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Isolation and Identification of Endophytic Fungi from *Aloe vera* Leaves and Chemical Analysis of the Alcoholic Extract of the Leaves Using HPLC, GC, and GC-Mass Devices

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Abstract

The study's goals were to separate and identify endophytic fungi from Aloe vera leaves by looking at their morphology and molecules, as well as to find the chemical compounds in the leaf extract by using HPLC, GC, and GC-Mass instruments. The results showed that 53 endophytic fungi were isolated from a total of 120 pieces of *A. vera* leaves, with a total colonization rate of 44.16%. The fungus *Aspergillus terreus* had a colonization rate of 14.16%; *Aspergillus niger* had a colonization rate of 13.33%; *Penicillium chermesinum* demonstrated a colonization rate of 6.66%; *Paecilomyces variotii* had a colonization rate of 2.5%; *Talaromyces radicus;* and *Aspergillus flavus* achieved a colonization rate of 1.66%. Finally, the fungi *Aspergillus quadrilineatus*, *Talaromyces verruculosus*, *Neoscytalidium dimidiatum*, *Alternaria solani*, and *Aspergillus niveus* achieved a colonization rate of 0.83%. The results of examining the alcoholic extract of the leaves using the HPLC device showed the presence of the chemical compounds aloin at a concentration of 125.39 ppm and aloe emodin at a concentration of 66.59 ppm. We looked at the leaf alcoholic extract with a GC machine and found a group of fatty acids. These included linoleic, oleic, palmitic, and stearic. The GC-MS test revealed a group of active compounds, including Heptane, 1-(ethenylthio), Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl), 2H-Pyran, 2-(3-butynyloxy) tetrahydro, 1,2-Cyclobutanedicarboxylic acid, 3-methyl-dimethyl ester and 4 (1H)-Pyrimidinone, 2-(propylthio). The presence of endophytic fungi from which effective enzymes or compounds can be isolated could probably have an important role in future medical and therapeutic uses. Also, the leaves of the *A. vera* plant have medicinal and therapeutic uses for many diseases.

Keywords: Endophytic fungi, Aloe, Identification, Chemical analysis, GC, HPLC,GC-Maas.

1. Introduction

Endophytes are microorganisms in the form of fungi and bacteria that exist within various tissues of the host plants and form a symbiotic relationship with their host in a highly competitive

environment. They live without the disease symptoms observed in plants [1]. Endophytes have unique functional and metabolic mechanisms that enable them to adapt to the specific environment within the plant, and at the same time, they can encode many bioactive substances. In addition, Endophytes have evolved with their host plants for a long period of time to produce some of the same metabolites with medicinal uses as those produced by their host plants [2].

Plant-associated endophytic fungi are a group of microorganisms that are rich in biodiversity and found within plant tissues or in the intercellular space. The production of secondary metabolites by endophytic fungi promotes the growth of their host plants and enhances their resilience to biotic and abiotic stresses. Medicinally important Phytochemicals that were once thought to only be produced by plants are now biosynthesized by Endophytic fungi [3].

Medicinal plants have shown great potential in biotechnology applications [4]. *Aloe vera* is one of the oldest medicinal plants in human history. It is known as the "healing plant" due to the presence of acemannan in its leaves, which possesses immunomodulatory, antitumor, bacterial, and fungal antagonistic properties [5]. The plant is used internally to combat most problems of the digestive system, such as constipation, poor appetite, colitis, irritable bowel syndrome, diabetes, peptic ulcers, and asthma. *A. vera* is used externally to treat skin irritations, burns, sunburn wounds, psoriasis, eczema, ulcers, acne, and dermatitis as it stimulates cell regeneration. It also has cytotoxic, anti-inflammatory, and antioxidant impacts, as well as affecting the heart and blood vessels [6].

There haven't been many studies on endophytic fungi isolated from medicinal plants in Iraq. These plants are important for medicine and cultured fungi are used to control plant pathogens biologically. The goal of this study was to isolate and identify endophytic fungi from the leaves of the A. vera plant and use PLC X, GC, and GC-MS to analyze the leaf alcoholic extract.

2. Materials and Methods

2.1. Plant samples collection

A. vera plant samples were collected during the period from June to July 2022 from different regions of Baghdad Governorate, Central Iraq. Plants that did not show pathological symptoms were selected and classified by a plant taxonomist (Assistant Professor Dr. Israa Karim Nasrallah) in the Department of Biology/College of Education for Pure Sciences (Ibn Al- Haitham).

2.2.Isolation of Endophytic Fungi

Leaves from the collected *A. vera* plants were cut with a sterile scalpel in the laboratory and immediately washed under tap water for 5 minutes to remove soil and dust particles, based on the method of [7] with some modifications. Following a one-minute sterilization process using 75% ethyl alcohol, the leaves underwent a three-minute soak in 2.5% sodium hypochlorite (NaOCl), three washes with sterile distilled water, and one final rinse. The leaves were placed on sterile filter paper and left to dry. Five square segments of approximately 1 cm^2 were cut from each leaf surface and placed using sterilized forceps into a Petri dish containing sterile PDA medium at approximately equal distances. The plates were incubated in the incubator at a temperature of 27 ± 2 °C for 7 to 10 days. During that period, the plates were examined daily in order to observe the growth of the endophytic fungus colonies. The growing fungi were purified on the cut parts of the

leaf by taking a part from the edge of the growing colony and placing it in a new petri dish containing the medium of PDA. The dishes were incubated at a temperature of 27 ± 2 °C for 7 days for the growth of fungal colonies. The number of isolated fungal colonies was recorded.

2.3. Identification of isolated endophytic fungi

The isolated fungi were identified phenotypically and molecularly.

