Antimicrobial activity, ergosterol content and phytochemical screening of Rorippa islandica (Oeder ex Murr.) and Carrichtera annua (L.)

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Abstract

The purpose of this study is to identify non-polluting substitute medicinal plants. This study interested by two medicinal plants (*Rorippa islandica* and *Carrichtera annua*) belonging to family Brassicaceae, to identify their bioactive constituents in their ethanol crude extracts using GC-MS and HPLC techniques, and evaluated *in vitro* their antifungal activity against five pathogenic fungi. GC-MS detected the existence of 50 and 55 compounds in *Rorippa islandica* and *Carrichtera annua* plant, respectively. The primary compound was 13-Docosenamide (20.54%) at *Rorippa islandica* and 2-Hydroxy-1-(Hydroxymethyl) Ethyl Stearate (9.74%) at *Carrichtera annua*, while HPLC identified 19 and 18 phenolic compounds in *Rorippa islandica* and *Carrichtera annua*, respectively. Gallic acid was the main compound in the two plants with concentration (3417.72 µg/g and 3733.98 µg/g), respectively. On the other hand, both plant extracts revealed potential antifungal activity, where the most promising effect of *Rorippa islandica* at concentration (10 mg/ml) was for ethanol 70% successive extract against *Colletotrichum gloeosporioides* with antimicrobial activity, MIC, and MFC (29±0.3 mm,7.8 µg/ml and 15.62 µg/ml), respectively. The most promising effect of *Carrichtera annua* L. at concentration (10 mg/ml) was for total extract against *Curvularia lunata* with antimicrobial activity, MIC and MFC (35±0.1 mm, 1.97 µg/ml and 3.9 µg/ml), respectively. A significant reduction in the ergosterol content of total and two ethanol (96% & 70%) successive fractions of two plants, the highest ergosterol reduction was in *Curvularia lunata* and *Colletotrichum gloeosporioides* with *Carrichtera annua* extract (49.93%, 47.7%), respectively. *Penicillium glabrum* and *Colletotrichum gloeosporioides* with *Rorippa islandica* extract (47.2%, 42.58%), respectively. The changes in cell morphology induced by total and ethanol (96% &70%) extracts of two plants were examined using AFM.

Introduction

Humans have long relied on biodiversity. Pesticides utilized extensively and improperly in the past, including by air spraying. These practices have caused many cases of acute or chronic toxicity in humans, contamination of the environment, increasing resistance inside the target plant and creating more harmful species. Because of these issues, authorities start defending human, animal and environmental health from the risks associated with pesticides so they may be used properly [1]. Also, fungal diseases have always been a major problem for crops. Chemical fungicides are typically used by growers to combat these kinds of illnesses. However, if the pre-harvest period is ignored, these products are harmful to both the surroundings and the consumer [2]. When Pesticides are of natural origin or based on living organisms [3]. They are called biopesticides. The evolution of ecologically friendly and safe integrated crop management (ICM) is attracting more interest and attention. The global trends today are for decreasing the use of chemical pesticides and emphasizing biopesticides particularly by the organic materials they contain. Plant extracts are substitutes and ways to offer comprehensive foliar pathogen control. They can respect the environment [4]. There are different mechanisms that underlie antimicrobial action of plant derived constituents. Phytochemicals can work by affecting cellular metabolism (cinnamaldehyde) or by rupturing microbial membranes (eugenol, carvacrol, and thymol). They can also regulate the manufacturing of biofilms (geraniol, carvacrol, thymol, etc.) and reduce microbiological generation of toxins (tannin, dihydroisosteviol, *etc*.) [5, 6]. Another mechanism of plant metabolites' antimicrobial action is that they are able to reduce the ergosterol content of fungal pathogen and causing leakage of protein and nucleic acids [7]. One of the dicotyledon families with the greatest commercial and scientific significance is the Brassicaceae (Cruciferae) family. Plants in the Brassicaceae family comprise a significant group of plants of great scientific interest due to the synthesis and accumulation of secondary metabolites known as glycosinolates (GSLs), sulfur-containing chemicals principally involved in plant defense against diseases and pests, in their tissues [8]. The products of GSL hydrolysis, particularly isothiocyanates (ITCs), have a variety of biological impacts. The most well-known and studied biological impact during the last 10 years has been the herbicidal, insecticidal, nematoidal, antifungal, and antibacterial actions [9]. This study was conducted to evaluate the effect of plant extracts of *R. islandica* (Oeder ex Murr.*)* and *C. annua* L. belonging to family Brassicaceae on *A. cerealis* MT808477 a phytopathogen causing leaf spot disease in tomatoes [10], *F. solani* OK464437 a phytopathogen causing disease (fusarium wilt) in a variety of agricultural crops and is preferred by warm, humid soils and high temperatures [11], *C. lunata* OM432028 a phytopathogen causing Curvularia leaf spot (CLS) is a prevalent foliar fungal disease of maize that is found all over the world, *P. glabrum* Op694171 a phytopathogen causing postharvest fruit rots in pomegranates (cv. Dente di Cavallo) 30 days after storage at 5°C [12] and *C. gloeosporioides* OP177948 a phytopathogen causing Anthracnose disease which resulted in huge damage to crops and fruit by presenting lesser yield or greater damage to plants [13]. Also, analyze their chemical composition to find non-polluting alternatives of fungicides.

Materials and Methods

Plant authentication and collection

Rorippa islandica (Oeder ex Murr.) and *Carrichtera annua* L. aerial parts (Family: Brassicaceae) were gathered in April-May 2022, North Western Woastal region (Marsa Matrouh), Egypt at the flowering stage. The plant specimen was verified in Herbarium by Dr. Omran Ghaly,

Researcher at Plant Ecology department, Desert Research Center, Cairo, Egypt. The code number *Rorippa islandica* (Oeder ex Murr.) (CAIH-1258-RPH) and *Carrichtera annua* L. (CAIH- 1084-R). Experimental and plant collection protocol was achieved after permission from the "Desert Research Center, Cairo, Egypt" (serial number of the protocol: MP 1841) and all processes comply with relevant institutional, national, and international guidelines and legislation.

Preparation and extraction

Every plant was spread out at room temperature and powdered with an electric grinder [14]. 30g ground samples of each plant powder, were soaked in ethanol 70% at room temperature in separate flask and shaken for 72 hours. The suspensions were filtered, and concentrated using a rotating evaporator at lower pressure (BűCHI VAC V-500) at 40 ℃ then dried in a 40 ℃ oven [15].

Fractionation by different organic solvent

Fifty-two grams of fine powder of each plant were put in the thimble and extracted successively three times with hexane, diethyl ether, chloroform, ethyl acetate, ethyl alcohol (96%) and ethyl alcohol (70%) for 72 hours. All solvent extracts were condensed using a rotary evaporator. The fractions were preserved in air tight brown bottle until further use [16].

Gas Chromatography-Mass Spectrometry (GC-MS)

The total ethanol extracts of *R.* (Oeder ex Murr.) and *C. annua* L. aerial parts were exposed to GC-MS analysis to identify some potent volatile and semi volatile components. Chemical analysis in each ethanol plant total extract after dissolved in chloroform was analysed by GC-MS using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 35°C and then increased by 3°C /min to 200°C hold for 3 min. increased to the final temperature 280°C by 3°C /min and hold for 10 min. The MS transfer line and injector were kept at 250°C and 260°C, respectively. At a constant 1 ml/min flow rate, helium was employed as a gas carrier. After a three-minute solvent delay, diluted samples of 1 µl were automatically injected using the AutosamplerAS1300 paired with GC in the split mode. In full scan mode, EI mass spectrawers were collected at 70 eV ionization voltages spanning the z40–1000 range. The temperature of the ion source was set at 200°C. The components were identified by comparing their mass spectra and retention durations to those in the NIST11 mass spectrum database and WILEY 09.

Qualitative and quantitative determination of the phenolic compounds using High Performance Liquid Chromatography (HPLC) technique

The HPLC analysis of total ethanol extract (70%) was carried out using an Agilent 1260 series. For the separation, the ZorbaxEclipsePlusC8 column (4.6mmx250mmi.d. 5µm) was utilized. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 mL/min made up the mobile phase. The following is how the mobile phase was sequentially coded in a linear gradient: 82% A for 0 minutes; 82% A for 0–1 minutes; 75% A for 1–11 minutes; 60% A for 11–18 minutes; 82% A for 18–22 minutes; and 82% A for 22–24 minutes. The multi-wavelength detector was seen at 280 nm. The injection volume of each sample solution was 5 µ. The temperature of the column was kept at 40°C.

Fungal strains

A. cerealis (MT808477), *F. solani* (OK464437), *C. lunata* (OM432028), *P. glabrum* (Op694171), and *C. gloeosporioides* (OP177948) were the standard fungal cultures to which the total crude extracts of ethyl alcohol (70%) and successive extracts (hexane, diethyl ether, chloroform, ethyl acetate, ethyl alcohol (96%) and ethyl alcohol (70%)) were obtained from Moubasher Mycological Center (AUMMC), Assiut University.

Media

Potato- dextrose agar medium (PDA) (g/L) was prepared according to instruction of manufacture by dissolving 39 g of PDA powder in 1 L of distilled water, they are properly mixed and dissolve to make the solution clear. Lastly, the solution is autoclaved at 121°C for 15 minutes. After autoclaving, the media is finally transferred into petri dishes, with roughly 25 mL in each dish. [17].

Potato- dextrose broth medium (PDA) (Liofilchem, Italy): The media was prepared according to instruction of manufacture by suspending 27 g of the media in 1 liter of distilled water then warming gently to dissolve completely. The media was sterilized by autoclave at 121℃ for

15 min [17].

Sabouraud's broth medium (g/l): Glucose, 20.0; Peptone, 10.0; KH_2PO_4, 1.0; and $MgSO_4$-$7H_2O$, 1.0 at PH 6.5 [18].

Standard drugs

The antifungal drug utilized in this study as control was Benozed 25%, obtained from Kafr El Zayat for Pesticides & Chemicals, Egypt. It was dissolved in distilled water.

Antifungal activity

All plant pathogenic fungi were cultivated on Potato Dextrose Agar (PDA) medium using the agar well diffusion method, which is commonly used to evaluate the antimicrobial properties of plants, and allowed to stand for 15 minutes. After adjusting the solution, agar plates should be inoculated with the fungal suspension within 15 minutes. The fungal strains were cultured in PDA media at 25°C. Using a loop, the sporulated fungus were removed off the agar slant and suspended in 10 milliliters of sterile water to create the inocula. To get rid of hyphae, the fungal solutions were filtered once using sterile gauze. The conidia suspensions that resulted were aggressively vortexed, and sterile distilled water was added to adjust the concentration to 105 CFU/mL. The entire dried agar surface is evenly streaked in three different directions. Allowing the agar surface to dry for no more than 15 min. Next, using a sterile cork borer or tip, a hole of 6 to 8 mm in diameter is aseptically punched. A volume of 100µL of the extract solution at the required concentration (10 mg/ml) is then added to the well. After placing the extract solution, plates should be refrigerated within 15 min after its have been disposed.Following 48 to 72 hours of incubation, the diameters of the inhibition zones (in millimeters) surrounding the wells should be measured to the closest full millimeter at the point where growth is noticeably reduced [19]. The experiment was done in triplicate.

