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Detection of Chemical Compounds (Secondary Metabolites) of Alcoholic Extract of Cladosporium Cucumerinum using Gas Chromatogram-Mass Spectrometry GC-MS

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ABSTRACT

The current study aimed to detect the chemical content of the active metabolites of the alcoholic extract (ethyl ester) of Cladosporium cucumerinum (endophytic fungi) isolated from the leaves of the olive plant Olea Europa, where gas chromatographic analysis of the alcoholic extract of Cladosporium cucumerinum showed many chemical compounds, including 7 The largest percentage was the compound undecenoic acid ethyl ester 10- which recorded the highest percentage as it was 40.28%, followed by the compound Choroundecanoic acid, chloromethyl at a rate of 39. 38%, where these two compounds constitute the majority of the chemical content of the alcoholic extract of the mushroom Cladosporium cucumerinum, followed by the compound Ricinoleic acid Which recorded a ratio of 11.77%, then the compound Heptadecanoic acid, ethyl ester, its percentage was 2.24, the fifth compound that was diagnosed was Behenic acid (methyl,19-methyl-eicosanoate) and its rate was 2.71%, and Methyl,12-hydroxy-9-octadecenoate At a rate of 2.22%, and Nitromethane at a rate of 1.37%

Introduction

Endophytic fungi are present in all plants and live inside their tissues without causing disease symptoms or infection. These fungi and other organisms have received great attention because they possess many compounds of importance in various aspects, such as the agricultural aspect, as they act on weeds of plants, suppressing their growth, as well as increasing the growth of The plant and its development, providing protection to the plant from attack by insects and pathogens, and obtaining nutrients from the soil and transferring them to the host plant, as these effective functions are important for agriculture, so they have proven to be a better alternative than traditional agricultural practices. Thus, it significantly reduces the use of agricultural chemicals such as fertilizers, fungicides, insecticides, and herbicides (White et al., 2019) and also acts as nutrients in plant roots (Mehta et al., 2019).

Endogenous fungi are a source of many intracellular and extracellular compounds that are biologically active against pathogens, as they act as biological control agents for many pathogens (Poveda, 2021). (Nisa et al., 2020; Sharma et al., 2020).

Most endophytic fungi have secondary metabolites, as indicated by studies, which have biological activity, such as terpenoids, phenols, alkaloids, steroids, and others (dos Santos et al., 2015; Deshmukh et al., 2015).

Among the endophytic fungi that have been isolated is the Cladosporium fungus, which is a cystic fungus Ascomycota (Abdallahhzadeh et al, 2020). Most of the species are living shoots, but some species were found to be a symbiotic relationship within the plant, and some species have the ability to produce compounds of medical importance, and they are also considered Among the biological control agents for plants (Adorisio et al., 2019; Khan et al., 2016), one of the identified species belonging to the genus Cladosporium is C.cucumerinum, which is a symbiotic relationship within the plant, in addition to having the ability to produce chemical compounds with Bioactivity against pathogens (Jasim, 2022).

Materials and methods.

Isolation of C.cucumerinum-1

The fungus was isolated by collecting the leaves of the olive plant from the farms of the Ishaqi, the village of Al-Rumailat, for the period from August to December 15, 2022, where the method of Araújo and his group (2002) was followed in isolating the internal fungi by taking the healthy leaves of the olive plant and washing them with tap water to remove dust and in order to eradicate On the microorganisms on the surface of the leaves, the leaves were sterilized by immersing them in 70% ethanol for one minute, then in sodium hypochloride (2-2.5% chlorine) for 4 minutes, then in 70% ethanol for 30 seconds, and then washed three times with water. Sterilized distilled leaves were then cut into small pieces of 0.5 cm in size, then transferred to Petri dishes containing potato medium, hard dextrose containing anti-chloramphenicol. The dishes were incubated in the incubator at a temperature of 25-28 o C for a day and checked daily for 30 days.

Diagnosis of fungus-2

Phenotypic diagnosis 2-1

The phenotypic diagnosis was made by observing the growth of colonies on solid dextrose potato media and observing the phenotypic characteristics of the color, shape of the colonies, colony strength, and the size of the fungal colonies. The shape of the colony was also observed from the opposite side of the plate (Ellis et al., 2007; Watanabe, 2002).

2Microscopic diagnosis-2

Microscopically, a sample of the fungal colony was taken by means of a Loop field vector and placed on a microscope slide containing methylene blue dye, mixed well, then fixed by heat and examined under a microscope with a magnification of 10X and 40X to note the fungal hyphae (Ellis et al., 2007). The microscopic characteristics of the hyphae, the size and shape of the conidia, and the branching of the conidia were observed, depending on the diagnostic key (Watanabe, 2002).