2.4. Phenotypic identification of isolated endophytic fungi

The growing fungi were identified morphologically through the phenotypic characteristics of the colonies, such as the colony's shape, colour, size, texture, and reflection color, as well as microscopic examination by light microscope of the somatic structures, such as the shapes of hyphae and spores or reproductive conidia. The appropriate taxonomic references were used [8-10]. The colonization frequency of endophytic fungal colonies was calculated according to the following equation:

Colonization frequency%=(number of pieces showing growth of endophytic fungus)/(total number of cultured pieces) x 100 [11].

2.5.The molecular identification of the isolated endophytic fungi

The isolated endophytic fungi were grown on Potato Dextrose Broth (PDB) medium for five days at a temperature of 25±2 °C. They were identified at the molecular level by using a Trans Gen Biotech-China identification kit to get the DNA from the isolated fungi and a thermal cycler for polymerase chain reaction (PCR). The Korean company Macrogen was responsible for the identification. DNA fragments amplified by the PCR were analyzed for the nitrogen base sequence of the targeted area, i.e., the Internet Transcripd Spacer (ITS) of the rDNA gene, using the ITS1 and ITS4 primers. The data were analyzed by comparing the results with the gene bank database using the BLAST tool program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Chemical analysis of the alcoholic extract of *A. vera* leaves

2.6.1. Detection of aloin – aloe emodin compounds in *A. vera* by means of high-performance liquid chromatography (HPLC) device

2.6.1.1. Extraction method of active compounds

After drying the leaves, they were ground to obtain a leaf powder for the purpose of preparing alcoholic extracts. *A. vera* leaf powder was soaked in a 60% ethanol solution for 24 hours. The solution and residue were isolated by centrifugation at 4000 rpm. A red-brown colloid containing leaf extract was obtained. Sulfuric acid and chloroform were added to the extracted materials, which were retrieved to remove the chloroform extract. This procedure was carried out three times. After evaporation of the chloroform, a yellowish-brown colloid was obtained as the crude extract. The stock solution was prepared by dissolving methanol [12].

2.6.1.2. HPLC analysis conditions

A High Performance Liquid Chromatography (HPLC) model from SYKAM Germany is equipped with gradient elution capability, an Ultraviolet spectrophotometer (UV, model S 3240)

as a detector, and an auto sampler model (S 5200). The column used in the analysis (C18 – ODS) (25cm * 4.6 mm). The mobile phase consisted of a ratio of water to acetonitrile (22%). The detection wavelength was 220 nm, and the flow rate was 1.0 mL/min. Each injection volume was 50 µL. The column temperature was maintained at ambient conditions.

After conducting the HPLC analysis, the standard compound aloe emodin was first injected into the HPLC device to determine the retention time. Then the standard compound aloin was injected into the device to determine its retention time. After the detection of the active standard compounds, the sample extracted from the leaves of *A. vera* was injected to determine the active compounds. To calculate the concentration of the compounds, the following equation was applied:

Substance concentration = (sample surface area x standard substance concentration)/(standard substance area) x (dilution times)/(sample weight) [13].

2.6.2. Determination of fatty acids in *A. vera* leaves using Gas Chromatography

2.6.2.1. Extracting lipids from the samples

Lipids were determined based on the AOAC method [14] using a lipid extractor (Soxholet).

2.6.2.2.Esterification of lipids

The sample was prepared according to the method approved by the AOAC [14], which is based on the esterification of lipids by interaction with methanolic potassium hydroxide. The latter was prepared by dissolving 11.2 g of potassium hydroxide in 100 ml of methanol. To a weight of 1 g of lipids, 8 ml of methanolic potassium hydroxide and 5 ml of hexane were added. The mixture was shaken quickly for 30 seconds, then left to separate into two layers. The upper layer (the hexane layer) containing the esterified fat was collected and injected into the device.

2.6.2.3. Chromatographic analysis of the sample

The fatty acid compounds were analyzed using a gas chromatography device (GC-2010, Shimadzu, Japan). An ionized flame detector (FID) and a capillary separation column (SE-30) with dimensions of 30m*0.25 mm were used, according to the following conditions: Temperature: Injection area 280°C, detector temperature 310°C, separation column temperature 120-290 (10°C/MIN), gas flow rate 100 Kpa [13].

After conducting gas chromatography analysis, the standard compounds of Linolinic, Linolic, Stearic, oleic, and then palmatic were injected into the GC sequentially to determine their retention time. To calculate the concentrations of compounds, the previous equation was applied.

2.6.3. Detection of organic compounds using GC-Mass spectrophotometry

2.6.3.1. Preparation of *A. vera* leaf extract for GC-Mass analysis

The extraction was carried out using a mixture of three solvents at a ratio of 5:1:1, v/v/v: ethyl acetate, hexane, and chloroform. A weight of 2g of the sample (*A.* vera leaf powder) was placed in a 100-ml beaker and 50 ml of the mixture was added to it. The new mixture was agitated for 5 minutes and then centrifuged. The supernatant was separated and warmed at 50°C for 30 minutes, agitating from time to time. The extract was filtered and placed in a small glass tube [15].

2.6.3.2. GC-MS assay of *A. vera* leaf extract

A mass spectrometer (7820A, Agilent Company, USA) was used in the laboratories of the Ministry of Industry and Minerals, Baghdad. A volume of 1µl of the previously prepared plant extract was injected. The mass of the organic compounds was obtained, and their spectrometers were compared with standard ones from the Mass spectral libraries NIST 11.L. The conditions for the analysis were as below:

Analytical Column: Agilent HP-5ms Ultra Ineit (30m Length × 250 µm Inner Diameter × 0.25µm Film Thickness). Injection volume: 1µl. Pressure: 11.933 psi. GC Inlet Line Temperature: 250C°. Aux heater temperature: 310 °C. Carrier Gas: 99.99%. Injector temperature: 250 °C. Injection Type: Splitless. and the oven program was: Temperature Ramp1 60 °C holds for 3 minutes; Ramp2 60 °C holds for 180 °C for 7 minutes; Ramp3 180 °C holds for 280 °C for 8 minutes; and Ramp4 280 °C hold for 3 minutes.

