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Research Article

Isolation and identification of phenolic compounds from *Eugenia caryophyllus* and study on its biological effect against *Macrophomina phaseolina*

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Abstract
Clove is one of the oldest and most famous spices. Its seeds resemble nails. It is used in medicinal fields, but its fungicidal activity is unknown. The aim of the study was to test the inhibitory activity of phenolic extracts of cloves against the pathogenic fungus *Macrophomina phaseolina.* The research was conducted under laboratory conditions to test the inhibitory ability of *Eugenia caryophyllus* phenolic extracts on the fungus *M. phaseolina*, which was partially diagnosed on strawberry plants based on Polymerase chain reaction (PCR) technique, in addition to its morphological and microscopic characteristics. The active compounds present The active compounds (Chlorogenic acid, Qurcetine, Gallic acid, Apigenin, Caffeic acid, Ferulic acid, Kaempferol, Rutin, Catechine) present in the extract of Industrial Methylated Spirit (IMS) separated from cloves were determined using High-performance liquid chromatography (HPLC). and active compounds of IMS phenols(Qurcetine**,** Apigenin**,** Ferulic acid**,** Kaempferol) **.**The inhibitory effect of phenols extracted from *E. caryophyllus* against *M. phaseolina* was tested. The results showed that the acetone extract and acetone phenols, which were used at concentrations of 10 and 15 mg/ml in PDA medium, had a significant effect on the growth of the fungus by 100%, as these fungi failed to grow. Formation of spores on Peptone dextrose medium (PDA) medium. While the percentage of inhibition of acetone phenols was 25% at a concentration of 5 mg/ml. As for the IMS extract and IMS phenols, it had a significant effect on the growth of the fungus at all concentrations used, but with different inhibition ratios, which increased directly with increasing concentration of the extract.

Keywords: *Eugenia caryophyllus* , Extract, High-performance liquid chromatography (HPLC) , *Microphobia phaseolina*, *Polymerase chain reaction* (*PCR*)

INTRODUCTION

Phenolic compounds are natural biologically active organic substances (Razem *et al*., 2022). They are produced in plants in part as a response to environmental and physiological stresses such as pathogens, insect attack, ultraviolet radiation, and plant wounding (Khoddami *et al*.,2013). Plant phenolic compounds are classified as simple phenols or polyphenols based on the number of phenolic units in the molecule. Thus, plant phenols include simple phenols, coumarins, lignins, lignans, phenolic acids and flavonoids (Soto-Vaca *et al*.,2012). Cloves are fragrant, dried, unopened flower buds of an evergreen tree 10-20 m high, belonging to the Myrtaceae family (Mashkor *et al*., 2015). In addition to the fact, the clove plant contains many effective compounds, including Vanillic acid Kaempferol (Bhowmik *et al*.,2012). It also contains many effective groups, including tannins, saponins, alkaloids and phenols (Kumar *et al*., 2012). Cloves are one of the main plant sources of phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids and hydroxyphenylpropenes. Eugenol is the main bioactive compound of cloves (Cortés-Rojas *et al*., 2014). Strawberry *Fragaria X ananassa Duch* belongs to the Rosacea family. It is a fruit with small fruits that are

widespread in regions of the world. Its fruits are characterized by their high nutritional value and good flavor because they contain many substances and nutrients in addition to vitamin C (Al–Handel *et al*., 2021). Strawberries are infected with many plant diseases, including root rot, which is caused by many soils' fungi *Fusarium oxysporum, Rhizoctonia fragariae, Pythium aphanidermatum, Phytophthora sp*, *Macrophomina phaseolina* (Al-Juboory *et al*., 2018). Soil-borne fungal pathogens play a major role in the root death of many important field and horticultural crops and often lead to the death of plants (Ehteshamul-Haque *et al*., 1993). Diseases of plant crops negatively affect the agricultural economy of many countries, depending on the severity of the diseases caused by the organism that causes the disease, and among the fungal pathogens transmitted through the soil is the fungus *M. phaseolina*. Infection of the roots with this fungus causes a reduction in the absorption of nutrients and results in rotting of the roots, which leads to the death of plants, as the fungus infects more than 500 species of plants (Dawar *et al*., 2008). The fungal extract is usually used in many studies, including on animals (Al-dulaimi and AL-Tarjuman, 2020), and plant and fungal extracts used to inhibit the growth of patgogenic bacteria and fungi instead of using the harmful chemicals (Al-dulaimi *et al*., 2020) The aim of the present study was to test the antifungal efficacy of clove flower bud extract against different pathogenic fungi *M. phaseolina*.