3-Prepare the alcoholic extract

The alcoholic extract was prepared using (ethyl acetate):

The method of Riose and his group (1987), modified from the basic method of the researcher Verpoorte et al, 1982), was adopted in the preparation of alcoholic extracts by crushing (10) g of the fungal sample in (100) cm3 of (ethyl acetate) with a concentration of (95%) in a bath Ice water, then the mixture was shaken well with a Stirrer, and left in the refrigerator for (24) hours, then the mixture was filtered through layers of gauze in order to get rid of the organic solvent, and the mixture was placed in the Rotary Evaporator, which works on the basis of evaporation under rarefied pressure And a temperature not exceeding 40 C, then the solvent was evaporated from the mixture and a layer of the extract was formed, which was dried with an air-dryer at a temperature not exceeding 40 C to preserve the active ingredients of the extract, and the samples were then preserved by freezing until used in the study.

4- Analysis using the GC-Mass device to detect the chemical compounds contained in C.cucumerinum.

The sample was analyzed using a Japanese Shimadzu (GCMS-QP2010Plus) device, by taking 20 microliters of the sample, supplemented with 5 ml of ethanol, and adjusting the injector of the device to 2 microliters of diluted sample using a capillary column type (InertCap 1 is a non polar column bonded 100% dimethylpolysiloxane with a length of 30 meters and the carrier gas is helium with a flow rate of (30 ML/min), the thermal program started at 50 percent with a fractionation ratio of 2:1 and this temperature was maintained for 1 minute after which the temperature was raised at a rate of 11 m per minutes until reaching a temperature of 180 °C, then fixed for a minute with a total holding time of 12 minutes. The mass spectra were recorded with a range of 40-800 m/z and an applied voltage of 72 ev. The chemical compounds extracted from the studied samples were identified by comparing the resulting mass spectra with the mass spectra available in the libraries available in the device program. (Meyer, Markus, 2016). Note that the method is developed and not fixed and varies according to the nature of the sample components.

Results and discussion

The chemical compounds of the alcoholic extract (ethyl acetate) of the mushroom Cladosporium cucumernium were analyzed and separated by the GC-Ms technique. The mass of each compound is shown in the form and according to what is found in Table (1)

Through the results of the analysis and separation of the components or compounds of the alcoholic extract of the

fungus used in the current study, 70 chemical compounds were diagnosed, of which 7 recorded the highest percentage, as shown in Table (1)

Where the compound -10-undecenoic acid ethyl ester was the highest, with a rate of 40. 28%, followed by the compound Choroundecanoic acid, chloromethyl, with a rate of 39. 38%, as these two compounds constitute the largest percentage of the alcoholic extract of the fungus, and the compound Ricinoleic acid, which recorded a rate of 11.77%, then Heptadecanoic acid, ethyl ester, its percentage was 2.24%, followed by the fifth compound, Behenic acid (methyl,19-methyl-eicosanoate), and its percentage was 2.71%, and it is one of the biologically effective compounds as antibacterial and antifungal. This is what was proven (Al-Qahtani and his group, 2018) In his study on cress seed oil (Lepidium), which proved the effectiveness of cress seed oil as an antimicrobial (candida sp, Klepsilla, E.coli, St.areus), he attributed the vital activity to the main compounds, including behenic acid. And compound Methyl,12-hydroxy-9-octadecenoate by 2.22%, and compound Nitromethane by 1.37%.

A study conducted by Gopinath and his group (2013) showed that the peaks shown by the analysis curves using the Gas Chromatogram Combined Mass Spectrometry (GC-Ms) technique indicate the nature of the chemical compounds present in the extract of the studied sample, as this technique works to isolate the chemical compounds at different times. And then diagnose them through their chemical composition, molecular weight and chemical formula, as these compounds are diagnosed through the office data stored in the device, so this is the first step towards understanding the chemical nature of these compounds, as internal fungi, especially those coexisting with medicinal plants, contain A store of chemical compounds from secondary metabolites, and this was proven through the current study, as a large group of chemical compounds were isolated and identified in the alcoholic extract of the fungus C. cucmerinum, which was proven by a previous study conducted by (Jasim, 2022), as it was proven that this fungus contains A high percentage of alkaloids as well as phenols through the study of estimating the total content of alkaloids and phenols, which was proven by the current study, where 19 alkaloid compounds and 6 phenolic compounds were identified, but they were in low percentages.