3. Results and Discussion

The results of the isolation of the endophytic fungi from *A. vera* leaves are shown in **Table 1.** The samples were obtained from a total of 120 pieces of leaves. The total number of isolated endophytic fungi was 53, with a total colonization frequency of 44.16%. The endophytic fungus *Aspergillus terreus* appeared with the highest colonization rate of 14.16%, i.e., 17 endophytic fungi, followed by *Aspergillus niger* with a colonization rate of 13.33% and 16 endophytic fungi, *Penicillium chermesinum* with a colonization rate of 6.66% and 8 endophytic fungi, and *Paecilomyces variotii* with a colonization rate of 2.5% and 3 endophytic fungi. Then, *Talaromyces radicus* and *Aspergillus flavus* showed a colonization rate of 1.66%, with two endophytic fungi for each. Finally, *Aspergillus quadrilineatus, Talaromyces verruculosus, Neoscytalidium dimidiatum, Alternaria solani,* and *Aspergillus niveus* demonstrated the lowest colonization rate of 0.83%, with one endophytic fungus for each.

The result of this study showed that all the isolated endophytic fungi belong to the phylum Ascomycota, and most of them belong to the genus *Aspergillus*, where the number of fungal isolates belonging to this genus amounted to 37 out of 53 total isolates. The results of this study are consistent with those of a previous study on endophytic fungi isolated from the leaves of *Moringa* sp. in terms of the dominance of fungal species belonging to the genus *Aspergillus* over the other isolated fungi, where the number of colonies belonging to this genus reached 46 out of 59 isolated total colonies [16]. Colonies of the endophytic *A. terreus* appeared yellow in color, and the fungal hyphae were translucent with septa (**Figure 1).** As for the *A. niger* colonies, they were black with white edges, while the fungal hyphae were transparent with septa (**Figure 2).** *Penicillium chermesinum* had colonies that appeared in a green to gray color, and the fungal filaments had septa (**Figure 3).** *Paecilomyces variotii* colonies were fast-growing and brownish-yellow or sandy in color, while the fungal hyphae divided transversely and the conidiophore had dense branches (**Figure 4).** As for the *Talaromyces radicus* colony, its color was olive green with light yellow edges, while the colony growth was slow and the fungal filaments were thin with septa (**Figure 5).** For *A. flavus*, its colonies appeared in an olive green color, while the fungal hyphae were transparent with septa (**Figure 6).** The colony of *A. quadrilineatus* appeared to be slow-growing, yellowish-green in color, with irregular edges, and the fungal filaments were translucent with septa (**Figure 7).** As for the color of the *Talaromyces verruculosus* colony, it appeared

grayish-green. The fungal hyphae had septa (**Figure 8).** *Neoscytalidium dimidiatum* had a fast-growing colony; its color was dark brown to black; the fungal hyphae were tortuous, irregular, branched, and with septa; and the conidia were in the form of chains with an oval to elliptical shape (**Figure 9).** *Alternaria solani* had a black-brown colony that was fast-growing. The fungal hyphae were branched and had septa. The conidia were large, sceptre-shaped, dark in color, and divided by septa (**Figure 10).** The last fungus, *A. niveus*, had white colonies in the form of aggregates, and the fungal filaments were transparent with septa (**Figure 11)**.

Table 1. Endophytic fungi isolated from A. *vera* leaves, with the number of colonies and the colonization frequency.

Isolated Endophytic fungi	Number of colonies	colonization Frequency %
Aspergillus terreus	17	14.16
Aspergillus niger	16	13.33
Penicillium chermesinum	8	6.66
Paecilomyces variotii	3	2.5
Talaromyces radicus	2	1.66
Aspergillus flavus	2	1.66
Aspergillus quadrilineatus	1	0.83
Talaromyces verruculosus	1	0.83
Neoscytalidium dimidiatum	1	0.83
Alternaria solani	1	0.83
Aspergillus niveus	1	0.83
total	**53**	**44.16**

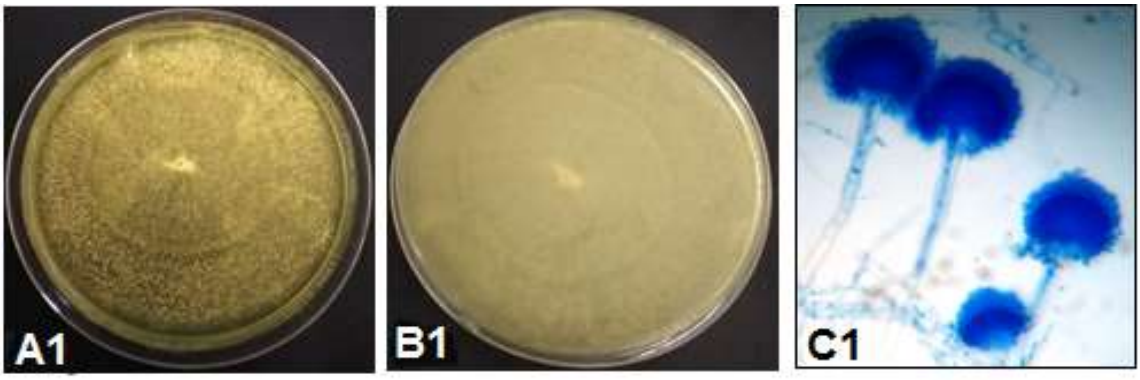

Figure 1. *Aspergillus terreus*: (A1) colony, (B1) Revers color, (C1) Microscopy 40x.