Determination of minimum inhibitory concentration (MIC) for pathogenic fungi

The MIC were determined using the broth microdilution technique for extracts or solvent fractions [20]. This technique is known as "microdilution because it uses tiny amounts of broth that are administered in sterile, plastic microdilution trays with conical or round bottom wells." There should be 0.1 mL of broth in each well. To prepare microdilution trays, make intermediate two fold dilutions of antifungal agent volumetrically in broth. To making serial two fold dilutions use one pipette for measuring all diluents and then for adding the stock antifungal solution to the first tube. For each subsequent dilution step, use a new pipette. Fill the plastic microdilution trays with the antifungal/broth solutions. Using a dispensing device and antifungal dilutions prepared in at least 10 milliliters of broth is the most practical way to prepare microdilution trays.The dispensing device then delivers 0.1 (± 0.02) mL into each of the 96 wells of a standard tray. At 25°C, the fungal strains were cultivated on Sabaroured Dextrose broth media. Using a loop, the sporulated fungus were removed off the agar slant and suspended in 10 milliliters of sterile water to create the inocula. To get rid of hyphae, the fungal solutions were filtered once using sterile gauze. Using a hemacytometer cell counting chamber, the resultant conidia suspensions were violently vortexed and adjusted by adding sterile distilled water to a concentration of 105 CFU/mL, which was confirmed by a serial dilution plate count. These fungal suspensions were diluted 1:5 using Sabaroured Dextrose Broth media to obtain 2×final suspensions. The final concentration of these conidial suspensions when mixed with antifungal solution was 104UFC/mL.

Using mold conidial suspensions made in sabaroured dextrose broth media and adjusted to a final concentration of (0.4–5) × 104 CFU/mL as previously described, MICs were calculated on round-bottomed 96-well plates. For 48 hours, the inoculation plates were incubated at 35°C. MICs were measured at 24 or 48 hours to determine the extract concentration that resulted in a 100% growth reduction when compared to the extract-free growth control well [21].

Determination of minimum fungicidal concentration (MFC)

The MFC was determined through subculturing of 10 µLcontent of microtitre plate well which is greater or equal to the lowest minimum inhibitory concentration on the sabouraud dextrose broth media and incubated for 24h. The Petri dish was evaluated for growth after a 24-hour incubation period, and the lowest concentration of extracts that showed no discernible growth was determined to be the minimal fungicidal concentration [22]. Three duplicates of the experiment were conducted.

Determination of ergosterol content in the plasma membrane using HPLC technique

Total intracellular sterols were extracted as reported by [23] with slight modifications. Briefly, A spore of each culture inoculated on PDA plate culture after 48-72h was used to inoculate 50 ml of Potato dextrose broth containing sub MIC µg of each extracts per ml. The cultures

were incubated for 72h with shaking at 25°C. After being centrifuged for five minutes at 3000 rpm to collect the stationary-phase cells, they were once again cleaned with sterile distilled water. The cell pellet's net wet weight was calculated. Three milliliters of 25% alcoholic potassium hydroxide solution (25 g of KOH and 35 ml of sterile distilled water, brought to 100 ml with 100% ethanol), was added to each pellet and vortex mixed for 1 min. After being moved to glass screw-cap tubes, cell suspensions were incubated for one hour in a water bath at 85°C. Tubes were left to cool to room temperature after incubation. After that, a mixture of 1 milliliter of sterile distilled water and 3 milliliters of n-heptane was added, and the liquid was vigorously vortexed for three minutes to extract the sterols. The heptane layer was stored at -20°C for up to 24 hours after being transferred to a glass screw-cap tube. Before analysis, a 20 µL aliquot of sterol extract was diluted five times in 100% ethanol. The HPLC system Agilent was set at a detection wavelength of 280 nm, and the detector was linked to Data Station Software. Chromatographic separation was performed using a 250 × 4.6 mm i.d. (5 µm particle size) C18 column, The mobile phase was 98% methanol. Samples were injected into the system and separated at 25°C. The injection volume was 20 µL, and the mobile phase was supplied at a flow rate of 1.0 mL/min. [23, 24].

Microscopic study of fungal morphology

The creation of three-dimensional images of the architecture of the cell surface under physiological settings has gained popularity throughout the past ten years. The features of the outermost cell surface are visible at molecular or nanoscale resolution, enabling direct observation of the components of the cell wall [25]. Atomic force microscope (AFM) [26]. AFM is a type of scanning probe microscopy (SPM) that has resolution on the fractions of a nanometer scale and is more than 1000 times greater than the optical diffraction limit. Using a mechanical probe, the data is obtained by "feeling" or "touching" the surface. Exact scanning is made possible by piezoelectric components, which allow for tiny but exact movements under (electronic) command. The AFM does not employ the nuclear force, despite its name. The changes at cell morphology induced by total and successive ethanol extracts of *R. islandica* (Oeder ex Murr.) and *C. annua* L. on the tested fungi were examined using AFM [27, 28]. Every image was captured in contact mode usin AFM of model wet. (SPM 9600) (Scanning probe microscope, Shimadzu made in Japan, Non Contact mode). The experiment was carried out by Micro analytical Center Cairo University

Results

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was used to identify the chemical components of each total ethanol extract investigation (*R. islandica* (Oeder ex Murr.) and *C. annua* L.). The primary chemicals and their retention durations were displayed in the results **(**Tables 1, 2 **and figures. 1, 2).**

In case of *R. islandica* (Oeder ex Murr.), GC-MS analysis showed that the total ethanol extract consists of fifty compounds, and the main compounds were 13-Docosenamide, (Z) (20.54%), 9-Octadecenamide (16.60%), Ç-Sitosterol (6.10%), Bicyclo (8.2.0) dodecane, 11,11-dimethyl (5.85%) and Hexadecanoic acid (3.81%) **(**Table 1**)**. On the other hand, the chemical constituents obtained from the total ethanol extract of *C. annua* L. consist of fifty four compounds, and the main compounds were 2-Hydroxy-1-(Hydroxymethyl) Ethyl Stearate (9.74%), Glycerol 1-palmitate (8.59%), Hexadecanoic acid (8.55%), Stigmast-5-en-3-ol (6.98%) and 3,7,11,15-tetramethylhexadec-2-en-1-ol (phytol) (4.64%) as indicated in **(**Table 2**)**

Identification of phenolic compounds using HPLC:

Using HPLC, the phenolic components of the ethanol extract of *R. islandica* (Oeder ex Murr.) and *C. annua* L. were estimated both quantitatively and qualitatively. Data presented in **(**Table 3**)** revealed that gallic acid represent the main compound in the two plant extracts *R. islandica* (Oeder ex Murr.) and *C. annua* L. with concentration of (3417.72 µg/g and 3733.98 µg/g, respectively), while cinnamic acid was the minor compound of both plants *R. islandica* (Oeder ex Murr.) and *C. annua* L. with concentration of (36.74µg/g and 7.82 µg/g, respectively). Also, data showed that the main compounds in *R. islandica* (Oeder ex Murr.) were gallic acid, syringic acid and pyro catechol with concentrations (3417.72 µg/g, 3326.94 µg/g and 1182.12 µg/g, respectively), while the main compounds in *C. annua* L. were gallic acid, pyro catechol and Naringenin with concentrations (3733.98 µg/g, 3511.85 µg/g and 2356.00 µg/g, respectively). Data revealed that rutin was found in *R. islandica* (Oeder ex Murr.) with concentration (75.02 µg/g), while not found in *C. annua* L.

Table 1
GC-MS analysis of ethanol extract of R. islandica (Oeder ex Murr.)

RT (min)	Compound Name	Chemical formula	MW	Area %
5.17	Dimethoxypropane	$C_5H_{12}O_2$	104	0.21
8.36	1,5-Hexadien-3-ol	$C_6H_{10}O$	98	0.27
9.13	S-Methyl methanethiosulfinate	$C_2H_6OS_2$	110	0.16
17.81	8-Nonene-1-nitrile	$C_9H_{15}N$	137	1.10
22.43	9-Decene-1-nitrile	$C_{10}H_{17}N$	151	0.25
24.20	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.62
34.19	2,5-Dimethoxy-4-ethylamphetamine	$C_{13}H_{21}NO_2$	223	0.36
36.58	Lactose	$C_{12}H_{22}O_{11}$	342	0.95
36.74	Ethyl à-d-glucopyranoside	$C_8H_{16}O_6$	208	0.96
37.36	D-Glucitol, 1-S-hexyl-1-thio-	$C_{12}H_{26}O_5S$	282	0.58
37.75	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	0.80
39.48	4-Chloro-2,5-Dimethoxyamphetamine	$C_{11}H_{16}ClNO_2$	229	0.60
41.80	1H-Indole-3-acetonitrile	$C_{10}H_8N_2$	156	0.78
43.35	8-Methyl-8-azabicyclo[3.2.1]oct-3-yl benzoate	$C_{15}H_{19}NO_2$	245	0.85
45.23	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.53
46.98	Bicyclo [8.2.0]dodecane, 11,11-dimethyl-	$C_{14}H_{26}$	194	5.85
48.88	2-Aminoethanethiol hydrogen sulfate (ester)	$C_2H_7NO_3S_2$	157	0.41
49.25	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.81
50.10	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.79
50.37	Ethanol, 2-(9-octadecenyloxy)-, (z)-	$C_{20}H_{40}O_2$	312	0.79
53.76	Phytol	$C_{20}H_{40}O$	296	3.69
54.40	Linolenin, 1-mono-	$C_{21}H_{36}O_4$	352	1.57
55.14	9-Octadecenamide, 12-hydroxy-, [R-(Z)]-	$C_{18}H_{35}NO_2$	297	3.68
55.28	Linolenic acid, ethyl ester	$C_{20}H_{34}O_2$	306	2.26
55.50	9-Octadecenoic acid (Z)-, ethyl ester (Ethyl oleate)	$C_{20}H_{38}O_2$	310	0.50
55.73	Oleic acid	$C_{18}H_{34}O_2$	282	0.47
56.69	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.47
56.86	2,3,4,5-Tetrahydroxypentanal	$C_5H_{10}O_5$	150	0.45
58.61	2,3-Dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	330	0.32
RT: Retention Time				
Mw: Molecular weight				

RT (min)	Compound Name	Chemical formula	MW	Area %
59.61	1,1-Diphenyl-1-(2-dimethylaminoethyl)-2-butanone	$C_{20}H_{25}NO$	295	0.55
60.93	9,12-Octadecadienoic acid (z,z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	1.18
61.28	9-Octadecenamide	$C_{18}H_{35}NO$	281	16.60
62.24	Stearamide (octadecanamide)	$C_{18}H_{37}NO$	283	2.22
64.82	Di-2-Benzothiazole disulfane	$C_{14}H_8N_2S_4$	332	0.61
65.93	Palmitin, 2-mono-	$C_{19}H_{38}O_4$	330	2.89
66.91	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	0.75
68.27	2,3-Dihydroxypropyl stearate	$C_{21}H_{42}O_4$	358	0.78
70.30	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	$C_{27}H_{42}O_4$	430	0.15
70.45	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.93
70.59	1,25-Dihydroxyvitamin D3, TMS derivative	$C_{30}H_{52}O_3Si$	488	2.14
71.45	Distearin	$C_{39}H_{76}O_5$	624	1.70
72.84	13-Docosenamide, (z)-	$C_{22}H_{43}NO$	337	20.54
73.98	6,8-DI-C-á-Glucosylluteolin	$C_{27}H_{30}O_{16}$	610	0.75
74.96	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal)	$C_{25}H_{34}O_7$	446	0.64
82.57	Campesterol	$C_{28}H_{48}O$	400	1.41
83.29	Stigmasterin	$C_{29}H_{48}O$	412	1.72
84.64	Ç-Sitosterol	$C_{29}H_{50}O$	414	6.10
85.49	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	0.51
86.14	3',4',7-Trimethylquercetin	$C_{18}H_{16}O_7$	344	0.49
87.81	Stearin, 1,3-dipalmito-2-	$C_{53}H_{102}O_6$	834	1.22
RT: Retention Time				
Mw: Molecular weight				

Table 2
GC-MS analysis of ethanol extract of *C. annua* L.

