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In Vitro Antimicrobial Activity and GC–MS Findings of the Gel of *Aloe vacillans* Forssk. of Abha Region, Saudi Arabia

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Abstract A great variety of weeds grow in the Abha area, Saudi Arabia, at more than 2700 m above sea level. The aim of this study was to evaluate the antimicrobial activities of various extracts of fresh and dry leaf gel of *Aloe vacillans* against eight clinical isolates of human pathogens. The chemical compounds present in the gel extract were identified and quantified by gas chromatography–mass spectrometry. Solvent extracts inhibited 62.5% of the examined microbes, whereas the fresh leaf extract was more potent and active against *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella oxytocam*, *Proteus mirabilis*, and *Candida albicans* compared to the dry leaf extract. No antimicrobial activities were observed against *Klebsiella pneumonia*, *Shigella flexneri*, and *Pseudomonas aeruginosa*. *Candida albicans* was the most susceptible pathogen based on its zone of inhibition. The maximum inhibitory activities were shown by the fresh gel of the chloroform extract against *M. luteus*, the methanol extract against *S. aureus*, the petroleum ether extract against *P. mirabilis*, *C. albicans*, and *K. pneumoniae*, and hot water extracts against *K. oxytocam*. Gram-positive bacteria and *C. albicans* were more susceptible than gram-negative bacteria. Compounds were identified and quantified; the major constituents of the gel were 1,3-dimethoxy-2-propanol and glycerol. Acetoglycerides, ethanol, 5-(hydroxymethyl)-2-furaldehyde, palmitic acid, butane, 1,2:3,4-diepoxy-,(.+/−.)-, 2-deoxy-D-galactose, butyl acetate, 2,2-dimethoxyethanol, diethyl phthalate, phytol, octadecanoic acid, gamma-linolenic acid, and stearic acid were present in low amounts. Therefore, because of its antimicrobial activities and useful phytochemical composition, *A. vacillans* leaf gel is a promising pharmaceutical drug for specific microbial infections and for improving health.

Keywords *Aloe vacillans* · Phytochemicals · Antimicrobial · Extracts · Gas chromatography–mass spectrometry

1 Introduction

Aloe vacillans Forssk. is a succulent plant that belongs to the Aloe family, which includes nearly 400 species worldwide. The plant is dominant in the desert because its leaves contain relatively large amounts of water under drought conditions while most other vegetation becomes dehydrated [1]. When the green skin is cut off, a yellowish substance is expelled containing a large amount of water and many other chemicals. The gel of the *A. vera* plant is composed of 99.3% water, 0.7% glucose, and many other constituents. These chemicals give the plant its distinct properties and can be used as skin care products, for cooling or treating various gastrointestinal disturbances, relieving thermal burns and advanced sunburn, stimulating wound healing, and promoting the body's immune system [1,2]. Although numerous studies have examined the therapeutic effect of *A. vera* gel, the medicinal use of *Aloe vacillans* [3,4] grown in Saudi Arabia remains unclear. Hence, in this study, the antimicrobial activities of both dry and fresh leaf extracts of *Aloe vacillans* were compared. In 1980s, few pathogens were resistant to medications. However, recent studies have shown that the number of drug-resistant microbes is increasing globally, although new antibiotics are being produced by the pharmacological industry [5]. For example, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella enteritidis*,

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Salmonella paratyphi, *Salmonella typhi*, *Enterococcus faecalis*, *Camplylobacter* spp., *Escherichia coli*, *Proteus* spp., *Proteus aeruginosa*, and *Candida albicans* have shown resistance to various drugs [6,7]. Natural products from plants are commonly used in traditional medicine to treat bacterial and *Candida* infections. Particularly, plants from the genus *Aloe* have several documented ethnobotanical uses in many cultures, including in ancient Egypt, Greece, and Rome to China and India, for healing various infectious diseases. For example, *Aloe vera* showed antiviral activities against herpes simplex virus type 2 [8], *Aloe hijazensis* showed activity against some hemagglutinating viruses [9], and *Aloe vera*, *Aloe marlothii*, and *Aloe ferox* showed skin permeation-enhancing effects [10]. Previously, the use of more than 90% of ethanol extracts of various *Aloe* species was evaluated in detail to determine their biological activities and understand how they prevent health problems [11,12]. The antibacterial and antifungal activities of some *Aloe* spp. are well documented [13]; however, this is the first study of the antimicrobial activities *of Aloe vacillans* Forssk. plants in the Abha area, Saudi Arabia. Here, the active chemical compounds were identified in the gel of *A. vacillans* plant extracts in vitro and characterized to determine their antimicrobial properties against a range of human pathogenic bacterial strains and *C. albicans*. We also compared the effects of various extraction methods for both analytical efficacy and possible biological applications.

