenyl-2-methoxy-	150	$C_9H_{10}O_2$

Figure 3: The various compounds found in *O. ficus-indica*.

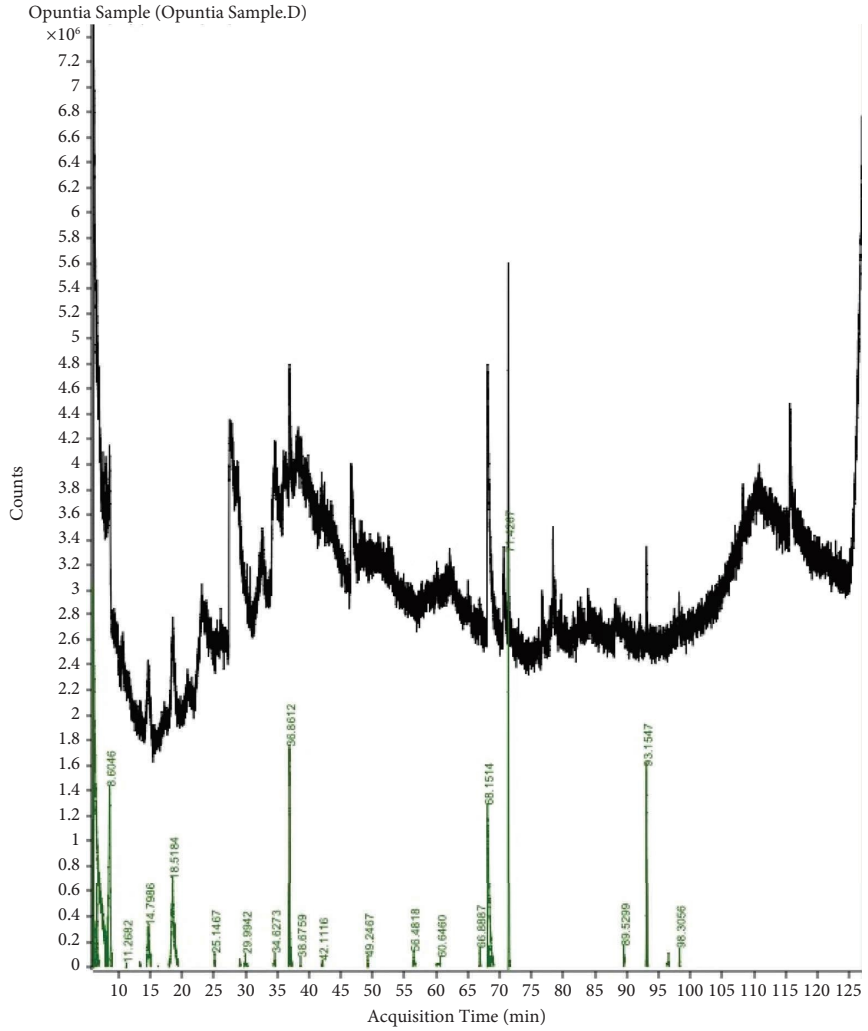
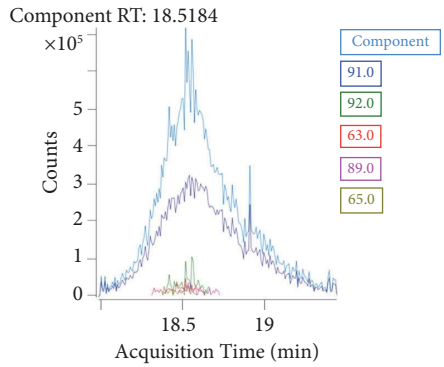
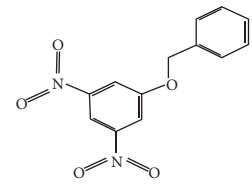
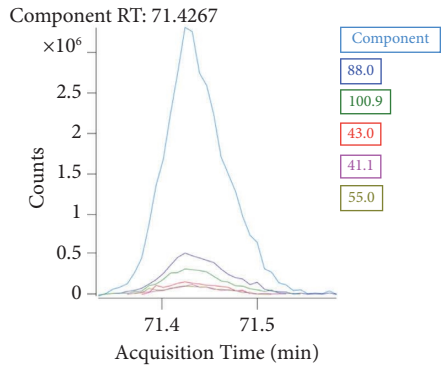
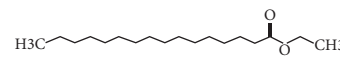
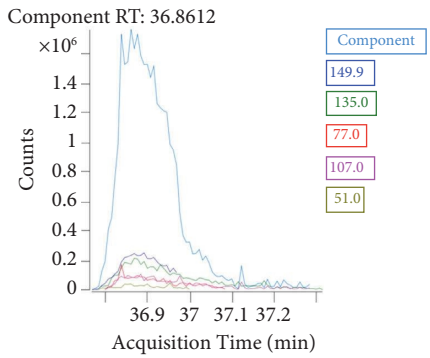
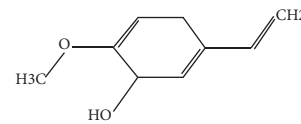
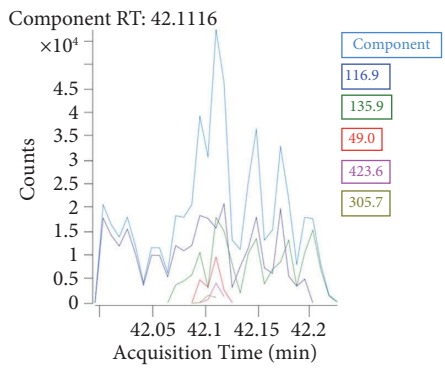
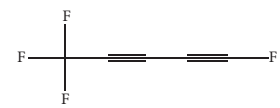