RT (min)	Compound Name	Chemical formula	MW	Area %
5.06	Propionaldehyde, dimethyl acetal	$C_5H_{12}O_2$	104	0.33
17.80	8-Nonene-1-nitrile	$C_9H_{15}N$	137	0.31
33.10	Acetone (1r)-(+)-camphor azine	$C_{13}H_{22}N_2$	206	0.27
33.30	3-(N,N-Dimethyllaurylammonio) propanesulfonate	$C_{17}H_{37}NO_3S$	335	0.62
36.53	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	256	0.65
37.33	A-mannopyranoside-1-methyl-2,3–4,6-di-butylboronate	$C_{15}H_{28}B_2O_6$	326	1.96
40.34	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	0.64
40.50	4-(1-Hydroxy-2-isopropyl-5-methylcyclohexyl)-3-butyn-2-one	$C_{14}H_{22}O_2$	222	0.72
40.83	2-Acetyl-3-(2-cinnamido) ethyl-7-methoxyindole	$C_{22}H_{22}N_2O_3$	362	0.42
43.98	Oleic acid	$C_{18}H_{34}O_2$	282	0.62
45.21	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268	1.23
45.46	2-Cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312	0.73
46.83	Stearic acid	$C_{18}H_{36}O_2$	284	0.24
46.99	2-Aminoethanethiolsulfuric acid	$C_2H_7NO_3S_2$	157	0.72
47.89	Methyl 16-hydroxy-hexadecanoate	$C_{17}H_{34}O_3$	286	0.24
48.14	Linolenin, 1-mono-	$C_{21}H_{36}O_4$	352	0.74
49.23	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.55
50.08	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.80
53.75	3,7,11,15-Tetramethylhexadec-2-en-1-ol (phytol)	$C_{20}H_{40}O$	296	4.64
54.38	Methyl 8-[2-((2-[(2-ethylcyclopropyl)methyl]cyclopropyl)methyl) cyclopropyl]octanoate	$C_{22}H_{38}O_2$	334	2.86
55.12	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	1.47
55.26	Linolenic acid, ethyl ester	$C_{20}H_{34}O_2$	306	1.67
55.62	Aqua cera	$C_{22}H_{44}O_4$	372	0.81
56.67	Ethyl octadecanoate	$C_{20}H_{40}O_2$	312	1.38
58.57	Di-2-benzothiazole disulfane	$C_{14}H_8N_2S_4$	332	1.06
59.60	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	1.17
59.82	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.64
60.91	Stearin, 1,3-di-	$C_{39}H_{76}O_5$	624	0.32
61.21	9-Octadecenamide	$C_{18}H_{35}NO$	281	3.56
65.60	Methyl 8-(7-hexyl-3,7-dihydro-4a(4H)-naphthalenyl)octanoate	$C_{25}H_{40}O_2$	372	1.65
65.92	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	8.59

RT (min)	Compound Name	Chemical formula	MW	Area %
66.90	3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	487	2.22
71.45	2-Hydroxy-1-(hydroxymethyl) ethyl stearate	$C_{21}H_{42}O_4$	358	9.74
72.79	18,19-Secoyohimban-19-oic acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15á,16E)-	$C_{21}H_{24}N_2O_3$	352	4.03
72.85	6,8-DI-C-á-Glucosylluteolin	$C_{27}H_{30}O_{16}$	610	3.26
74.95	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal)	$C_{25}H_{34}O_7$	446	1.28
75.48	N-(2-{4,5-dimethoxy-2-[2-phenylethenyl]phenyl }-3-phenylpropyl)-n,ndimethylaminehydrochloride	$C_{27}H_{32}ClNO_2$	437	0.88
76.45	3',4',7-Trimethylquercetin	$C_{18}H_{16}O_7$	344	3.13
80.26	Cholest-5-en-3-ol (3á)-	$C_{27}H_{46}O$	386	1.31
80.96	1,5-Dimethoxy-2,4-bis(3-methylphthalidyl) benzol	$C_{26}H_{22}O_6$	430	1.09
81.20	3-(Tetradecanoyloxy)-2-[(trimethyl) oxy]propyl myristate	$C_{34}H_{68}O_5$	584	0.81
81.49	Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1058	0.13
81.81	Flavone 5,7-oh,3',4'-ome	$C_{17}H_{14}O_6$	314	0.51
82.34	3-Hydroxyspirost-8-en-11-one	$C_{27}H_{40}O_4$	428	0.38
82.55	Ethyl 3,7,12-trihydroxycholan-24-oate	$C_{26}H_{44}O_5$	436	2.44
82.83	Palmitin, 1,2-di-	$C_{35}H_{68}O_5$	568	0.92
84.40	3-[(Z)-2-Phenylethenyl] cholestan-2-one	$C_{35}H_{52}O$	488	0.31
84.63	Stigmast-5-en-3-ol	$C29H_{50}O$	414	6.98
86.13	Methyl glycocholate, 3TMS derivative	$C_{36}H_{69}NO_6$	695	1.76
86.73	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	0.87
87.80	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	430	2.50
91.01	4a-phorbol-12,13-didecanoat	$C_{40}H_{64}O_8$	672	0.36
91.08	H-purin-6-amine, [(2-fluorophenyl) methyl]-	$C_{12}H_{10}FN_5$	243	1.73
92.15	28 lidbpzoyraxili-qinsgfpzsa-n	$C_{32}H_{39}NO_{10}$	597	0.27

RT: Retention Time

Mw: Molecular weight

Table 3
Phenolic composition of ethanol extract of *R. islandica* (Oeder ex Murr.) and *C. annua* L. using HPLC technique.

	Compound name	*R. islandica* (Oeder ex Murr.)		*C. annua* L.	
RT. (min)		Area	Conc. µg/g	Area %	Conc. µg/g
3.59	Gallic acid	773.20	3417.72	844.75	3733.98
4.29	Chlorogenic acid	43.95	297.10	52.63	355.73
4.46	Catechin	23.57	268.79	101.16	1153.44
5.56	Methyl gallate	52.80	136.69	27.31	70.69
5.81	Coffeic acid	269.62	1101.98	47.87	195.64
6.31	Syringic acid	887.37	3326.94	43.89	164.56
6.78	Pyro catechol	161.76	1182.12	480.55	3511.85
7.03	Rutin	9.35	75.02	N.D.	N.D.
7.22	Ellagic acid	27.73	121.98	171.65	755.08
8.73	Coumaric acid	122.32	225.86	31.96	59.01
8.99	Vanillin	552.28	1042.28	43.34	81.80
9.75	Ferulic acid	17.70	53.65	298.98	906.09
10.41	Naringenin	173.99	830.05	493.84	2356.00
11.89	Rosmarinic acid	25.69	140.45	7.31	39.95
15.92	Daidzein	16.43	47.58	5.22	15.13
17.37	Querectin	41.50	262.50	15.72	99.43
19.29	Cinnamic acid	39.79	36.74	8.47	7.82
20.42	Kaempferol	83.13	273.54	8.30	27.32
21.19	Hesperetin	28.45	73.18	32.46	83.51

N.D.: Not Detected

Antifungal study

Fungal strains

The total crude extracts ethyl alcohol (70%) and successive extracts (hexane, diethyl ether, chloroform, ethyl acetate, ethyl alcohol (96%) and ethyl alcohol (70%) were tested against standard fungal cultures *A. cerealis* (MT808477) **(Fig. 5)**, *F. solani* (OK464437) **(Fig. 6)**, *C. lunata* (OM432028) **(Fig. 7)**, *P. glabrum* (Op694171) **(Fig. 8)** and *C. gloeosporioides* (OP177948) **(Fig. 9)**.

Screening of antifungal activity using agar diffusion method

The antifungal activity of the crude extracts of the two plants under investigation was assessed using the inhibitory zone diameter and benozed 25% as a positive control; results showed that the susceptibilities of tested fungi are variable. Also, successive extracts for two plants, *R. islandica* (Oeder ex Murr.), *C. annua* L. with hexane, diethyl ether, chloroform, ethyl acetate, ethyl alcohol 96% and ethyl alcohol 70% were used for the extraction of most active compounds from two plants aerial parts.

Total ethanol extracts of *R. islandica* (Oeder ex Murr.) showed the maximum inhibitory effect. The most sensitive species was *C. gloeosporioides* by inhibition zone diameter (21 ± 0.1 mm), followed by *A. cerealis, C. lunata* and *P. glabrum* (20 ± 0.1, 17 ± 0.1 and 16 ± 0.1 mm, respectively) while *F. solani* was the most resistant species with inhibition zone (12 ± 0.2 mm) **(**Table 4**)**. Ethyl alcohol 96% was the best successive solvent used to extract antifungal compounds from the two plants since it inhibited the growth of all tested pathogenic fungal species. While ethyl alcohol 70%, extract of *R.islandica* (Oeder ex Murr.) was active against *C. gloeosporioides, P. glabrum* and *A. cerealis* (29 ± 0.3, 18 ± 0.2 and18 ± 0.1mm, respectively) but has no activity against other tested organisms.

On the other hand, the crude extracts, the total ethanol extract of *C. annua* L. exhibited the highest antifungal activity, where *C. lunata* was the most sensitive fungal species with inhibition zone diameter of (35 ± 0.1 mm) followed by *C. gloeosporioides, A. cerealis* and *P. glabrum* (28 ± 0.1, 22 ± 0.1 and 22 ± 0.2 mm, respectively). While the minimum activity was recorded against *F. solani* with inhibition zone diameter of (17 ± 0.1 mm). while ethyl alcohol 70% successive solvent of *C. annua* L. was active against *C. lunata, C. gloeosporioides, F. solani* and *P. glabrum* (27 ± 0.1, 22 ± 0.3, 17 ± 0.2 and 15 ± 0.2 mm, respectively) but has no activity against *A. cerealis*. On the other hand, results in **(Table, 4)** showed that chloroform and ethyl acetate solvents of *Carrichtera annua* L. were active only against *A. cerealis* (19 ± 0.1 &14 ± 0.1mm, respectively*)* but have no activity against other tested organisms.

Table 4
Antifungal activity of plant extracts against some tested fungal species using agar well diffusion method.