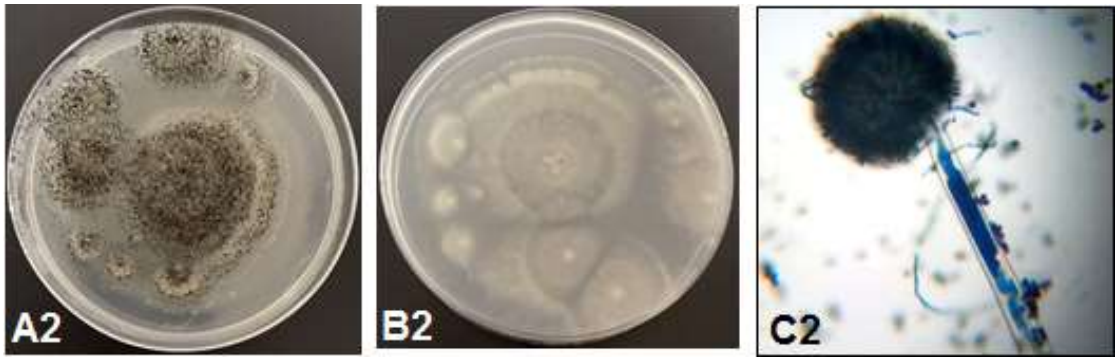

Figure 2. *Aspergillus niger*: (A2) colony, (B2) Revers color, (C2) Microscopy 40x.

Figure 3. *Penicillium chermesinum*: (A3) colony, (B3) Revers color, (C3) Microscopy 40x.

Figure 4. *Paecilomyces variotii*: (A4) colony, (B4) Revers color, (C4) Microscopy 40x.

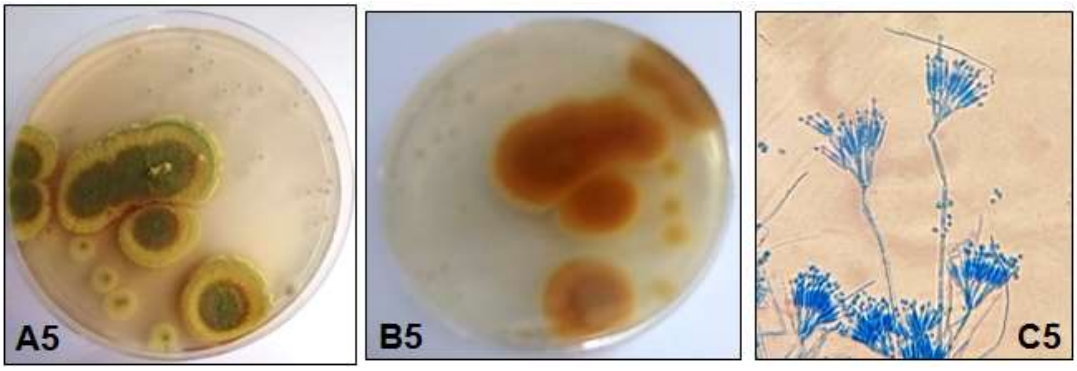

Figure 5. *Talaromyces radicus*: (A5) colony, (B5) Revers color, (C5) Microscopy 40x.

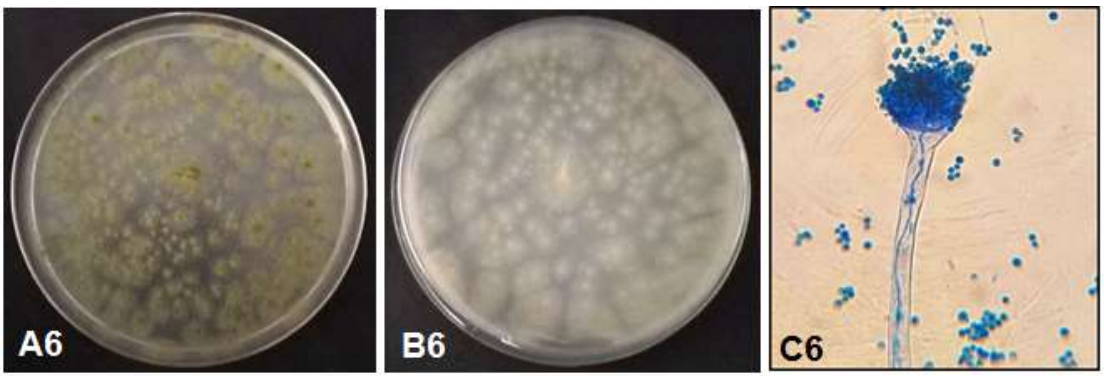

Figure 6. *Aspergillus flavus*: (A6) colony, (B6) Revers color, (C6) Microscopy 40x

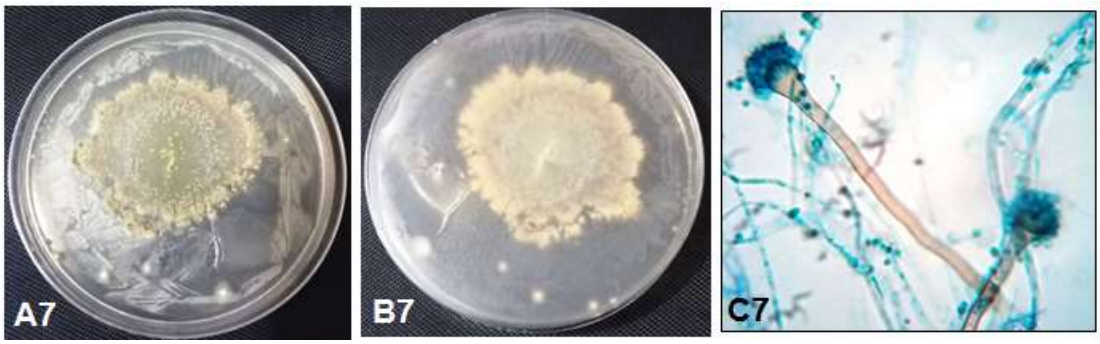

Figure 7. *Aspergillus quadrilineatus*: (A7) colony, (B7) Revers color, (C7) Microscopy 40x