In addition to the aforementioned compounds shown in Table (1), which constituted the largest percentage of the chemical content of the extract, which are likely to be biologically effective as a result of previous studies in which C.cucumerinum extract proved its biological activity against pathogens, as in the Yehia study. and his group, 2020), where he attributed the reason for the biological activity to the fungus containing active substances, And a study (Khan and his group, 2020), which proved that the Cladosporium extract has an antibacterial effect.

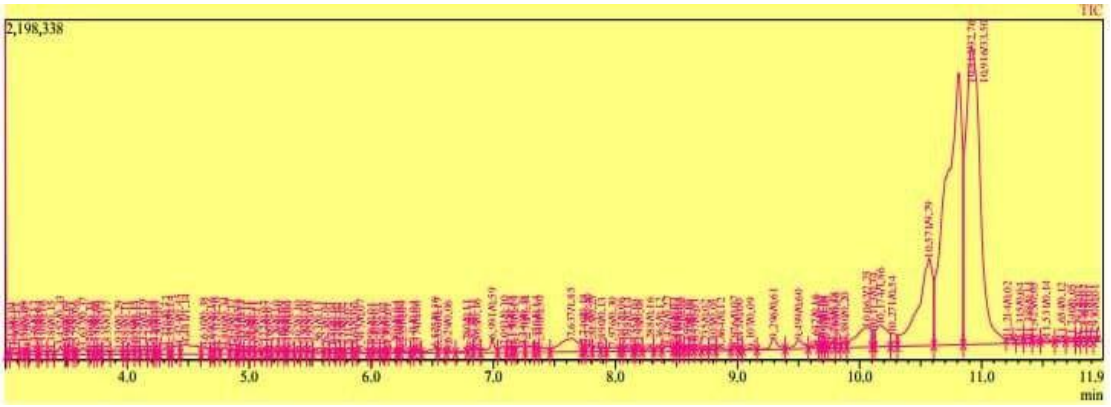

Figure (1): Chromatogram analysis of the model by GC-Ms

Table (1) shows the materials separated and identified in the model by GC-Ms

S	Rt detention time	Area%	molecular weight M.W	Molecular formula	compound name
1	4.48	1.37	61	CH3NO2	Nitromethane
2	7.63	2.22	312	C19H36O3	Methyl,12-hydroxy-9-octadecenoate
3	10.06	2.71	340	C22H44O2	Methyl,19-methyl-eicosanoate (behenic acid)
4	10.17	2.24	298	C19H38O2	Heptadecanoic acid,ethyl ester ()
5	10.57	11.77	298	C18H34 O3	Ricinoleic acid
6	10.81	39.38	268	C12H22CL2O2	Chloroundecanoic acid,cloromethtl ester
7	10.91	40.28	212	C13H2402	10-undecenoic acid,ethyl ester