Inhibition zone diameters (mm)						
Tested fungal strains\ Plant extract		***A. cerealis***	***F. solani***	***C. lunata***	***P. glabrum***	***C. gloeosporioides***
R. islandica (Oeder ex Murr.)	Total crude extract	20 ± 0.1	12 ± 0.2	17 ± 0.1	16 ± 0.1	21 ± 0.1
	Hexane	NA	NA	NA	NA	NA
	Diethyl ether	NA	NA	NA	NA	NA
	Chloroform	NA	NA	NA	NA	NA
	Ethyl acetate	NA	NA	NA	NA	NA
	Ethyl alcohol (96%)	25 ± 0.1	13 ± 0.2	20 ± 0.1	20 ± 0.1	23 ± 0.1
	Ethyl alcohol (70%)	18 ± 0.1	NA	NA	18 ± 0.2	29 ± 0.3
C.annua L.	Total crud extract	22 ± 0.1	17 ± 0.2	35 ± 0.1	22 ± 0.1	28 ± 0.1
	Hexane	NA	NA	NA	NA	NA
	Diethyl ether	NA	NA	NA	NA	NA
	Chloroform	19 ± 0.1	NA	NA	NA	NA
	Ethyl acetate	14 ± 0.1	NA	NA	NA	NA
	Ethyl alcohol (96%)	13 ± 0.1	15 ± 0.2	25 ± 0.1	20 ± 0.1	18 ± 0.1
	Ethyl alcohol (70%)	NA	17 ± 0.2	27 ± 0.1	15 ± 0.2	22 ± 0.3
Control	Benozed 25%	18 ± 0.1	16 ± 0.1	45 ± 0.2	33 ± 0.3	39 ± 0.1
NA: No activity						
±: Standard deviation						

Determination of the minimum inhibitory concentration (MIC)

The MIC of most active plant extracts against tested fungal species was determined using broth microdilution assay. According to results showed at **(**Table 5) *C. annua* L. total ethanol extract has the lowest MIC value against *C. lunata* (1.97 mg/ml). Also, the lowest MIC value of successive extracts was for ethyl alcohol 70% of *R. islandica* (Oeder ex Murr.) against *C. gloeosporioides* (7.8 mg/ml) and also for ethyl alcohol 70% successive extract of *C. annua* L. against *C. lunata* (7.8 mg/ml)

Determination of the minimum fungicidal concentration (MFC)

Concerning the MFC, the two plant extracts and standard drug killed the tested fungal species at concentrations higher than MIC values. According to the results showed at **(Table, 5)** *C. annua* L., total ethanol extract has the lowest MFC value against *C. lunata* (3.9 mg/ml), while ethyl alcohol (96%) of *R. islandica* (Oeder ex Murr.) successive extract had MFCs values ranging from (31.25–500 mg/ml). *A. cerealis* and *C. gloeosporioides* were the most sensitive tested fungi species followed by *C. lunata* and *P. glabrum*, then *F. solani.*

Table 5
MIC and MFC values of total and the most active plants extract on tested fungal species

Samples		Activities	mg/ml				
			A. cerealis	*F. solani*	*C. lunata*	*P. glabrum*	*C. gloeosporioides*
R. islandica (Oeder ex Murr.)	Total crude extract	MIC	31.25	250.00	125.00	125.00	62.50
		MFC	62.50	1000.00	500.00	500.00	125.00
	Ethyl alcohol (96%)	MIC	15.62	250.00	31.25	31.25	15.62
		MFC	31.25	500.00	62.50	62.50	31.25
	Ethyl alcohol (70%)	MIC	62.50	NA	NA	62.50	7.80
		MFC	125.00	NA	NA	125.00	15.62
C. annua L.	Total crude extract	MIC	62.50	125.00	1.97	62.50	15.62
		MFC	125.00	250.00	3.90	125.00	31.50
	Ethyl alcohol (96%)	MIC	250.00	125.00	15.62	15.62	62.50
		MFC	500.00	250.00	31.25	62.50	125.00
	Ethyl alcohol (70%)	MIC	NA	125.00	7.80	125.00	15.62
		MFC	NA	250.00	15.62	250	15.62
Control	Benozed 25%	MIC	62.50	125.00	0.90	3.90	1.90
		MFC	125.00	250.00	1.90	7.90	3.90

NA: No activity

Determination of ergosterol content in the plasma membrane

Ergosterol is an essential functional component of the plasma membrane. The effect of sub- inhibitory concentration of ethyl alcohol (96% & 70%) extracts of the two plants under investigation on this vital content is showing in **(Table, 6 and Fig. 10)**. Following exposure to inhibitory amounts of total and ethyl alcohol (96% and 70%) extracts of two plants under research, a decrease in the total cellular ergosterol content of the five fungal species was found in comparison to the control. The highest ergosterol reduction was recorded in cells of *C. lunata* and *C. gloeosporioides* with *C. annua* L. extract (49.93%, 47.70%), respectively. Also, *P. glabrum* and *C. gloeosporioides* with *R. islandica* (Oeder ex Murr.) extract (47.20%, 42.58%), respectively.

Table 6
Reduction percent of ergosterol content in tested fungal species by total and ethyl alcohol (96% &70%) extracts of two plants under investigation

Plants	**Samples\ Tested Fungi**	**Control**	**Total extract**	**R%**	**Ethyl alcohol (96%)**	**R%**	**Ethyl alcohol (70%)**	**R%**
R. islandica (Oeder ex Murr.)	**A. cerealis**	17.00	10.40	38.80	NA	NA	NA	NA
	P. glabrum	19.51	NA	NA	11.90	39.00	10.30	47.20
	C. gloeosporioides	14.23	12.09	15	10.04	15.39	8.17	42.58
C. annua L.	**C. lunata**	22.15	18.10	18.28	14.17	36.00	11.09	49.93
	C. gloeosporioides	14.23	7.44	47.70	10.51	26.14	9.70	31.83

R%: Percent of reduction

NA: No Activity

Observing morphological changes under AFM

The changes in cell morphology induced by total and ethyl alcohol (96% &70%) extracts of two plants under investigation were examined using (AFM). AFM images give not only qualitative information of biological sample but also quantitative measurements at nanometer level. Therefore, it easy to make accurate comparison between treated and untreated samples. Since AFM is capable of providing a precise 3D map of the cell surface in the X, Y and Z dimentions on a sub micrometer scale, the Z axis (Z hight) value can be used to describe the effect of total extracts of two investigated plants against all tested fungi and ethyl alcohol (96% &70%) extracts against the most effected fungi. Variations in the Z hight of untreated and treated fungi with total extracts were apparent from the 3D images in **(Fig., 11).** The Z hights and roughness of untreated and treated *A. cerealis, F. Solani, C. lunata, P. Glabrum* and *C. Gloeosporioides* with total extract of *Rorippa islandica* (Oeder ex Murr.), *Carrichtera annua* L. appeared in **(Table, 7).** As showed Z hights of untreated fungi (*A. cerealis, F. Solani, C. lunata, P. Glabrum* and *C. Gloeosporioides*) were found to be (79, 88, 91, 83 and 94) nm, respectively and after treatment with *R. islandica* (Oeder ex Murr.) were found to be (134.5, 30.9, 71.2, 330 and 27) nm, respectively and for *C. annua* L. (104.9, 1.3, 55.3, 108.4 and 42.1) nm, respectively. According to the showed result, it appeared that roughness of all tested treated fungi with the two total plant extracts under investigation were lower than roughness of untreated tested fungi except for *P. Glabrum* the roughness of it before treating (8.25 nm) was lower than roughness of it after treating with the two total plant extracts of *R. islandica* (Oeder ex Murr.) and *C. annua* L. (24.17 and 11.57) nm respectively. Also, Variations in the Z hight of untreated and treated fungi with the best of successive extracts (ethyl alc. 96% and ethyl alc. 70%) were apparent from the 3D images in **(Fig. 12).** The Z hights and roughness of untreated and treated *C.lunata* and *C. Gloeosporioides* with the best successive extracts (ethyl alc. 96% and ethyl alc. 70%) of *Rorippa islandica* (Oeder ex Murr.) and *Carrichtera annua* L. appeared in **(Table, 8).** As showed Z hights of untreated fungi (*C. lunata* and *C. Gloeosporioides*) were found to be (91 and 94) nm respectively and after treatment of *C. Gloeosporioides* with *R. islandica* (Oeder ex Murr.) (ethyl alc. 96% and ethyl alc. 70%) were found to be (67and 78) nm respectively and after treatment of *C. lunata* with. *Carrichtera annua* L. (ethyl alc. 96% and ethyl alc. 70%) was found to be (75 and 117.5) nm, respectively.

Table 7

Morphological changes under (AFM) in tested fungal species by control and treated five tested fungi with total ethanol(70%) extracts of the two plants under investigation.

Fungal strains	Control		*Rorippa islandica* (Oeder ex Murr.)		*Carrichtera annua* L.	
	Z hight nm	Roughness	Z hight Nm	Roughness	Z hight Nm	Roughness
A. **Cerealis**	79.00	16.48	134.5	8.29	104.9	7.96
F. Solani	88.00	13.69	30.90	3.11	1.30	0.13
C. lunata	91.00	15.84	71.20	7.47	55.3	9.50
P. Glabrum	83.00	8.25	330.00	24.17	108.40	11.57
C. Gloeosporioides	94.00	16.52	27.00	3.94	42.10	4.50

Table 8

Morphological changes under (AFM) in tested fungal species by control and treated five tested fungi with successive extracts of the two plants under investigation

Samples\ Tested fungi	Control		*R. islandica* (Oeder ex Murr.)				*C. annua* L.			
			Ethyl alcohol (96%)		Ethyl alcohol (70%)		Ethyl alcohol (96%)		Ethyl alcohol (70%)	
	Z hight	Roughness	Z hight	Roughness	Z hight	Roughness	Z hight	Roughness	Z hight	Roughness
C. lunata	91.00	15.84	-	-	-	-	75.00	16.70	117.50	11.50
C. Gloeosporioides	94.00	16.52	67.00	6.80	78.00	8.70	-	-	-	-

Discussion

When everyone has physical, social, and financial access to enough food that is safe and nourishing, there is food security. At the moment, this activity has been adversely affected by the results of applying fungicides to traditional agricultural production systems in order to combat crop diseases. Chemical fungicides have frequently been employed to control these diseases, although this practice is linked to adverse environmental effects, possible pesticide exposure for humans, and residue deposition on fruits. However, the frequent emergence of disease resistance has diminished the efficacy of synthetic fungicides. Hence there is a great demand for safer, alternative and effective

chemotherapeutic agents [29, 30]. Currently, the search for natural products with novel uses, particularly related to pest management is very active. Plant extracts with antibacterial properties and containing a range of secondary metabolites, such as alkaloids, quinones, flavonoids, glycosides, saponins, tannins, and terpenoids, have piqued interest in the field of plant disease control. The content of these bioactive compounds varies by plant species based on environmental conditions and the pathosystem [30, 31]. In the past, pesticides have been widely and inappropriately used, such as spraying by air. These practices have caused many cases of acute or chronic toxicity in humans, contamination of the environment, increasing resistance in the target plant and creating more harmful species. Because of these issues, authorities start defending human, animal and environmental health from the risks associated with pesticides so they can be used properly [1]. When pesticides are of natural origin or based on living organisms [3] they are called bio pesticides. The development of ecologically friendly and safe integrated crop management (ICM) is attracting more interest and attention. Global trends of today promote the use of biopesticides and decrease the usage of chemical pesticides.

In an effort to manage severe fungal plant diseases, environmentally friendly antifungal chemicals are being developed, the extracts of two plants belonging to the same family collected from North western coastal reagion (Marsa Matrouh) were tested against five pathogenic plant fungal species.

GC-MS examination of the ethanol extract in this investigation revealed the presence of bioactive chemicals, primarily hydrocarbons, oxygenated compounds, and derivatives of benzene. This result might indicate that the active compounds which are found in the aerial part of the two plants are mainly non-polar in their nature like terpene, hydrocarbons and sterols and this explains the exceedingly ability of high polar solvents to extract high concentrations of these bioactive molecules [32].