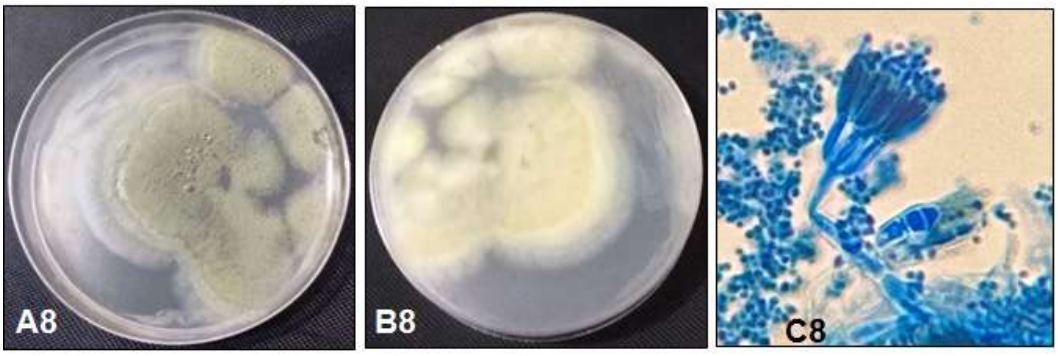

Figure 8. *Talaromyces verruculosus*: (A8) colony, (B8) Revers color, (C8) Microscopy 40x

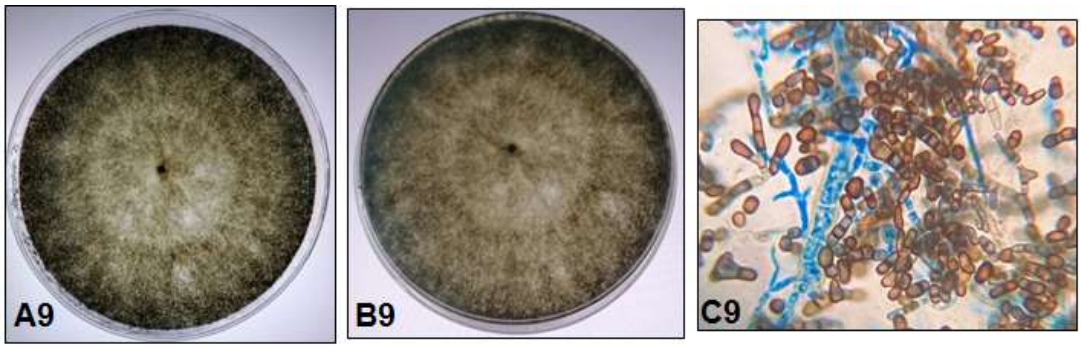

Figure 9. *Neoscytalidium dimidiatum*: (A9) colony, (B9) Revers color, (C9) Microscopy 40x

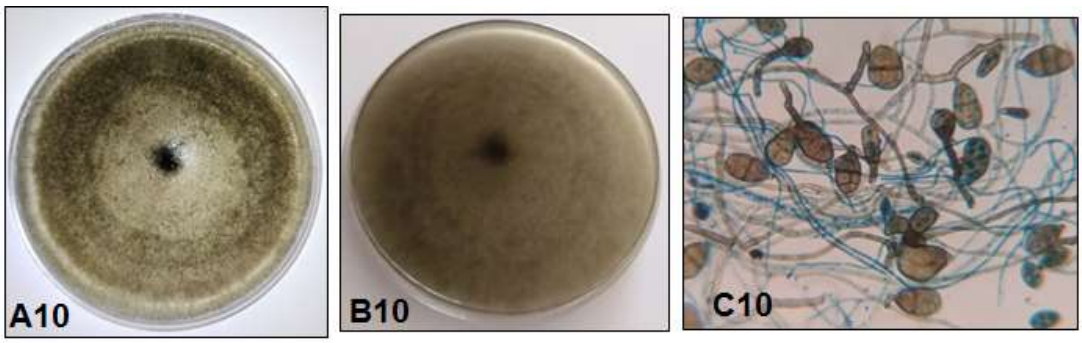

Figure 10. *Alternaria solani*: (A10) colony, (B10) Revers color, (C10) Microscopy 40x.

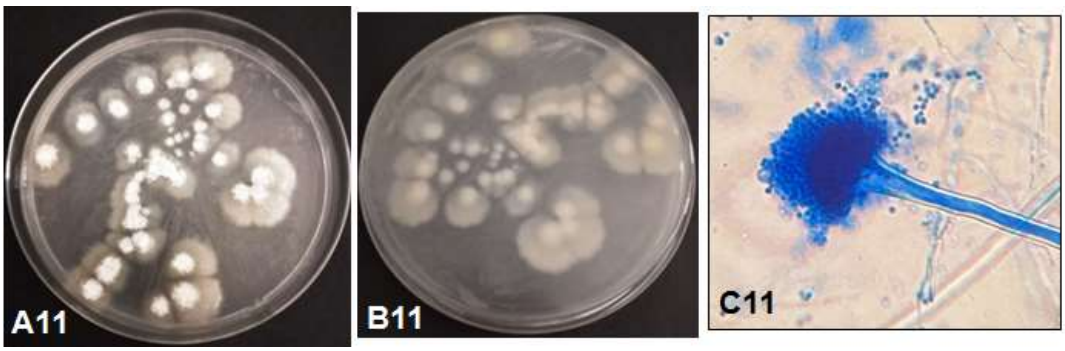

Figure 11. *Aspergillus niveus*: (A11) colony, (B11) Revers color, (C11) Microscopy 40x

3.1. Molecular identification of endophytic fungi

The results of the electrophoresis of the DNA of the fungal samples after conducting the PCR reaction showed the appearance of 11 bands of DNA from the samples. They were compared to the marker ladder, which ranges from 100 to 1500 base pairs (bp), and the molecular size of the bands was estimated at 550 base pairs (**Figure 12)**.