2 Materials and Methods

2.1 Samples

Freshly cut *A. vacillans* leaves were harvested from Wadi Al-Beeh village, Abha city, Saudi Arabia, in October 2015, and a voucher sample was stored in the herbarium of the Biology Department, King Khalid University. The *A. vacillans* inner leaf gel extract (AVLGE) was collected and stored at −20 °C for gas chromatography (GC)–mass spectrometry (MS).

2.2 Material

All diagnostic standard and organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.3 Solvents Extractions

AVLGE was collected and either air-dried completely at room temperature (≈25 °C) and then ground into a fine powder using a mortar and pestle and electric grinder, while fresh extracts were prepared by grinding the samples for solvent extraction. To obtain the AVLGE dry leaf extract, 25 mL methanol, chloroform, petroleum ether, acetone, diethyl ether, hot water (≈99 °C), and cold water (≈24 °C) were added separately to 7 g of ground leaf gel in a conical flask. For fresh extraction, the required solvent was added to 14 g of ground fresh AVLGE. All conical flasks were placed in a rotary shaker instrument at 370 RPM at 25 °C for 48 h to allow complete extraction of the chemical compounds from the plants. The extracts were filtered using WHATMAN® filter paper and dried in an oven at 57 °C for 72 h for complete solvent evaporation [14,15]. The residue was weighed and then dissolved in 3 mL sterile dimethyl sulfoxide (DMSO), placed in a rotary shaker at 190 RPM at 19 °C for 72 h, and stored at 4 °C until further use [16].

2.4 Pathogenic Strain Preparation

Eight clinical isolates of human pathogens including two gram-positive (*Staphylococcus aureus* and*Micrococcus luteus*), five gram-negative (*Klebsiella oxytocam*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Proteus mirabilis*), and one candida (*C. albicans*) strains were obtained from the Microbiology Laboratory, Biology Department, Faculty of Science and Microbiology Laboratory, Faculty of Medicine, King Khalid University, Kingdom of Saudi Arabia. All clinical isolates were first subcultured in nutrient broth and incubated at 29 °C for 24 h, except for *C. albicans* which was incubated for 48 h [14,15,17].

2.5 In Vitro Antimicrobial Assay

Evaluation of the antimicrobial activity of each solvent extract was accomplished by using the agar well-diffusion method [15,17,18]. All extracts were tested for their antibacterial and anticandidal activities against *S. aureus*, *M. luteus*, *K. oxytocam*, *K. pneumonia*, *P. aeruginosa*, *S. flexneri*, *P. mirabilis*, and *C. albicans.* Twenty milliliters of sterilized nutrient agar medium was poured into disposable sterile petri dishes (100 × 15 mm) and stored at 24 °C until the media had solidified. All plates were inoculated with the microbes using a sterile loop. A well 6 mm in diameter was made in each plate of the agar media using a sterile cork-borer, and the well was filled with 100 µL dry or fresh plant extracts. The inoculated plates were incubated for 60 min at 24 °C to allow the extract to diffuse into the agar well followed by incubation at 29 °C. DMSO was used as a negative control, while synthetic baneocin dissolved in DMSO and Miconaz gel were used as positive controls for bacterial strains and *C. albicans*, respectively. The experiments were repeated three times, and the inhibition zones formed on the media were measured with a transparent ruler.

2.6 GC–MS

A PerkinElmer GC Clarus 500 system composed of an AOC-20i auto-sampler and gas chromatograph interfaced to a mass spectrometer (Waltham, MA, USA) was used to characterize and quantify individual chemicals found in the methanol extract of the leaf gel of *A. vacillans* [19,20]. For detection, an ionization energy of 70 eV in the electron ionization system was applied in electron impact mode. As a carrier, helium gas (99.999%) was applied at a constant flow rate of 1 mL/min and the injection volume was 2 μL (split ratio of 10:1) [21]. The spectrum of unknown components was compared with that of known components stored in the data bank of The National Institute of Standards and Technology library. The name, molecular weight, and molecular formula of unknown compounds were determined from relative peak heights.