The present results were in agreement with results reported by **Raveendran Ramya, 2021**[33] who showed that bioactive substances including fatty acids, steroids, alkaloids, terpenoids, vitamins, and heterocyclic compounds were present in the ethanolic leaf extract of *Hellenia speciosa*, according to GC-MS analysis. The main chemicals that may contribute to biological activities including antioxidant, anti-microbial, anti-cancer, anti-diabetic, and anti-inflammatory properties are octadecanoic acid, n-hexadecanoic acid, caryophyllene, ar-tumerone, piperine, and squalene.

13-Docosenamide is a primary fatty amide, identified as major compound only in *R. islandica* (Oeder ex Murr.) where **Medeiros Caroline dos Reis et al**., **(2019)** [34] revealed that according to **Pradheesh et al**. **(2017)** [35], 13-docosenamide, (Z)-, was one of the compounds found in the ethanolic extract of the medicinal plant *Pisonia grandis* R. Br. that demonstrated antifungal and antibacterial properties against *Aspergillus niger* and *Staphylococcus aureus*, respectively. β -Sitosterol (beta-sitosterol) is one of several phytosterols (plant sterols) with chemical structures similar to that of cholesterol identified in *R. islandica (Oeder ex Murr.)* where **Bhagat akshi et al**., **(2019)**[36] reported that the result of their studies are in confirmatory with **Aslam et al**., **(2010)** [37] who observed that *Azadirachta indica* leaf extract inhibited the fungal growth because of the presence of certain secondary metabolites (quercetin and β-sitosterol) in the leaves that possess antimicrobial properities .

Walters Dale et al., **(2004)** [38] revealed that in higher plants, linolenic and linoleic acid are substrates for the production of a range of trihydroxy oxylipins, which are known to possess antifungal activity e.g., 9(S), 12(S), 13(S)-trihydroxy-10(E)-octadecenoic acid, which is produced in plants infected with the rice blast fungus *Magnaportha grisea* and has been shown to exert antifungal activity against it.

Sharaf Mohamed H., (2022) [39] showed that a few key compounds were present like 9-octadecenoic acid (Z)-; methyl ester methyl stearate; 9,12-octadecadienoic acid (Z, Z)-; 2-hydroxy-1-(hydroxymethyl) ethyl ester; and 9,17-octadecadienal, (Z)-, according to GC–MS analysis can inhibit the microbial growth in the crude extract of *Aspergillus nidulans*, *Aaspergillus fumigatus*, and *Aspergillus flavus* and this is according to **El-Fayoumy et al**., **2021** [40].

Hexadecanoic acid (palmitic acid) appeared in our results as one of main compounds of two plants of our study (*Rorippa islandica* (Oeder ex Murr.) and *Carrichtera annua* L.) and it is the most common saturated fatty acid found in animals, plants and microorganisms and this was explained by **Tulika Tyagi and Mala Agarwal, (2017)** [41] as they found that n-Hexadecanoic acid as the comman compound in the leaves of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) and E 11-Hexadecanoic acid, ethyl ester act as antifungal.

The current results also concurred with those of other earlier research by **Yuan et al**. **(2012)** [42], which showed that all benzene compounds had antifungal activity against *Fusarium oxysporum*. Benzothiazoles phenol and 2, 3-trimethylphenol totally stopped *F. oxysporum* from growing, and that these compounds' antifungal properties seemed to be connected to their low benzene contents. The practically complete antagonistic actions of alcohol, aldehyde, ester, ether, and naphthyl compounds on *F. oxysporum* may result from both the reduction of pathogen mycelial groth and the inhibition of spore germination.

Cerqueira Sales MD et al., 2015 [43] reported that types of plant extract that have antifungal properties and may be used to manage phytopathogenic fungi. In the current investigation, ethanol extract and fractions showed a great antifungal activity against all tested fungi, this may be due to high antifungal compounds content found in these extracts. This is in line with the findings of **Saha D. et al. (2005)** [44], who discovered that ethanol and aqueous extracts showed either 100% or greater than 90% inhibition of spore germination of all fungal pathogens, making it the most promising plant among those whose extracts were determined to be advantageous. Since the solubility of secondary matabolities is strongly dependent on the polarity of the solvents, the variable degrees of inhibition zone seen when employing different extracts may be caused by the solvent's polarity [45]. The increased solubility of the active antimicrobial components in ethanolic extracts may be the cause of their inhibitory potentials. This result was consistent with that of **Ayyasamy et al. (2012)** [46], who found that ethanolic extracts of *Pleurotus florida* had superior inhibitory activity.

Dodecane is an oily liquid n-alkane hydrocarbon found in the present study in *Rorippa islandica* (Oeder ex Murr.) plant, and this was in accordance with the study by **Adeleyaet al., (2010)** [47] who reported that natural alkanes detected in GC-MS analysis of natural cure concoction Epa-Ijebu was found to have potent antifungal activity.

In the present study it was found that gallic acid is the main active phenolic compound found in the two investigated plants, and it was known that phenolic acids have great antifungal activity as the study represented by **Li et al., (2017)** [48] who revealed Gallic acid [C6H2 (OH)3COOH] is a trihydroxybenzoic acid, a natural polyphenol compound, found in several plant species, and has been shown to have antifungal and antibacterial properties. Also, **Nguyen et al., (2013)** [49] showed that gallic acid exhibited strong antifungal activity against *Fusarium solani*.

Plants in the present study, these results were in agreement with the result obtained by **(Ahmed et al., 2015)** [50] who reported that the six flavonoids have been isolated from the butanol extract of air dried herb of *A. maurorum* Medic. These compounds were identified as kaempferol, quercetin, quercetin 3-O-α- rhamnoside, kaempferol-3-O-β- glucoside, quercetin – 3-O-β- gluciside and isorhamnetine – 3-O-β- rutinoside.The antifungal activity of *A.maurorum* Medic plants could be explained due to their high flavonoids contents. The possible mechanism of the antimicrobial action may be related to viability inhibition of the tested organisms by *A.maurorum* Medic extract due to the loss of their ability to bind to DNA. This finding implied that the medicinal extract from *A. maurorum* might work by preventing DNA replication and cell division [51].

Ergosterol is an important component of the fungal cell membrane, and it is crucial for fungi to maintain the fluidity and permeability of their cell membranes [52]. In our experiment, aquantitative determination of the ergosterol content of the test organisms was adopted as an indicative effect of the plant extract on the ergosterol of the test organisms. This result suggested that the plasma membrane is considered as an ideal target of two investigated plants extracts. The results were in agreement with **(Brilhante et al., 2016)** [53] who found that tyrosol caused leakage of protein and nucleic acids, suggesting the effect of

tyrosol on both, cellular and nuclear membrane. Tyrosol inhibits ergosterol synthesis, as demonstrated by the reduced amount of ergosterol recovered from fungal strains after exposure to high tyrosol concentrations, according to the results. Terpenes have been demonstrated to change the permeability and fluidity of the lipid bilayer membrane by penetrating the fungal cell wall and gaining access between the fatty acid chains that comprise it. The cell wall may break down and adhere to host surfaces less well as a result of these alterations, in addition to other effects include disruption of the plasma membrane, loss of cell content, cytoplasmic coagulation, and cell lysis [54].

According to research by **Cuenot S. and Bouchara JP. (2018)** [55], fungal pathogens' surfaces mediate attachment to host tissues, immune system identification or evasion, and the development of disease [56, 57]**)**. Both the molecular interactions and surface composition of many bacteria are important for the spread of infection [56, 58]. (Glyco) proteins, lectins, polysaccharides, lipids, and other macromolecules covering the cells are some of the components that mediate the properties of the pathogen surfaces **[57, 58, 59)**. Understanding how these molecules interact with their surroundings and how they structurally organize at the fungal surface is one of the main challenges. With its capacity to image biological cell surfaces under physiological conditions with nanoscale resolution and to probe cell surface properties with piconewton sensitivity, AFM has become a crucial tool in nanobiotechnological research over the past ten years [60, 61]. In this study, AFM data topographyic image indicated that total ethanol extract of *R. islandica (Oeder ex Murr.)* and *C. annua* and the best successive extracts (ethanol 96% and ethanol 70%) of them caused morphological changes in the surfaces of *A. cerealis*, *F. solani*, *C. lunata, P. glabrum* and *C. gloeosporioides*. So it may be that ethanol extract of plants had attached to the surfca of fungi and enter the cell wall leading to cell wall deformation. This was in line with **Kim, S.H., and Vujanovic, V. (2018)** [62], who reported that the general dryness or desiccation of the fungal hyphal surface shape or topography with roughness at different media and exposure durations was assessed using AFM in tapping mode.

The hyphal surface of *Sphaerodes mycoparasitica* in potato dextrose broth (PDB) showed the change in the morphology from soft to hard surface and roughness from low to high.

Conclusion

The present study demonstrated that ethanol extracts of the aerial part of two plants*R. islandica* (Oeder ex Murr.)and *C. annua* L.have varying antifungal activity against tested fungal pathogens. Additionally, there was a difference in the antifungal activity between the plant solvent extracts, which may have resulted from variations in the phytocompound concentrations of the different secondary metabolites included in the extract.

This could suggest that probably certain phytochemicals exhibit their antimicrobial action only with other phytoconstituents in a synergistic way. It is therefore recommended the synergistic use of plant extracts which might solve the problem of chemical fungicides and emphasizing biopesticides especially by the natural substances that they contain and treat the emerging disease caused by fungal pathogenic species.

As all the plants investigated in the present work are common in Egypt, the recovery of their compounds is high and these species may be exploited as potent fungicides for many plant diseases.

The potential antifungal activity of ethanol extracts of *R. islandica* (Oeder ex Murr.)and*C. annua* L.against plant pathogenic fungi may be explained on the basis of, the presence of these bioactive phytoconatituents. Therefore, these bioactive constituents need further multipronged research to implement its use as a natural fungicide for treating plant diseases instead of chemical harmful pesticides.

Declarations

Funding Declaration

No funding.

Author Contribution

H.Y. wrote the main manuscript text and M.A. and S.A. prepared figures and tables. N. M. and E. I. reviewed the manuscript

Data Availability

All data generated or analysed during this study are included in this published article.