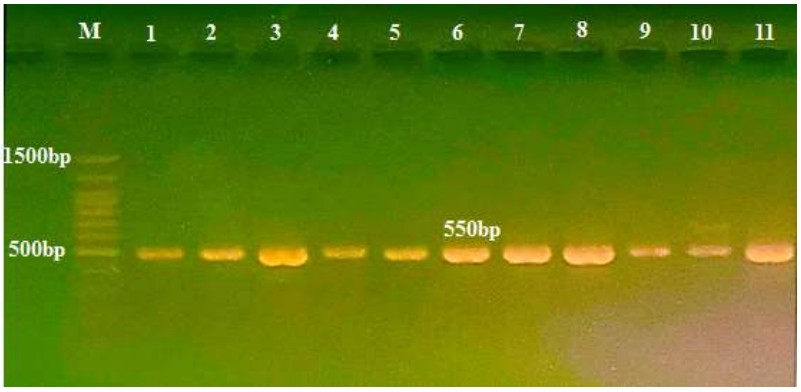

Figure 12. Electrophoresis of the PCR product of endophytic fungi samples isolated from A. *vera* leaves using 1.5% agarose under a voltage of 70 volts for 1.5 hours, photographed with ultraviolet rays. The first column represents the marker ladder.

It was found that between 92% and 99% of the nitrogen base sequences of the ITS region of the rDNA gene of fungal isolates matched those of global isolates. This is shown in **Table (2).** The fungus *A. terreus* had a 94% match rate with the global isolate. With a total of 17 isolates, its nucleotide sequences were deposited in the Genome Bank under the accession number LC743583. This species was followed by *A. niger* with a match rate of 99% and 16 isolates; its nucleotide sequences were deposited under the accession number LC743859. It was followed by *Penicillium chermesinum*, with a match rate of 99% and 8 isolates; its nucleotide sequence was deposited under accession number LC743582. It was followed by *Paecilomyces variotii*, with a match rate of 94% and 3 isolates; its nucleotide sequence was deposited under accession number LC743738. As for the two fungi, *Talaromyces radicus* and *A. flavus*, they had a match rate of 96% and 92%, respectively, with two isolates each, and their nucleotide sequences were deposited under accession numbers LC743858 and LC743860, respectively. Finally, the fungi *A. quadrilineatus, Talaromyces verruculosus, Neoscytalidium dimidiatum, Alternaria solani,* and *A. niveus* showed matching percentages of 99%, 95%, 98%, 99%, and 99%, respectively, with one isolate for each. The nucleotide sequences of these fungi were deposited under accession numbers LC743861, LC743737, LC743864, LC743862, and LC743863, respectively.

The results of this study are in agreement with those of [17], where the ITS region of the rRNA gene was used to identify the fungal isolates of *A. terreus* and *A. flavus*. The ITS is a special region for the classification of fungi because it retains evolutionary constraints [18].

Table 2. Endophytic fungi isolated from *A. vera* leaves when matched with the National Center for Biotechnology Information (NCBI) database. The results shown are number of isolates, match percentage, and band size, along with accession numbers in the Gene Bank.

Isolated Endophytic Fungi	Number of Isolates	Match Percentage	Size of the Band (bp)	Global Isolates Accession Number in Gene Bank	Accession Number in Gene Bank
Aspergillus terreus	17	94 %	592	KF669499	LC743583
Aspergillus niger	16	99 %	541	MT597436	LC743859
Penicillium chermesinum	8	99 %	531	KM278124	LC743582
Paecilomyces variotii	3	94 %	565	OM455486	LC743738
Talaromyces radicus	2	96 %	577	EU818697	LC743858
Aspergillus flavus	2	92 %	598	MF113270	LC743860
Aspergillus quadrilineatus	1	99 %	508	MG786524	LC743861
Talaromyces verruculosus	1	95 %	548	MW958038	LC743737
Neoscytalidium dimidiatum	1	98 %	542	OW987723	LC743864
Alternaria solani	1	99 %	523	OM236769	LC743862
Aspergillus niveus	1	99 %	549	OM980255	LC743863
Total	53				

This study is consistent with many studies in that the endophytic fungi isolated from the *A. vera* under study had been previously isolated from other plants; the endophytic fungus *A. terreus* was isolated from many plants such as *Moringa* sp. [16], *Myrtus communis* [19], and *Ficus elastica* [17]. The endophytic fungus *A. niger* was isolated from several plants, such as *Ziziphus spina* [20] and *Eucalyptus camaldulensis* [21]. *Penicillium chermesinum* was isolated from *Panax notoginseng* [22]. As for *Paecilomyces variotii*, it was isolated from the roots of a group of plants in the Cabo de Gata Natural Park in Spain [23]. *T. radicus* was isolated from *Cucumis dipsaceus* [24]. *A. flavus* was isolated from the Sidr plant *Ziziphus spina* [20], the soybean *Glycine max* [25], *Albizia lebbeck* [26], and *Dysoxylum gotadhora* [27]. Isolation of *A. quadrilineatus* was reported from the castor plant *Ricinus communis* [28]. *T. verruculosus* was isolated from *Schima wallichii* [29]. *Neoscytalidium dimidiatum* was isolated from the Egyptian plant *Hyoscyamus muticus* [30]. *Alternaria solani* was isolated from *Aconitum transsectum* [31]. Finally, the fungus *A. niveus* was isolated from the sunflower [32].