2.7 Data Analysis

Raw data of the antimicrobial effect of gel extracts of *A. vacillans* against tested pathogens were analyzed and compared using GraphPad Prism 5 software (GraphPad, Inc., La Jolla, CA, USA) using two-way analysis of variance.

3 Results

3.1 Inhibitory properties of various extracts of *A. vacillans*

Figure 1 shows the inhibition activities of various extracts obtained from the dry and fresh gel of *A. vacillans* against various human pathogenic microbes. The results revealed that the different solvent extracts affected 62.5% of the tested panel of microbial species. The antimicrobial activity of the extracts can be attributed to structural variations in the precipitated bioactive components from *A. vacillans* plants. The fresh extract was more potent than the dry extract against most of the tested microbes. The methanol extract of *A. vacillans* fresh gel showed excellent activity against *S. aureus* at 0.7 g/mL with an inhibition zone of 2.05 ± 0.21 cm, followed by the acetone extract (1.64 ± 0.06 cm), while the chloroform extract showed the lowest zone of inhibition of 1.17 ± 0.01 cm. The dry extracts showed the same trend in activity against *S. aureus* at 0.7 g/mL, but with 18.5% lower activity than the fresh extract; the methanol extract showed the highest activity (1.28 ± 0.07 cm) followed by the acetone extract (1.08 ± 0.18 cm), while the chloroform extract showed the lowest activity (0.88 ± 0.18 cm). None of the extracts of both the dry and fresh gel of *A. vacillans* plants showed inhibition activities against *K. pneumonia*, *P. aeruginosa*, or *S. flexneri*. Cold water extracts of the dry and fresh gels of *A. vacillans* leaf showed no antimicrobial activity against the tested gram-negative or gram-positive bacteria or *C. albicans*. Aqueous extracts with hot water from the gels of dry and fresh leaves inhibited 2 of the 8 (25%) microbes with average zones from 2.40 ± 0.40 cm for fresh gels to 1.96 ± 0.05 cm for dry gels against *K. oxytocam* and from 0.97 ± 0.05 cm for fresh gels to 0.76 ± 0.02 cm for dry gels against *P. mirabilis*. Chloroform extracts showed moderate antibacterial activities against *P. mirabilis*, with zones of inhibition ranging from 1.40 ± 0.10 cm for fresh gels to 0.78 ± 0.01 cm for dry gels, while methanol and petroleum ether extracts showed nearly the same activities ranging from 1.13 ± 0.12 to 1.17 ± 0.15 cm for fresh gels and 0.75 ± 0.04 to 0.74 ± 0.03 cm for dry gels, respectively. Chloroform, petroleum ether, acetone, and diethyl ether extracts from either fresh or dry gels showed strong antimicrobial activity against *C. albicans*, with zones of inhibition of 2.56±0.57 cm for fresh gels and 2.10 ± 0.09 cm for dry gels. Chloroform, acetone, and diethyl ether extracts from fresh and dry gels exhibited strong antibacterial activity against *K. oxytocam*, with zones of inhibition ranging from 2.13 ± 0.15 cm for fresh gels to 1.63 ± 0.06 cm for dry gels. Methanol extracts from fresh and dry leaf gels showed no activities against *C. albicans* or *K. oxytocam*. Cefoxitin (30 μg), which was used as a positive control against all tested human pathogenic microbes, showed a zone of inhibition, while DMSO did not.

3.2 Phytocomponents in the Gel Leaf of *A. vacillans*

Identification and characterization of the active phytocomponents in the methanol extract of the gel leaf of *A. vacillans* are illustrated in Fig. 2. Phytocomponent retention time, the percent of peak area, molecular weight, and molecular formula are presented in Table 1. Fifteen peaks were identified in the GC–MS chromatogram, among which the most common compounds found in the leaf gel of *A. vacillans* were 1,3-dimethoxy-2-propanol (41.275%) and glycerol (14.751%). The gel contained relatively small amounts of acetoglycerides (8.44%), ethanol (6.7202%), 5-(hydroxymethyl)-2-furaldehyde (6.34%), palmitic acid, (4.57%), butane, 1,2:3,4-diepoxy-,(.+/−.)-(3.27%), 2-deoxy-D-galactose (3.01%), and butyl acetate (3.18%). 2,2-Dimethoxyethanol, diethyl phthalate, phytol, octadecanoic acid, gamma-linolenic acid, and stearic acid were identified as minor ingredients.