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Figures

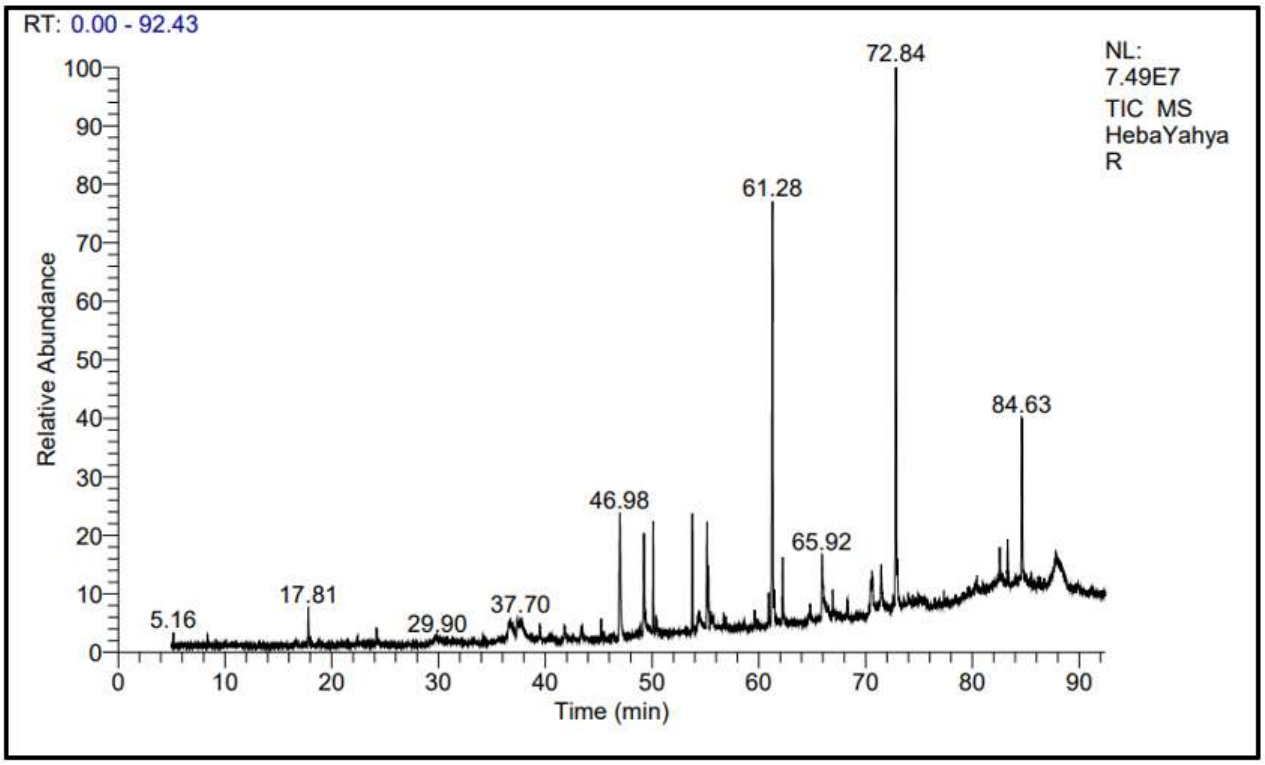


Figure 1

GC-MS analysis of *R. islandica* (Oeder ex Murr.)

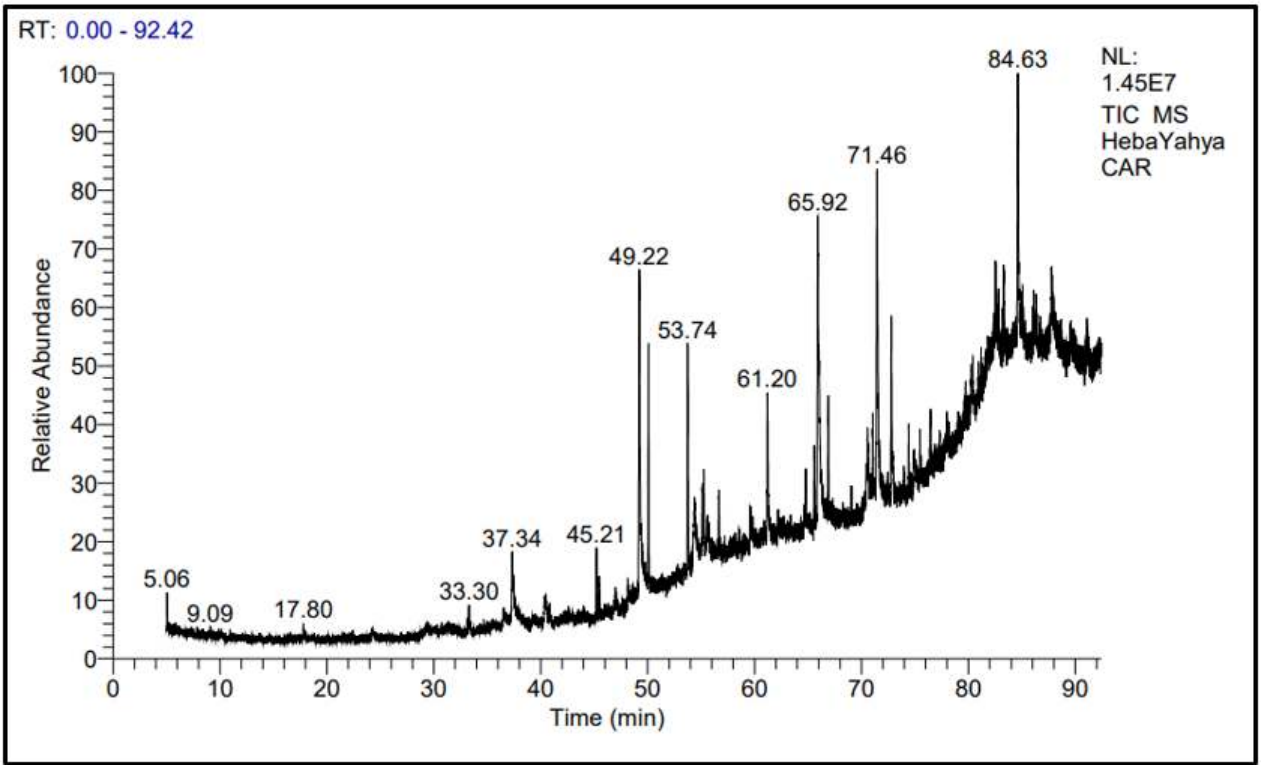


Figure 2

GC-MS analysis of ethanol extract of *C. annua* L.

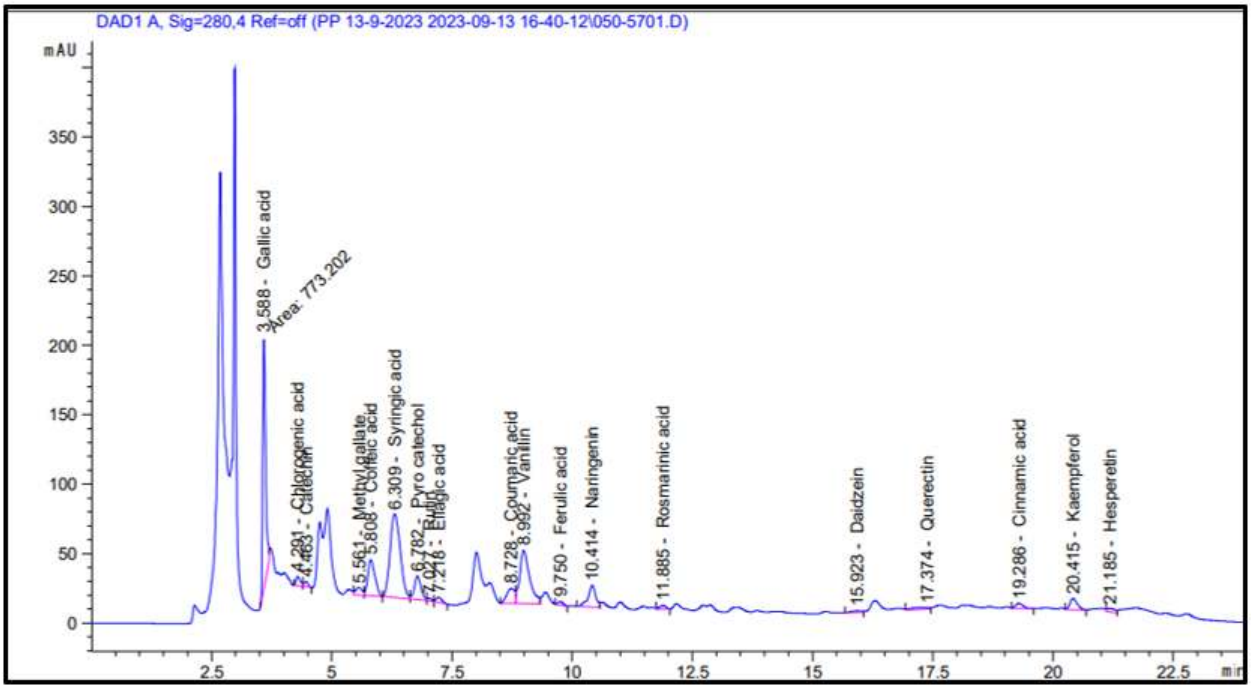


Figure 3

HPLC analysis of ethanol extract of *R. islandica*(Oeder ex Murr.)

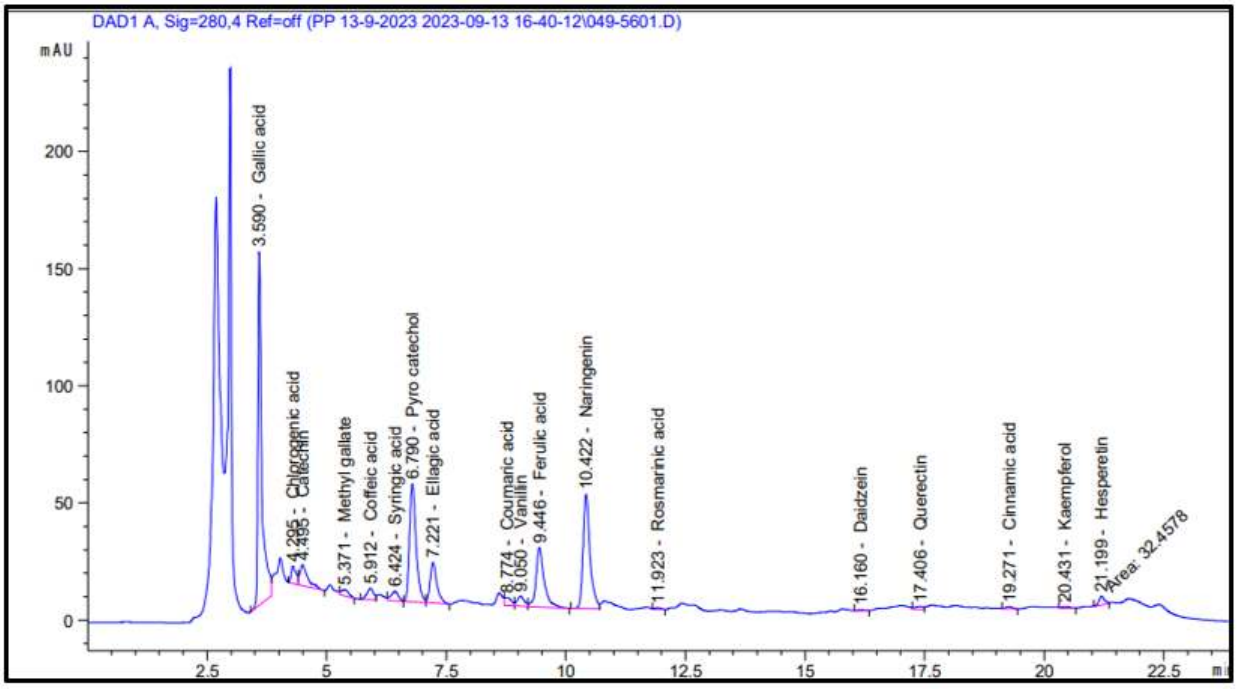


Figure 4

HPLC of ethanol extract of *C. annua* L.