Figure 4: Continued.

Compound name	GC MS Analyses	Structure
1-(Benzyloxy)-3,5-dinitrobenzene		
Hexadecanoic acid, ethyl ester		
Phenol, 5-ethenyl-2-methoxy-		
1,3-Pentadiyne, 1,5,5,5-tetrafluoro-		

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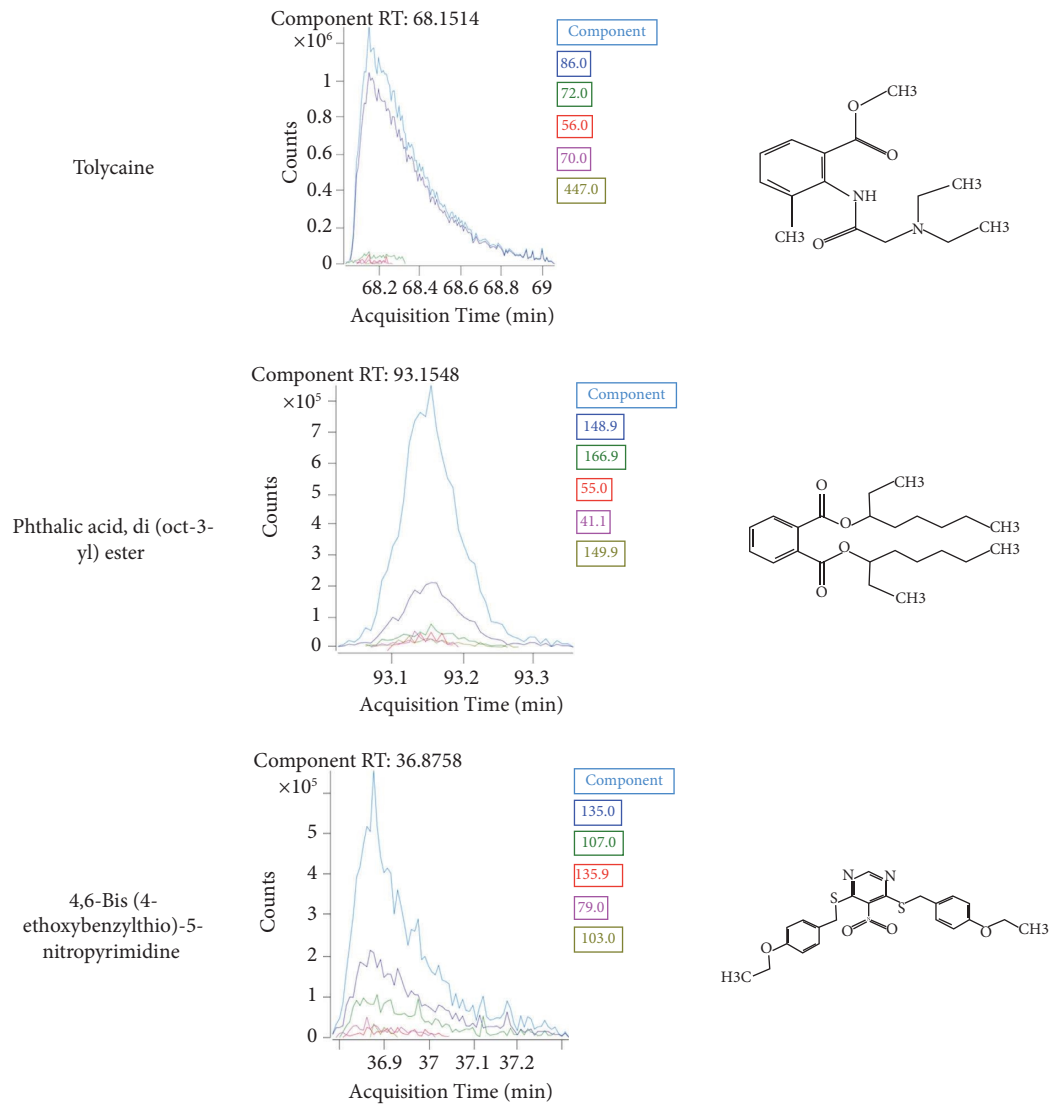