4 Discussion

A significant ($P < 0.05$) susceptibility pattern was observed with the extracts of *A. vacillans* gel against gram-positive bacteria, including *S. aureus* and*M. luteus*, and two gram-negative bacteria, *K. oxytocam* and *P. mirabilis*, as well as *C. albicans*; however, *K. pneumonia*, *P. aeruginosa*, and *S. flexneri* showed resistance. This susceptibility pat-

tern of the tested plant extracts to these specific microbes may be exploited for therapeutic purposes in chemotherapy in humans and other animals. The susceptibility of these pathogenic human microbes strongly suggests that the compounds can be utilized against emerging microbes that are multidrug-resistant to synthetic antibiotics [22–24]. Numerous strains of methicillin-resistant *S. aureus* appear to be resistant to all available antimicrobials [25,26]. Synthetic carbapenems are commonly regarded as the last line of chemotherapeutic agents used to treat infectious diseases caused by highly antimicrobial-resistant organisms such as *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacteriaceae*, which produce extended-spectrum β-lactamase or plasmid-mediated AmpC β-lactamase. The prevalence

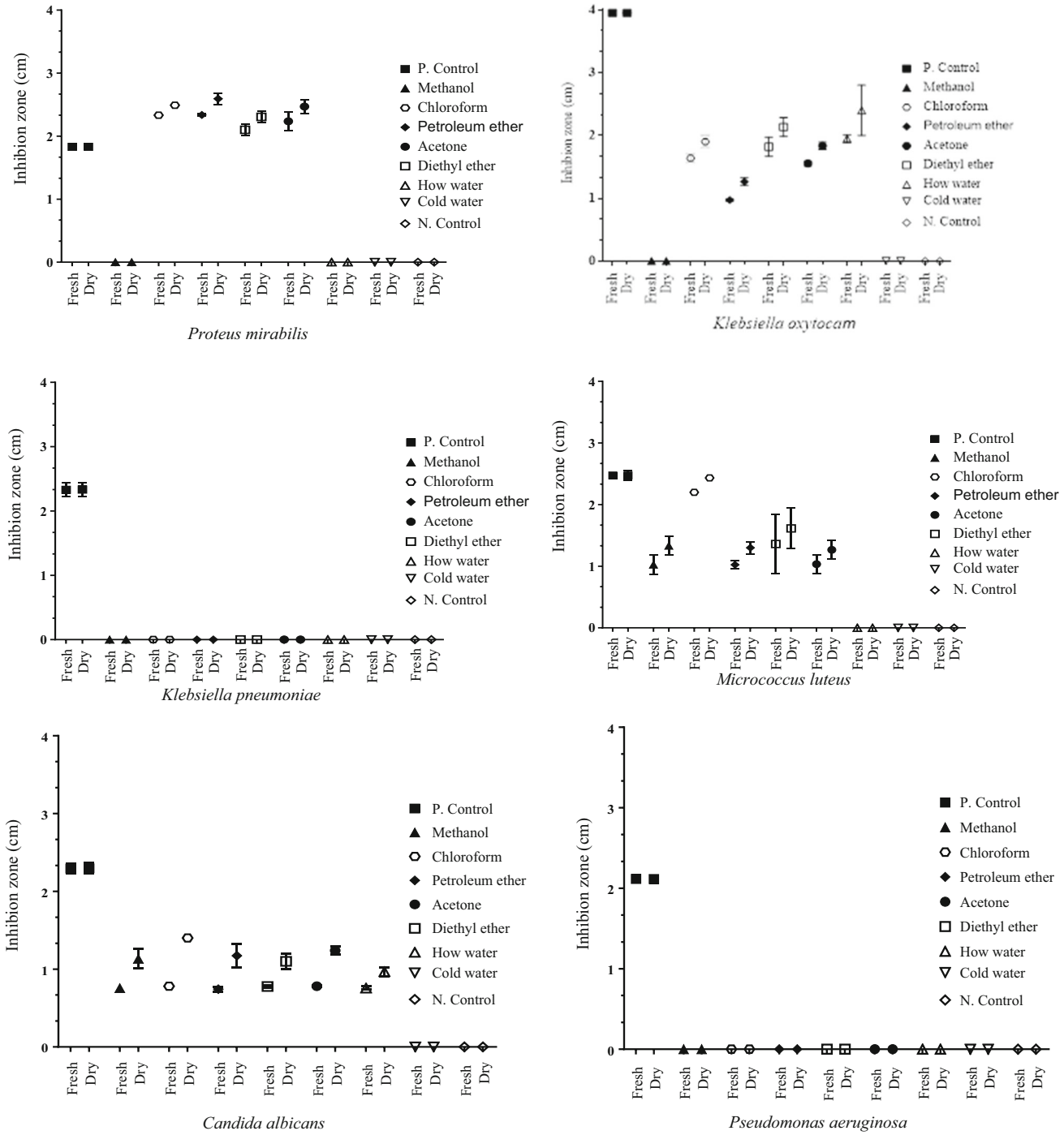

Fig. 1 Antimicrobial activities of fresh and dry gels of *A. vacillans*. Mean value ± SD, $n = 3$ (clearance zones of inhibition (in centimeters)) including a well 6 mm in diameter