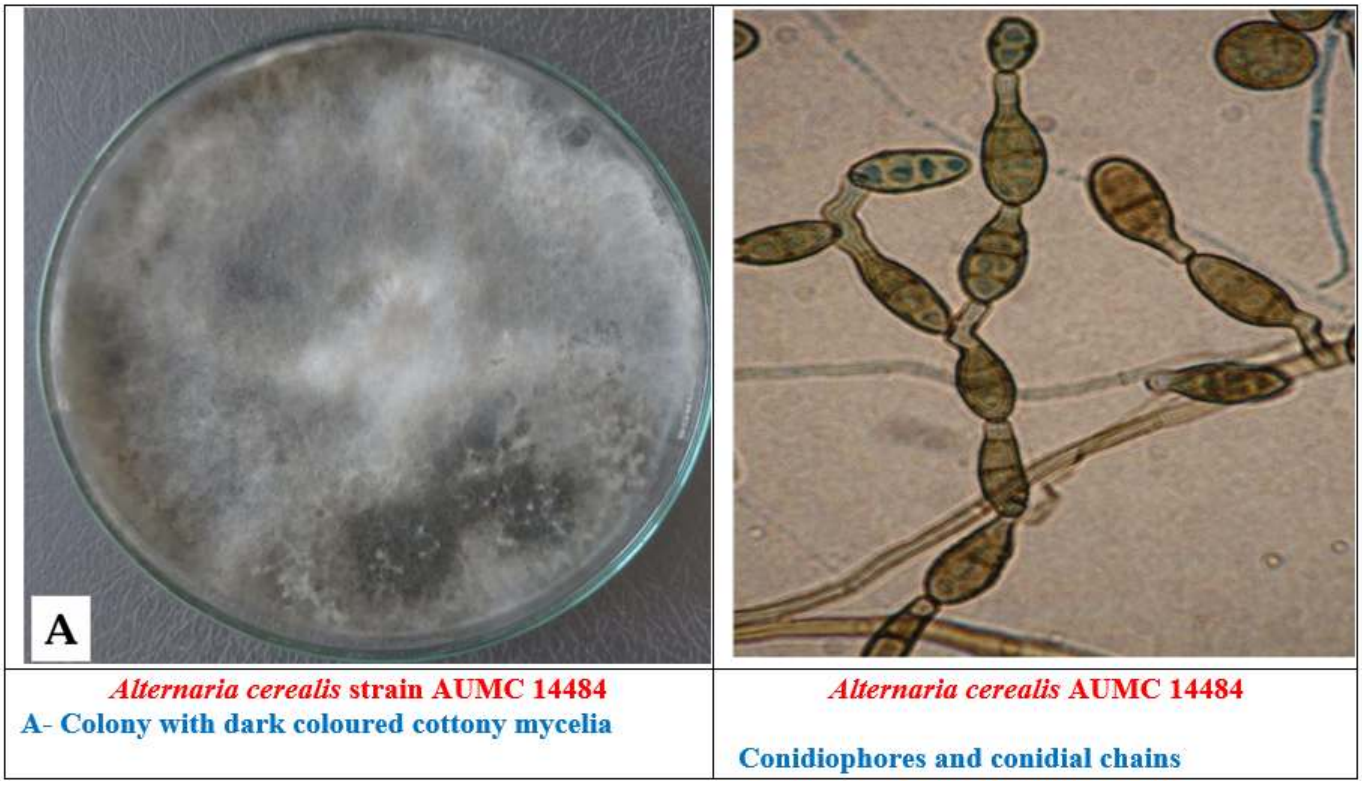


Figure 5

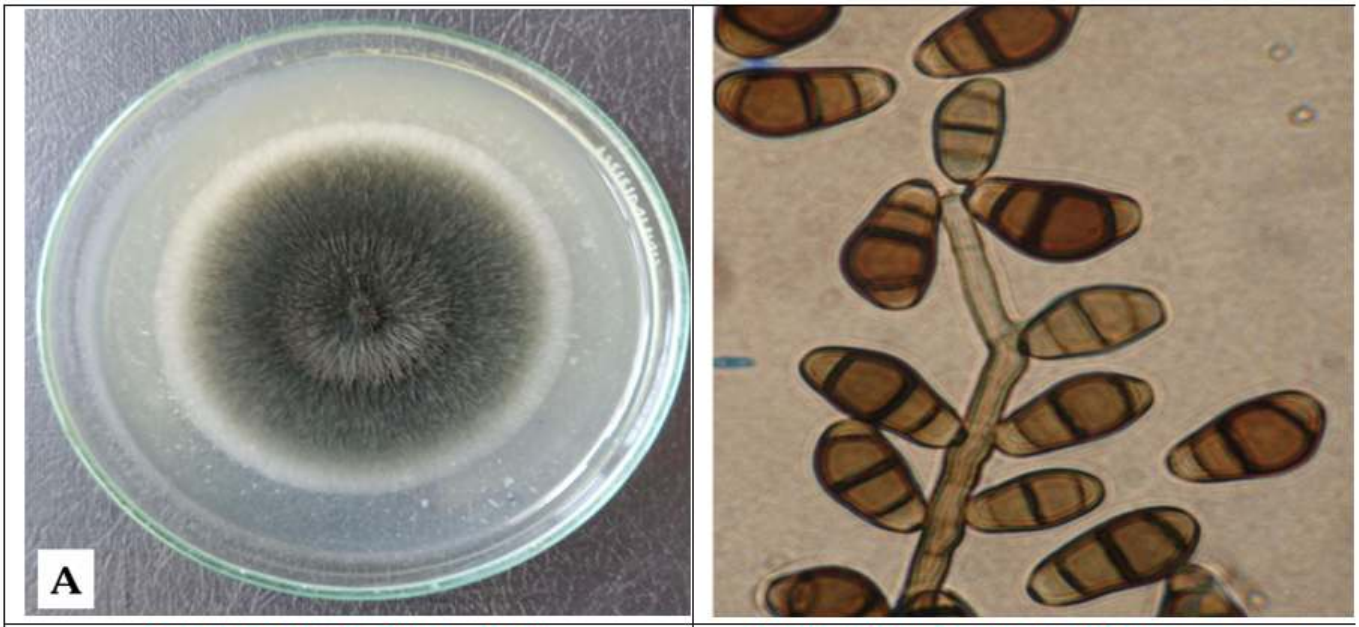
Alternaria cerealis(MT808477)

***Fusarium solani* AUMC 15119**
A. Colony on Potato sucrose agar after 7 days at 28°C

***Fusarium solani AUMC 15119* Hyphae with long phialide producing a cluster of microconidia**

Figure 6

Fusarium solani (OK464437)

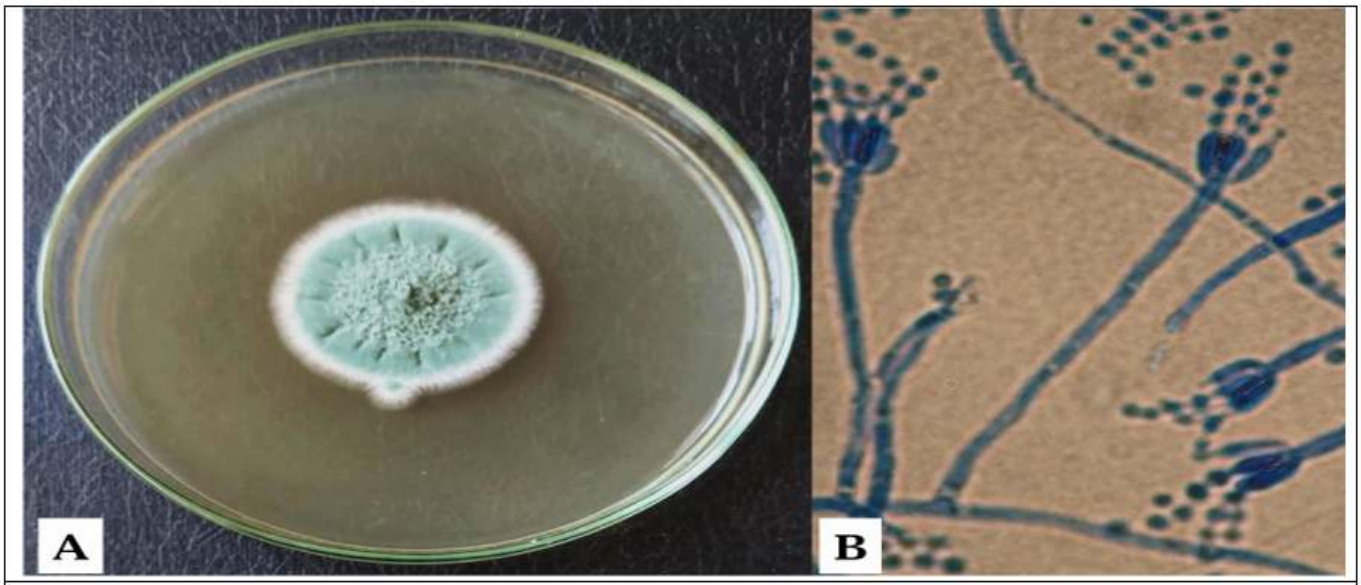


Curvularia lunata AUMC 15458
A- Dark colony on Potato sucrose agar after 7 days at 28°C

Curvularia lunata AUMC 15458 Pigmented conidiophore producing dark 3 septate conidia

Figure 7

Curvularia lunata (OM432028)



Penicillium glabrum AUMC 15597
A- Bluish-green colony on Czapek`s yeast agar after 7 days at 28°C
B- Conidiophores bearing ampuliform phialides and conidial chains.

Figure 8

Penicillium glabrum(Op694171)

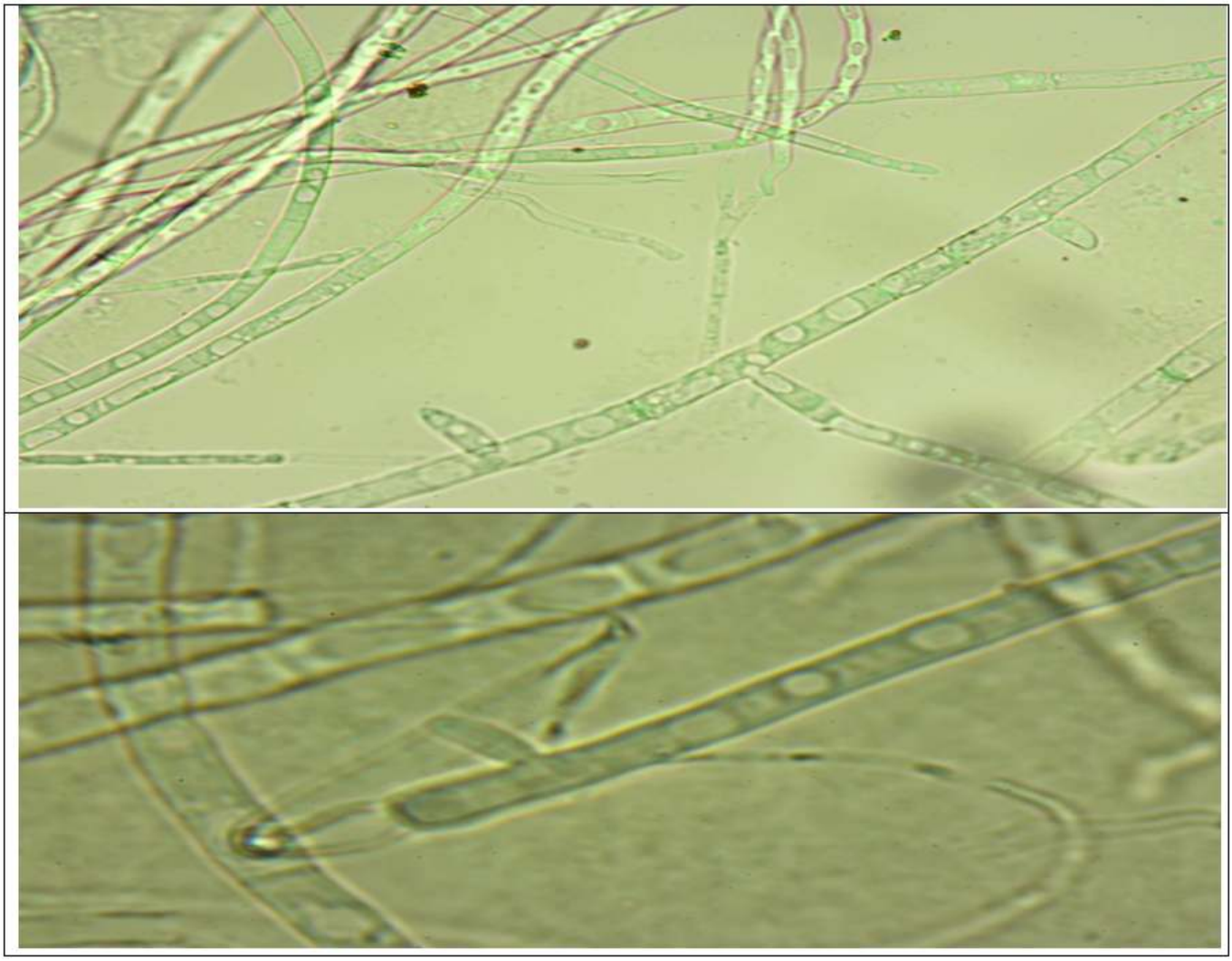


Figure 9

Colletotrichum gloeosporioides (OP177948)