Figure 4: Active metabolites in *O. ficus-indica* extract identified by GC–MS. Base peak chromatogram of *O. ficus-indica* methanolic extract (A) and identified secondary metabolites. A: The GCMS chromatogram of the A. vera ethanolic extract.

Table 8: Biological activities of *A. vera* compounds.

No	RT	Compound	Match factor	Biological activity
1	18.6693	Phthalic acid, di(2-propylpentyl) ester	92.4	Antimicrobial [30]
2	11.9689	Hexadecanoic acid, ethyl ester	91.8	Antioxidant, nematicide, hypocholesterolemic, pesticide, luubricant, antiandrogenic, flavor, hemolytic [22, 23]
3	11.4707	Lidocaine	89.4	Anaesthetic, antiarrhythmic (DrugBank accession number DB00281)
4	15.3492	Tributyl acetylcitrate	88.1	Anticancer, antimicrobial activity [28]
5	9.6893	Pyrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione	77.3	Antimicrobial, antibacterial, antifungal, anti-inflammatory, antitumor, antioxidant, antiviral, anti-HIV agents, antiasthmatic, anticoagulant. [24]
6	30.6763	2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester	71.2	Acidifier, arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity [32]
7	11.1200	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	71.2	Antimicrobial, α-Glucosidase inhibition, and the in vivo hypoglycemic effect. [29]
9	8.8667	Pyrrole, 2-methyl-5-phenyl-	66.0	Antimicrobial, inti-inflammatory, antitumor [27]
10	24.9162	Benzamide, 2-methoxy-*N*-benzyl-*N*-phenethyl	65.6	Antifungal and antibacterial. [26]

Table 9: Biological activities of *O. ficus-indica* compounds.

No	RT	Compound	Match factor	Biological activity
1	71.4267	Hexadecanoic acid, ethyl ester	80.6	Antioxidant, nematicide, hypocholesterolemc, pesticide, lubricant, antiandrogenic, flavor, and hemolytic [22, 23]
2	68.1514	Tolycaine	78.8	Reduce the pain of injection, use in dental injection. (DrugBank)
3	93.1548	Phthalic acid, di(oct-3-yl) ester	72.1	Antimicrobial and antifouling. [31]
4	93.1547	Bis(2-ethylhexyl) phthalate	67.1	Antibacterial and antifungal agent. [25, 28]

resistance in humans. The plant extracts with known antimicrobial properties can be of great importance for therapeutic treatments. *O. ficus-indica* has been used traditionally for controlling many different pathogenic bacterial infections [37].

Endophytic fungi isolated from *A. vera* (*Penicillium* sp., *Aspergillus* sp., and *F. oxysporum*) showed a moderate inhibition zone when treated with methanol extract [38]. The *A. vera* extracts showed antibacterial activity against both Gram-positive and -negative isolates, while the leaf extracts did not show any such activity [4]. Ethanolic extract of *A. vera* inhibited the growth of *E. coli*, *S. aureus*, and *C. albicans* with zones of inhibition of 6, 5, and 4 mm, respectively, while aqueous extract had zones of inhibition of 6, 4, and 3 mm, respectively [7]. The methanolic extract of *A. vera* inhibited the growth of *E. coli* (3 mm) only.

A. vera extracts showed greater antibacterial activity against Gram-positive bacteria as compared to Gram-negative bacteria [6]. With respect to individual pathogens, the ethanolic extract showed greater inhibition than the methanolic extract, while significantly lower inhibition was observed with acetone extract. *A. vera* distilled extract was effective against *P. aeruginosa* [5]. *A. vera* sterol extract had great antifungal activity against *A. niger*, *A. terreus*, and *Penicillium* sp. and antibacterial activity against *S. aureus*, *E. coli*, and *S. typhimurium* [39]. *A. vera* extract has antimicrobial activity against *S. aureus*, *E. coli*, and *Shigella* [40]. Both *A. barbadensis* juice and gel inhibited the growth of *C. albicans*, *E. coli* and *P. fluorescens* better than that of *A. arborescens* [41].