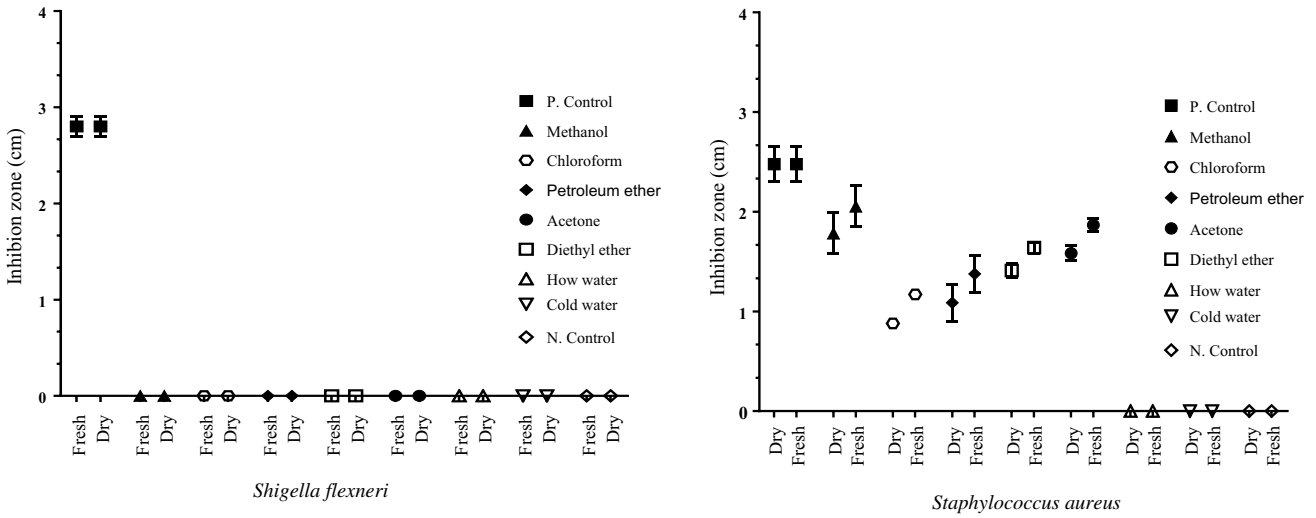

Fig. 1 continued

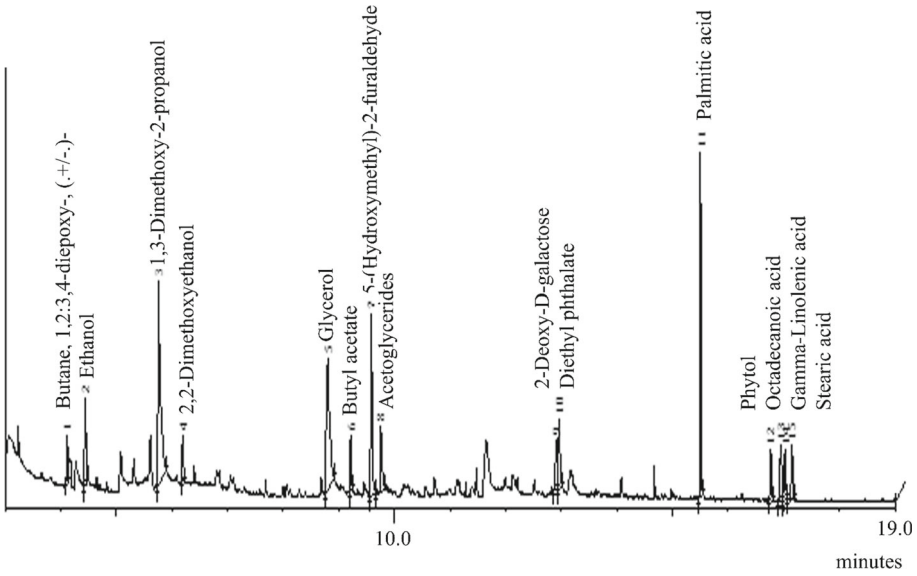

Fig. 2 Typical GC–MS chromatograph with identified chemicals from the gel of *Aloe vacillans*

of carbapenem-resistant gram-negative microbes has dramatically increased recently [27]. Extracts prepared using cold water showed no activity against any of the tested microbes, while hot water extracts exhibited activity only against *K. oxytocam* and *P. mirabilis*. These results agree with those of a previous study by Bojlul et al. [28] who found through gene expression analysis that the cold water extract of *Ficus vesiculosus* plant contains various immunomodulatory bioactive compounds. Previous studies have shown that solvent extraction produces more potent compounds than water extraction [29]. They explained that the high volatility of organic solvents causes the precipitation of more bioactive chemicals from the plant sample than aqueous extractions; our results showed similar trends. The chloroform, petroleum ether, acetone, and diethyl ether extracts showed potent activity against pathogenic *C. albicans* compared to their activities against other tested microbes. Most antimicrobial agents are soluble in polar and nonpolar solvents such as acetone, chloroform, petroleum ether, and diethyl ether rather than in water [14]. In this study, because of the higher sensitivity of *C. albicans* to the gel extract of *A. vacillans*, there are likely additional substances present that were not identified by GC–MS. These compounds may