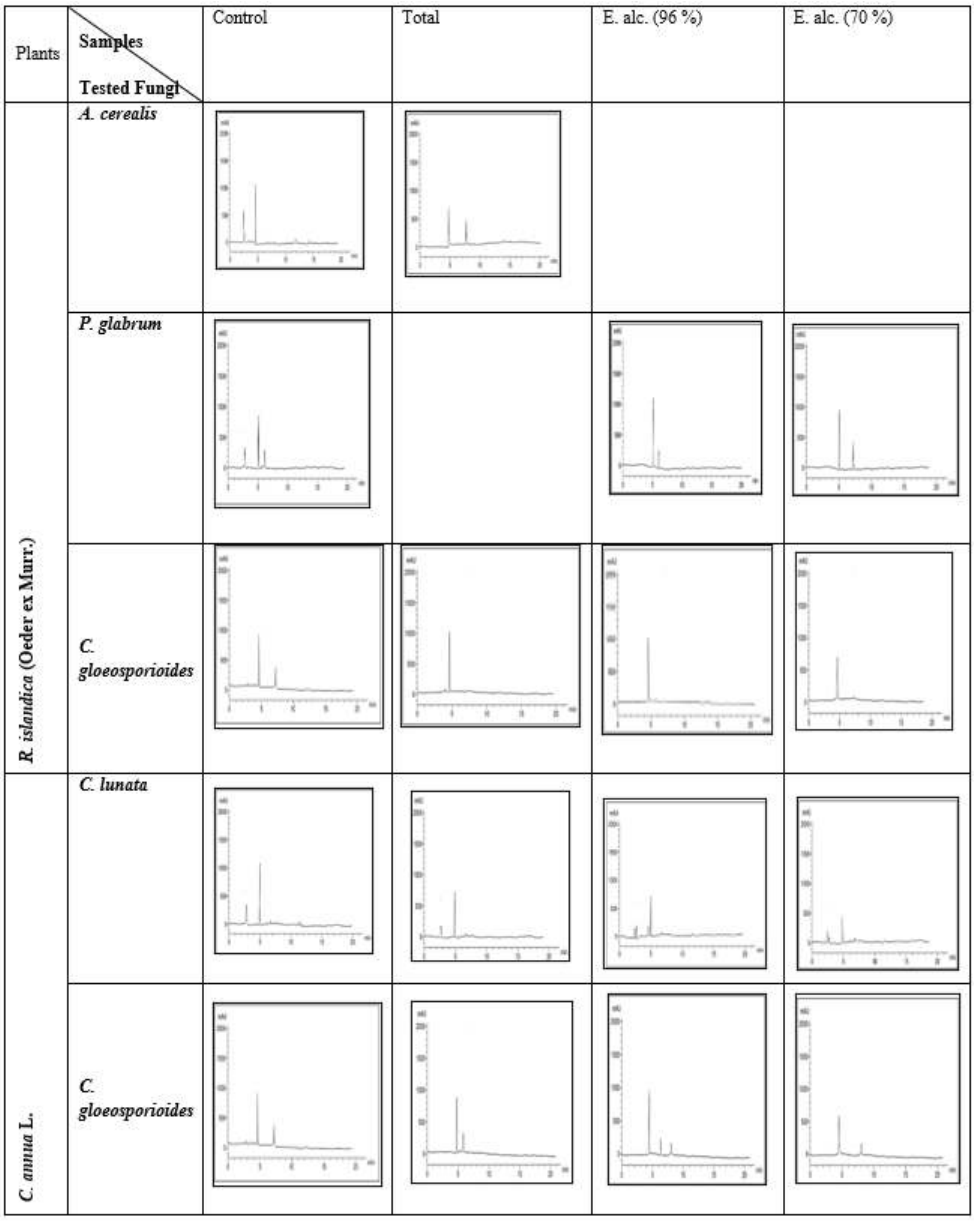


Figure 10

Charts of ergosterol content in tested fungal species and by total and ethyl alcohol (96% &70%) extracts of two plants under investigation

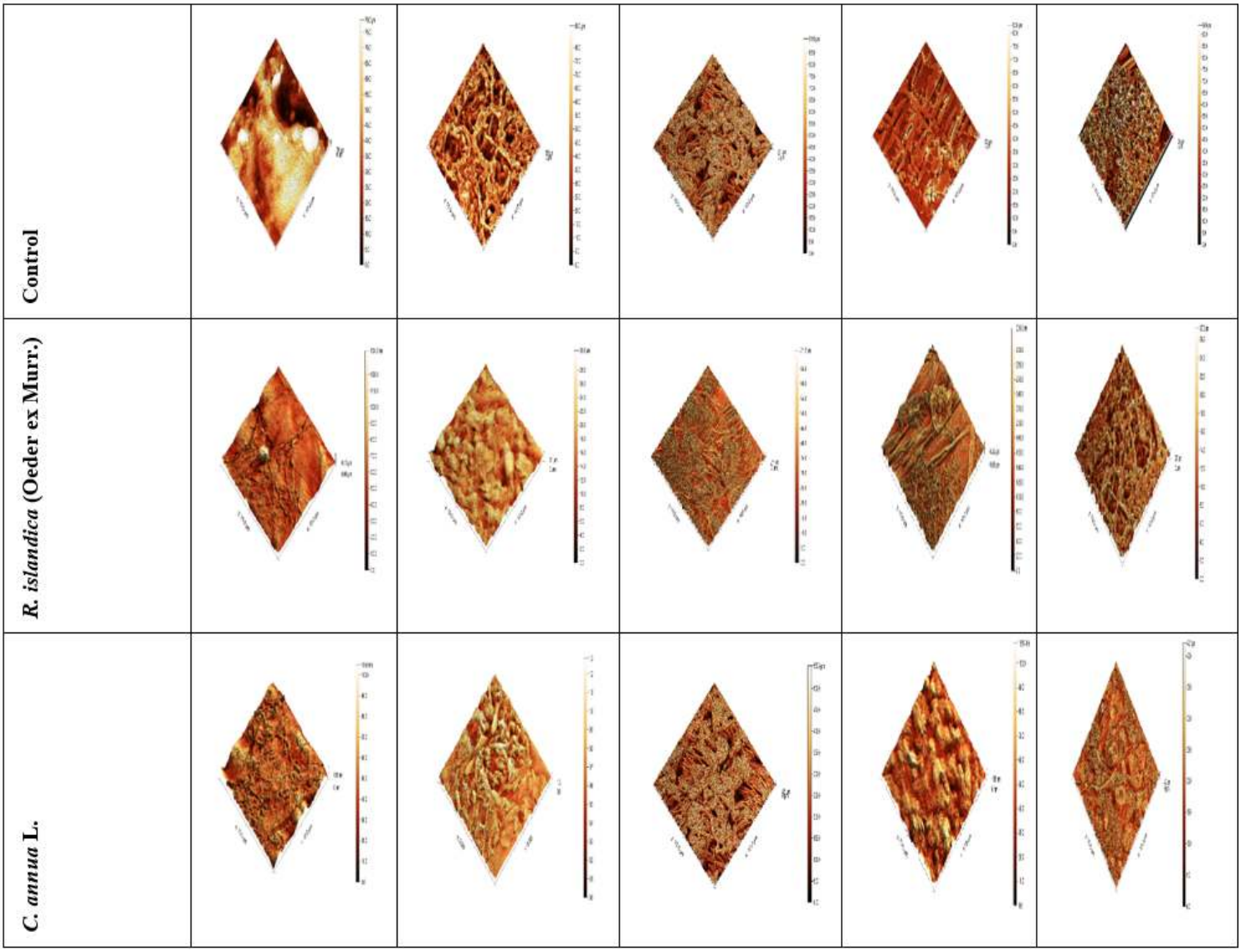


Figure 11

Topographic images of control and treated five tested fungi with total ethanol (70%) extracts of the two plants under investigation

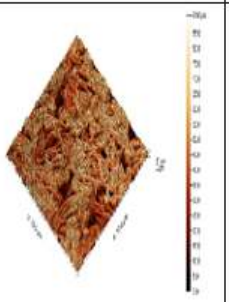
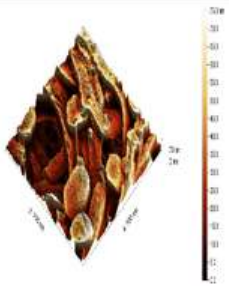
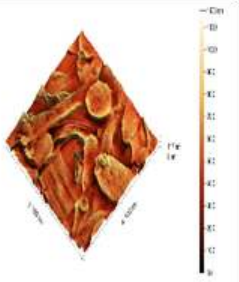
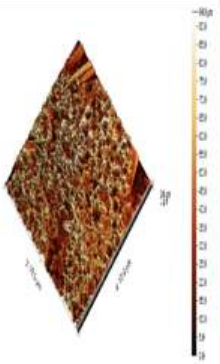
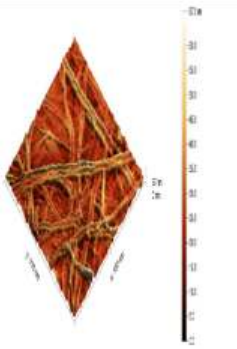
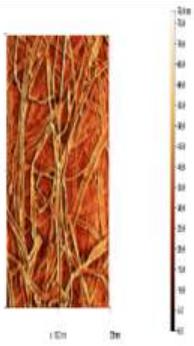
C. annua L.			
Strains	**Control**	**Ethyl alc.(96%)**	**Ethyl alc. (70%)**
C. lunata			
***R. islandica* (Oeder ex Murr.)**			
C. Gloeosporioides			

Figure 12

Topographic images of control and treated five tested fungi with successive extracts (ethyl alcohol 96%& ethyl alcohol 70%) of the two plants under investigation