The study does not agree with Sahani *et al*. [33], who isolated two endophytic fungi that were not isolated in the current study, which were *Cladosporium* sp. from the roots of *A. vera* and *Nigrospora* sp. from the leaves of the plant. Also, the study does not agree with Nigam & Jampani [34], who isolated the endophytic fungi of *Penicillium pseudostromaticum, Fusarium solani, Mycelia sterilia,* and *Gliocladium fimbriyatum* from the *A. vera* plant, which were not isolated in the current study.

3.2. Detection of aloin , aloe emodin compounds in *A. vera* leaves by HPLC

After conducting the HPLC analysis of *A. vera* leaf extract, the results showed the presence of several peaks in the extracted sample. The results showed that the retention time of the standard compounds matched some of the peaks in the sample, as shown in **Figure 13**. The results also

showed that the sample contained aloin and aloe emodin, and their concentrations are shown in **Table 3**.

Table 3. Active compounds in *A. vera* leaf extract, their retention time, and concentrations as tested by HPLC device.

Peak	Retention time (min)	Concentration (ppm)	Chemical compound
1	3.80	125.39	aloin
2	6.15	66.59	aloe-emodin

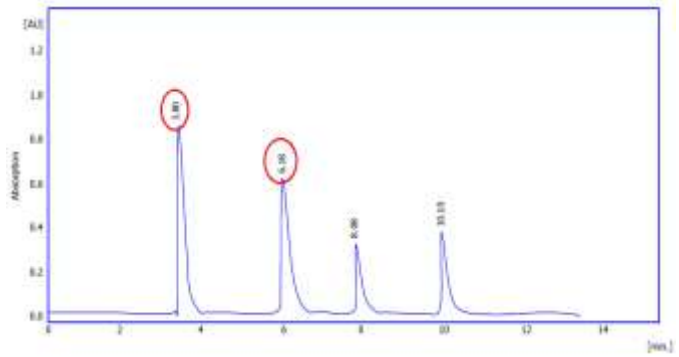

Figure 13. Chromatographic analysis of the active compounds in *A. vera* leaf extract and their retention time as measured by HPLC device.

This study is consistent with [35] *A. vera* has traditionally been used to treat skin infections (burns, cuts, insect bites, eczema) and digestive problems due to its anti-inflammatory, antimicrobial, and wound-healing properties, the most active compounds responsible for these activities are aloin and aloe emodin, in addition to aloesin, emodin, and acemannan. This study also agrees with Borges & Hoefel [36] in vitro and in vivo studies revealed that compounds derived from *A. vera* (aloin- aloe emodin) had satisfactory results with regard to antitumor activity in different human cancer cell lines. Photodynamic therapy (PDT) mediated by aloin and aloe emodin shows potential for clinical development as an effective and safe new photosensitizer for the treatment of skin cancer [37].

Aloe emodine exhibits several pharmacological effects, including antiviral, anti-inflammatory, anticancer, anti-plasmodium, and hepatoprotective activities [38]. These pharmacological properties lay the foundation for the treatment of many ailments, including sepsis, Alzheimer's, influenza virus, infections, glaucoma, malaria, cirrhosis, type 2 diabetes, psoriasis, growth disorders and many cancers. However, several studies reported adverse effects of aloe emodine, including hepatotoxicity and nephrotoxicity, which are of worldwide concern [39].

3.3. Determination of fatty acids in *A. vera* leaves by Gas Chromatography

After conducting a gas chromatography analysis of *A. vera* leaf extract, the results showed the presence of several peaks in the extracted sample. The results showed that the retention time of the standard compounds matched some of the peaks in the sample, as shown in **Figure 14**. The results also showed that the sample contained fatty acids, and the concentrations of those acids are shown in **Table 4**.

Table 4. Fatty acids in *A. vera* leaf extract, their retention times and concentrations as analyzed using GC device.

Peak	Retention time (min)	Concentration %	Amino acid
3	2.493	50.49	linoleic
6	3.894	15.88	oleic
8	4.468	0.65	linolenic
14	6.429	1.89	stearic
15	7.299	13.56	palmitic

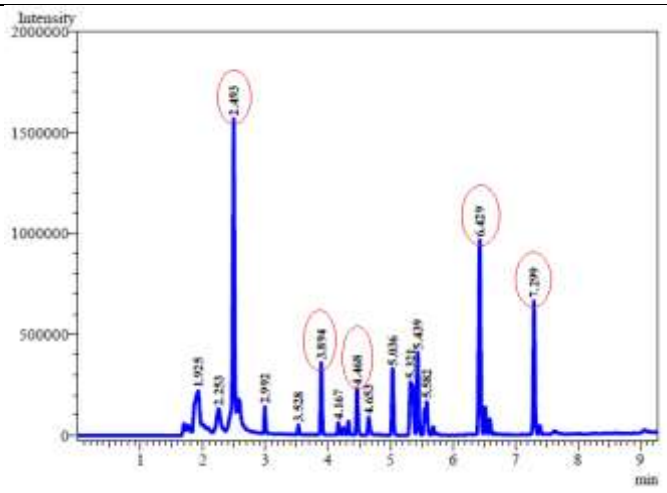

Figure 14. Chromatographic analysis of fatty acids in *A. vera* leaf extract and their retention times as analyzed using GC device.

The study is consistent with [40] linolenic, linoleic, and palmitic fatty acids have been extracted from the *A. vera* plant, in addition to other compounds. The study is also consistent with [41], who showed that linolenic, linoleic, palmitic, oleic, and stearic fatty acids, along with some other acids, were extracted from the *A. vera* plant.