A. vera extracts showed low antifungal activity against the *Aspergillus* and *Penicillium* strains and strong activity against the *Alternaria spp2* strain [42]. The cactus pear fruit (*O. ficus-indica)* extracts clearly showed positive action against the bacterial and fungal species [43]. However, the extract did not show any activity against *A. niger* for more than 2 days, unlike *C. albicans*, which can resist the inhibitory activity of the extract for periods longer than 2 days. Good antibacterial activity of the *O. ficus-indica* skin fruit extracts has been revealed against both Gram-positive and Gram-negative bacterial isolates [44]. However, the extraction of *A. vera* showed low antifungal activity on the *Aspergillus* strains and *Penicillium* strains and strong activity against the *Alternaria spp2* strain [42]. *A. niger* showed a large inhibition zone with the alcohol and aqueous extracts of *A. vera*, and no zone was observed with *C. albicans* [45].

GC–MS was conducted using the National Institute Standard and Technology (NIST) database. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The essential oil composition of the skin, pulp, and seeds from the *O. ficus-indica* fruits was analyzed by hydrodistillation (HD) followed by gas chromatography–mass spectrometry (GC–MS) to investigate the compounds in processed fruit [20]. Prickly pear has a high bioactive potential, being an important source of bioactive compounds and an excellent source of dietary antioxidants [46]. The antioxidant and antimicrobial compounds highlight the importance of *O. ficus-indica* as a crop [20]. Twelve compounds were identified from *A. vera*, including hexadecenoic acid, octadecanoic acid, tricosane, 1-octadecanol, and trace amounts of sterols [39]. The ethanolic and aqueous extracts of the bagasse (byproducts of *A. vera* processing leaves) have shown antioxidant and antifungal activity [47].

The antioxidant properties of *A. vera* flowers could have an important application in the food industry, providing an income for farmers if the flowers are considered as a byproduct rather than a residue. Natural products with antioxidant properties have been extensively utilized as health-promoting products and natural additives in the food industry [48]. *A. vera* compounds have anticancer and antimicrobial activity [19].

We investigated the activity of 18 and 34 compounds from *A. vera* and *O. ficus-indica*, respectively. The studied compounds showed biological activity similar to the results reported earlier, with similar antioxidant, antifungal, antibacterial, antiviral, and anti-inflammatory properties. These plants may offer a new source of antibacterial and antifungal agents with significant activity against infective microorganisms [3]. However, our results should be verified through further investigations on the antimicrobial activity of these cacti-like plants and other aspects. Based on the results obtained in this research, those types of polyphenols compounds obtained in *A. vera* and *O. ficus-indica* may be a potential target in the future to explore antifungal and microbial benefits for humanity.

5. Conclusions

Extracts from cacti and cacti-like plants possess antifungal and antibacterial activity against various pathogens. These extracts can be used as natural alternatives to synthetic antifungal treatments in fungi management. GC–MS clearly shows more polyphenols in *A. vera* extract as phthalic acid di(2-propylpentyl) ester and hexadecenoic acid ethyl ester, and 1-(benzyloxy)-3,5-dinitrobenzene and phenol, 5-ethenyl-2-methoxy in the *O. ficus-indica* extract. These compounds have a biological activity such as antimicrobial and antioxidant, which means that these plant extracts can be used in

medical and cosmetic products against fungi and bacteria. In the future study, we would like to use the HPLC for both plant extracts and study the phenol, flavonoids, and alkaloids compounds.

Abbreviations

NC-endophytes:	Nonclavicipitaceous endophytes
C-endophytes:	Clavicipitaceous endophytes
DSE:	Dark septate endophytes'
WHO:	World Health Organization
MRSA:	Methicillin-resistant *Staphylococcus aureus*
GC-MS:	Gas chromatography-mass spectrometry
PDA:	Potato dextrose agar
PDB:	Potato dextrose broth
EDTA:	Ethylenediamine tetracetic acid
Tris:	(Hydroxymethyl) aminomethane
TAE:	(Tris-acetate-EDTA) buffer
NaCl:	Sodium chloride
BLAST:	Basic local alignment search tool
PCR:	Polymerase chain reaction
ITS:	Internal transcribed spacer
UV:	Ultraviolet
NCBI:	National Center for Biotechnology Information
CF:	Colonization frequency
NIST:	National Institute Standard and Technology
HD:	Hydro distillation.

Data Availability

All datasets generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All authors have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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