MATERIALS AND METHODS

The study's methods included two main directions: separation and identification of active plant compounds from the buds of cloves, isolation, and identification of fungi pathogenic to strawberry plants and partial identification of the fungus.

First trend: analysis of plant compounds

The flower buds of *Eugenia caryophyllus L.* were obtained from the local market in the city of Mosul in the spring 2022, packaged in polyethylene bags and transferred to the laboratory , classified by taxonomists (Sultan,2020).

Preparation of dry clove extracts

The extract was prepared using a continuous extraction apparatus using acetone solvent and Industrial Methylated Spirit(IMS) solvent according to the methodology of the researcher (Al-Daody,1998)

Separation and purification of phenols from *Eugenia caryophyllus* by acid hydrolysis

The process of acid hydrolysis was carried out according to (Herborne,) 1973 to separate phenols in their free forms from the sugar units associated with them, as the phenols are present inside the plant in the form of glycosides. 2 ml each of acetone and IMS extracts was taken separately and 50 ml of hydrochloric acid was added to it. A thermal sublimation was performed at 100 ºC for 1 hour. The solution was cooled in a separating funnel, 15 x 2 mL of ethyl acetate was added to it, and the top layer was isolated and dried with anhydrous magnesium sulfate. The bottom layer was discarded. Ethyl acetate was evaporated using an RVE device. Free phenols are kept in sterile sealed vials and stored in the refrigerator until used and diagnosed using HPLC (Sultan *et al*., 2020).

High Performance Liquid Chromatograph (HPLC)

The process of identifying the phenolic compounds separated from the flower buds of the clove plant in Baghdad was carried out after the acid hydrolysis of phenolics, using an HPLC device of the type Shimadzo, LC - 2010 AHT, with membrane filters of diameter 0.1 µm, wavelengths of 320 nm, the flow speed of 1 ml/min, and a mobile phase. (HO: Methanol) (15:85) As for the separating column, it was of the C18 type, with dimensions of 4 x 240 mm and at a temperature of 30° C, which was supplied by an American company, Macherey– Naged (Najm *et al*., 2022).

Second direction: Isolation and identification of pathogenic fungi of strawberry plants and molecular identification of the fungus

Isolation of pathogens associated with strawberry

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Isolation of pathogens associated with strawberry

The fungi accompanying the roots of strawberry were isolated, on which symptoms of infection were observed, such as poor growth, yellowing of the vegetative system, and rotting of the root system, as the infected samples were collected from some greenhouses from the College of Agriculture and Forestry / University of Mosul. The sample was taken in a closed bag to the laboratory to isolate the fungus. The roots were washed well with running water to remove dust and impurities. Then they were cut into small pieces (1-5.0 cm), sterilized with a sodium hypochlorite solution with a concentration of 1% NaOCl for two minutes, and washed. With sterile distilled water and dried using sterile filter paper, all pieces were transferred to a Petri dish containing antibiotic-added PDA medium and incubated at a temperature of 25 ± 2 °C for five days. The fungi were purified by transferring part of the end of the fungus colony to another plate containing the food medium (PDA) and it was done under the same conditions (Agrios,2005, Al -Ameri *et al*.,2018). *M. phaseolina* was isolated based on taxonomic keys (Ellis,1971, Leslie *et al*.,2006, Pitt *et al*.,2009).