be separated from the gels by other chemical analytical methods to produce various useful chemical compounds. The identification of a new agent from plant sources against *C. albicans* is very important, as it was reported that among all Candida species, *C. albicans* can change from a non-harmful yeast to harmful fungi causing numerous clinical infections, typically candidiasis [30]. Additionally, *C. albicans* is a member of the mucosal flora and can cause mucosal disease with a high morbidity rate by invading epithelial cells and causing severe tissue dam-

Table 1 GC–MS analysis of methanol extract of *Aloe vicillans* leaves

No.	Compounds	Rt time	% Area	Molecular weight	Chemical formula
1	Butane, 1,2:3,4-diepoxy-,(. + / − .)-	4.117	3.2799	86.08	$C_4H_6O_2$
2	Ethanol	4.439	6.7202	46.06	C_2H_6O
3	1,3-Dimethoxy-2-propanol	5.761	41.275	120.14	$C_5H_{12}O_3$
4	2,2-Dimethoxyethanol	6.191	1.2973	106.12	$C_4H_{10}O_3$
5	Glycerol	8.808	14.751	92.094	$C_3H_8O_3$
6	Butyl acetate	9.215	3.1819	116.16	$C_6H_{12}O_2$
7	5-(Hydroxymethyl)-2-furaldehyde	9.587	6.3404	126.11	$C_6H_8O_3$
8	Acetoglycerides	9.759	8.4456	1742.5	$C_{100}H_{188}O_{22}$
9	2-Deoxy-D-galactose	12.930	3.0106	164.16	$C_6H_{12}O_5$
10	Diethyl phthalate	12.967	1.9293	222.24	$C_{12}H_{14}O_4$
11	Palmitic acid	15.510	4.5737	256.42	$C_{16}H_{32}O_2$
12	Phytol	16.763	1.3371	296.53	$C_{20}H_{40}O$
13	Octadecanoic acid	16.946	1.2188	284.47	$C_{18}H_{36}O_2$
14	Gamma-linolenic acid	17.024	0.8529	278.43	$C_{18}H_{30}O_2$
15	Stearic acid	17.148	1.7852	284.48	$C_{18}H_{36}O_2$