Alpha Linolenic Acid (ALA) offers a variety of pharmacological properties, such as treatment of obesity and diabetes and cardiovascular preservation, along with anti-inflammatory, antioxidant, and anticancer properties, while also protecting the nervous system and improving memory [42]. Linoleic acid (LA) consumption reduces the incidence of cardiovascular disease and type 2 diabetes [43]. Conjugated linoleic acids have anticancer, antimutagenic, and antioxidant activities [44]. Oleic acid (OA) exhibits immune-modulating properties and has the potential for the treatment and prevention of various types of disorders, such as cardiovascular diseases, autoimmune diseases, skin injuries, cancer, and metabolic disorders [45]. The use of palmitic acid (PA) as an immunostimulant at low concentrations has been shown to protect *Danio rerio* (zebra fish) from the spring viremia of the carp virus (SVCV) and lead to a reduction in mortality [46]. Stearic acid was used to produce Triazoles, Thiadiazoles, and Thiadiazines, which showed antibacterial activities against two types of Gram-negative bacteria and two types of Gram-positive bacteria [47].

3.4. GC-MS analysis of *A. vera* leaf extract

GC-MS was also used to analyze *A. vera* leaf extract. **Figure 15** indicates the presence of 20 peaks that identify the presence of 20 active compounds. **Table 5** shows the compounds obtained.

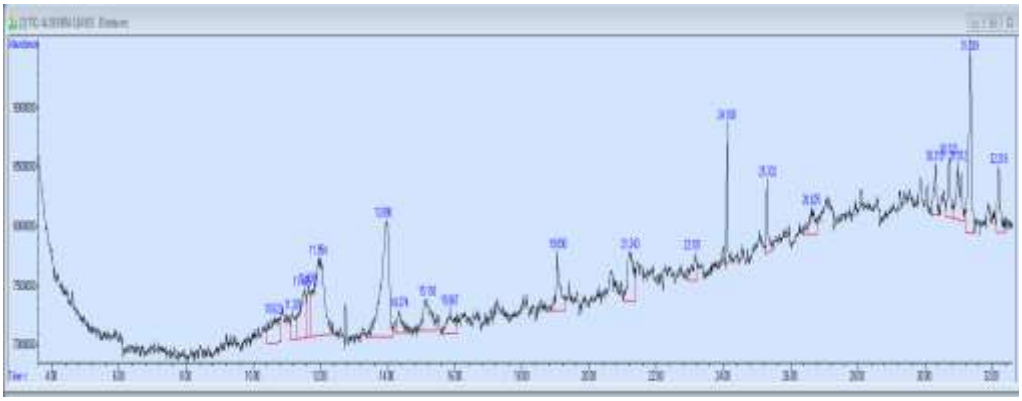

Figure 15. Chromatographic analysis of the active compounds in *A. vera* leaves extract and their retention times as measured using GC-MS device.

Table 5. The active compounds of *A. vera* leaf extract, the retention times, and the area percentage as analyzed by GC-MS.

Peak	Retention time (min)	Area %	Chemical compound
3	11.463	4.99	2H-Pyran, 2-(3-butynyloxy)tetrahydro
6	13.890	17.19	Heptane, 1-(ethenylthio)-
7	14.376	2.28	4-Spirohexanone, 5,5-dichloro-
8	15.195	4.61	1,2-Cyclobutanedicarboxylic acid, 3-methyl- , dimethyl ester
12	23.104	2.28	cis-Aconitic anhydride
13	24.107	2.93	Phenol, 2,2'-methylenebis[6-(1,1-d imethylethyl)-4- methyl-
18	31.012	4.54	4(1H)-Pyrimidinone, 2-(propylthio)
19	31.335	9.48	Ethanedicarboxamide, N-allyl-N'-(2 ,5-dimethylphenyl)-

2H-Pyran, 2-(3-butynyloxy) Tetrahydro is one of the compounds obtained from GC-MS analysis of *Hertia cheirifolia* leaf soil at a concentration of 0.20%. The oil showed antibacterial activity against *Pseudomonas aeruginosa*, S. *aureus*, E. *coli*, and *Klebsiella pneumonia* [48]. From the compound Heptane, 1-(ethenylthio)-, new aminomethoxy derivatives were produced and showed antifungal activity against *A. niger* and *Candida tropicalis* and against *P. aeruginosa* [49]. This compound was among the compounds produced by GC-MS analysis of the aqueous extract of *Allium sativum* and had a concentration of 1.57% [50]. 4-Spirohexanone, 5,5-dichloro- was among the plant compounds identified by GC-MS analysis of wheat grain *Triticum* sp., and its concentration was 1.11% [51]. Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl), was one of the volatile organic compounds obtained from GC-MS analysis of Chinese fir plant, and its concentration was 0.38% [52]. Conjugation of cis-Aconitic anhydride with chrysin led to an improvement in the anti-cancer activity of chrysin against human breast cancer cells [53]. Phenol, 2,2'-methylenebis [6-(1,1-d imethylethyl)-4-methyl- is one of the compounds obtained from the GC-MS analysis of *Lycopersicum esculentum*, *Ocimum basilicum*, and *Prunus mahaleb* oils. At a concentration of 0.19% in tomato, 0.05% in basil, and 0.11% in wild cherry, the oils showed different antibacterial activities against some types of bacteria [54].

4. Conclusions

A. vera, or the healing plant, is one of the oldest medicinal plants whose leaves have anti-bacterial, anti-fungal, and anti-tumor properties. The results of the examination of the alcoholic extract of the leaves showed that it contains many active compounds that have efficacy against microorganisms and cancer. Thus, we would like to draw attention to the possibility of using these

compounds for medical and therapeutic purposes. These compounds differ according to the environment in which the plant is found.

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Conflict of Interest

There are no conflicts of interest.

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