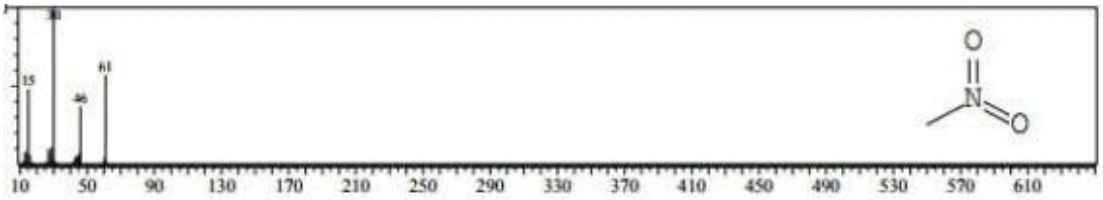

Figure (2): The mass spectrum of Nitromethane

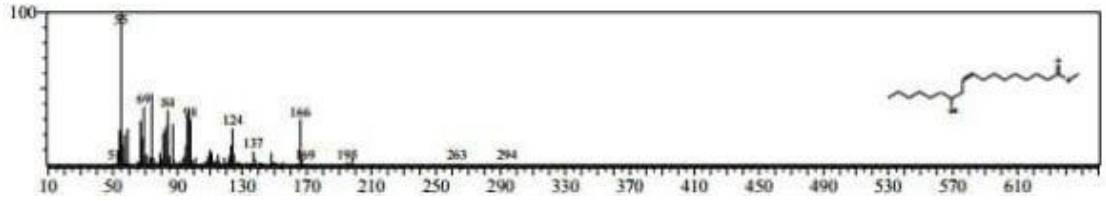

Figure 3: The mass spectrum of Methyl,12-hydroxy-9-octadecenoate

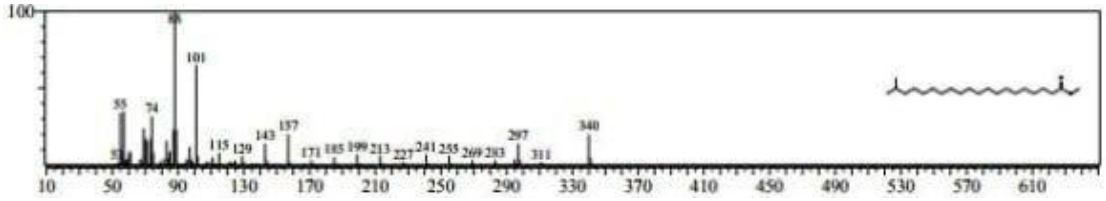

Figure (4): the mass spectrum of the compound Methyl,19-methyl-eicosanoate(behenic acid)

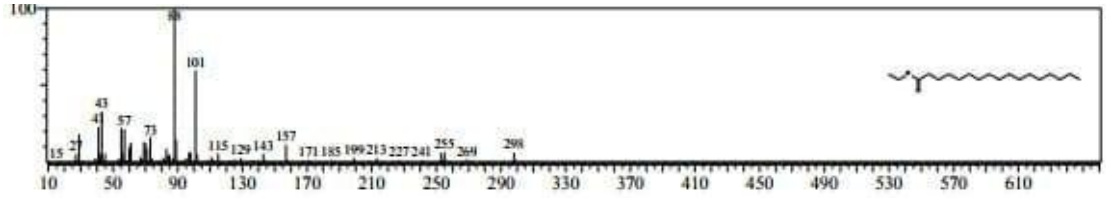

Figure (5): the mass spectrum of the compound Heptadecanoic acid, ethyl ester

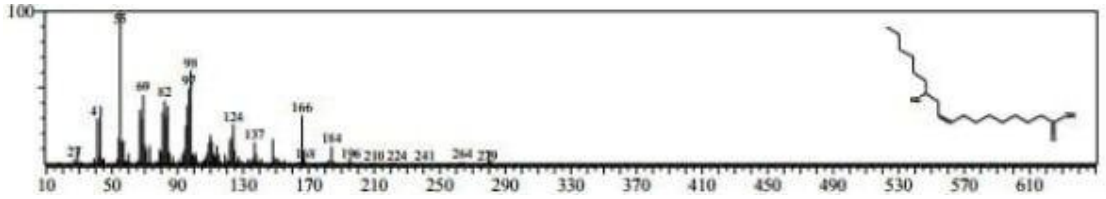

Figure (6): the mass spectrum of the compound Ricinoleic acid

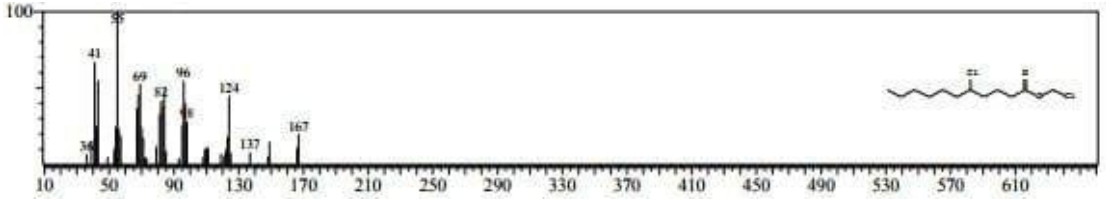

Figure (7): the mass spectrum of the compound Chloronedecanoic acid, chloromethtl ester

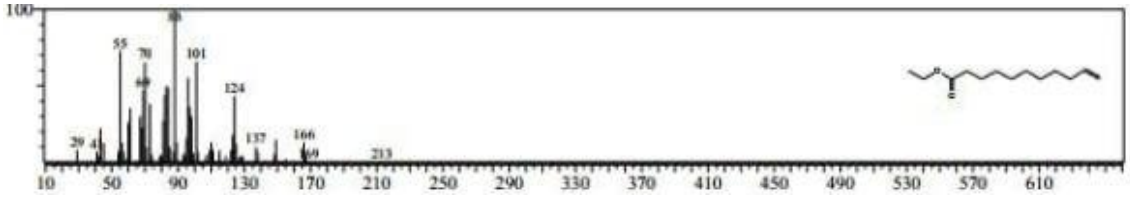

Figure (8): the mass spectrum of the compound -10-undecenoic acid, ethyl ester

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