age [31,32]; this study recommends superficial application of aloe gel on the infected skin, which may inhibit such infections. Wendakoon et al. [33] and Moustafa and Alrumman [14] reported that both the solvent type and sample extraction conditions greatly affect the degree of antimicrobial activity. Generally, the maximum antibacterial properties of the chloroform extract against *M. luteus*, methanol extract against *S. aureus*, petroleum ether extract against *Proteus mirabilis*, *C. albicans*, *K. pneumonia*, and hot water extracts against *K. oxytocam* were more effective with the fresh gel than the dry gel. This may be because drying degraded the active chemicals or/and volatile oils in the plant. Previous studies showed that antibacterial activity was clearly decreased after drying, for example, in*Zingiber officinale* and *Lippia gracilis* plants [34,35]. Although, Abascal et al. [36] claimed that freeze drying is effective for extraction, it alters the concentration of the active components. Analysis of the inhibitory effects revealed that no tested extracts were active against the gram-negative bacteria *K. pneumonia*, *P. aeruginosa*, and *S. flexneri*, but some were active against *K. oxytocam* and *P. mirabilis*. Similarly, Wattanastcha et al. [37] observed that the same chemical compounds, *viz.* citronellal inhibited the growth of*S. aureus* but not *E. coli*. In the present study, gram-positive bacteria were more susceptible to the gel extract than gram-negative bacteria. These results agree with those of previous studies showing that the effects were related to the structure of the cell envelope, complexity of the gram-negative bacteria cell wall, charge in the surface, and other factors [38,39]. This may explain why the methanol extract was not effective against some specific bacteria strains, while other extracts showed activity depending upon the ability to disrupt the cell wall and cytoplasmic membrane. Chemical analysis by GC–MS revealed 15 different compounds in the gels of *A. vacillans* extracts, and nearly all or their derivatives have been shown to have biological activity. For example, a study evaluating the inhibitory effect of wiping intraoral phosphor plates with tissue paper containing ethanol or 2-propanol alcohols contaminated with *C. albicans* and *Streptococcus oralis* showed that ethanol completely eliminated *C. albicans* and *S. oralis* while 2-propanol did not inhibit all microorganisms tested [40], supporting the antimicrobial activities in the aloe gel extract against *C. albicans*. The antimicrobial effects of ethanol against *Listeria monocytogenes* (gram-positive bacterium) were evaluated, and it was found that up to 1.25% ethanol did not stop growth, but growth was strongly inhibited at concentrations up to 5% [41]. This value is in excellent agreement with our results, which were obtained with ethanol concentrations of 6.7202% in the aloe gel extract; thus, the gel can be used as an antiseptic for wounds. Glycerol is an important moisturizing agent; it also prevents the stratum corneum (SC) stage transition, shows keratolytic effects by degrading desmosomes, influences the protective capacity of the skin against irritation and entrance of foreign substances through the SC, plasticizes the SC, diminishes tissue scattering, settles skin collagen, and increases the healing rate [42–44], indicating that this gel can be used as a skin protective agent. Products containing butyl acetate showed stronger antimicrobial effects toward *P. aeruginosa*, *S. aureus*, *Escherichia coli*, *C. albicans*, and *Trichophyton rubrum* [45]. It was reported that bis-(ethylhexyl) phthalate isolated from *Streptomyces bangladeshiensis* had antimicrobial inhibitory activities against gram-positive bacteria and pathogenic fungi, and isolates from *Pongamia pinnata* Pierre leaf showed antiviral inhibition activity against White Spot Syndrome Virus of *Penaeus monodon* Fab [46,47]. Palmitic and stearic acids showed potential antimicrobial

activities against a range of pathogenic bacteria and fungi [48]. Gamma-linolenic acid refers to the omega-6 fatty acids present in vegetable and plant foods. While young, healthy persons can synthesize gamma-linolenic acid, a large percentage of people are unable produce insufficient amounts, likely because of diet, alcohol consumption, smoking, viral infection, medical conditions, or aging [49]. It remains unclear whether *A. vacillans* exhibits beneficial effects when included in the diet. A recent study showed that *A. vera* promotes immune system function, maintains blood sugar levels, and reduces redness and swelling [50]. Therefore, *A. vacillans* plants should be further evaluated to examine these possibilities and test their toxicity and carcinogenicity.

5 Conclusion

Aloe vacillans is a very important medicinal plant grown as a weed in Abha area, Saudi Arabia. The plant contains antimicrobial compounds against specific pathogenic microbes. The bioactive compounds present in *A. vacillans* can be utilized to develop natural antibiotics from plant resources that may be exploited as phytomedicines for various infections, particularly those associated with a high mortality rate. The bioactive chemicals found in the gel extract, such as gamma-linolenic acid, are phytopharmacologically important and thus can be used to improve health and fitness.

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