Table 1. Nitrogen bases sequence with universal primers ITS1- ITS4

Primer	Sequence
Forward	TGAATCATCGACTCTTTGAACGC
Revers	TTTCTTTTCCTCCGCTTATTGATAT

Molecular diagnosis of an elected local isolate of *M. phaseolina*

After growing the mushroom isolates using the Single Spore Technique and obtaining a pure fungal culture emerging from a single spore, the purely grown mushroom isolates were used to extract deoxyribonucleic acid (DNA) isolated from strawberry fruit infected with the pathogenic fungus *M. Phaseolina* and conducted after This is a Polymerase Chain Reaction (PCR) test and a nucleotide sequencing reaction by sending pure and newly grown mushroom isolates to the biotechnology laboratories of the Scientific Research Office DNA / Mosul**.**

DNA extraction

DNA of *M.phaseolina* isolate was extracted using Genomic DNA mini Kit (Geneaid) according to the manufacturer's instructions.

Gel Electrophoresis

The DNA was carried over and detected , a 1% agarose gel was prepared,0.5 g of agarose powder was dissolved in (50) ml of X1 TBE and 3 microliters of red safe dye were added. That was done using a heat source with continuous stirring until it boils and left to cool to a temperature of (50-60) ˚C. Then the gel solution was poured into the tray of the relay device, after installing the wells comb at the edges of the gel, taking into account that the pouring was done quietly to avoid the formation of bubbles, and if they were formed, they are removed using a pipette, then the gel was left until it solidifies.Then the tray was placed in the electrophoresis basin containing an appropriate amount of X1 TBE solution, after which the comb was gently lifted. To prepare Migration samples (Khoddami et a., 2013) microliters of DNA sample mixed by (Al-dulaimi *et al*.,2020) microliters of loading solution. After that, the device was operated, and then the gel is photographed under ultraviolet light using a gel documentation device to be able to see the DNA bundles, as well as Polymerase Chain Reaction (PCR) reaction products (AL-TARJUM AN *et al*.,2017 ; Al-Tarjuman *et al*., 2020).

Polymerase Chain Reaction:

Eugenia program conditions are mentioned in Table 2.

Preparation of extra

Preparation of different concentrations of acetone and IMS extracts, acetone phenol extract and IMS phenol extract of *E. caryophyllus* to use them in studying their biological effects on *M. phaseolina* fungus was as

follows:

The stock solution of *E. caryophyllus* extract was prepared by (Mitscher) method using the dilution method by dissolving 4 g of the prepared extract in 100 ml organic solvent (DMSO). The following concentrations (5,10,15) mg/ml for each of *E. caryophyllus* extracts were prepared. It was kept in sterilized bottles in the refrigerator until use (Mitscher *et al*.,1972) .

Testing the inhibitory effect of *Eugenia caryophyllus* extracts on *M. phaseolina*

Concentrations of 5, 10 and 15 mg/ml were prepared for each of the studied plant extracts, and they were added to dishes containing PDA medium that were sterilized and cooled to a temperature of 50 °C, with three replicates for each extract, except for the comparison treatment, where sterile distilled water was added. Inoculated the center of each plate with a disc from the edge of a fresh culture of *M. phaseolina* with a diameter of 0.5 mm, the dishes were incubated at a temperature of 25 ± 2 °C, and after the growth of the fungus in the comparison treatment reached the edge of the dish, the average diameters of the fungal colonies were calculated by measuring the average of two perpendicular diameters, and the percentage of inhibition was calculated as in the equation(Lavanya *et al*., 2022).

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Testing the inhibitory effect of *Eugenia caryophyllus* extracts on *M. phaseolina*

Concentrations of 5, 10 and 15 mg/ml were prepared for each of the studied plant extracts, and they were

Table 2. Thermocycling program conditions

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	45 sec.	
3.	Annealing	55	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1

added to dishes containing PDA medium that were sterilized and cooled to a temperature of 50 °C, with three replicates for each extract, except for the comparison treatment, where sterile distilled water was added. Inoculated the center of each plate with a disc from the edge of a fresh culture of *M. phaseolina* with a diameter of 0.5 mm, the dishes were incubated at a temperature of 25 ± 2 °C, and after the growth of the fungus in the comparison treatment reached the edge of the dish, the average diameters of the fungal colonies were calculated by measuring the average of two perpendicular diameters, and the percentage of inhibition was calculated as in the equation(Lavanya *et al*., 2022)

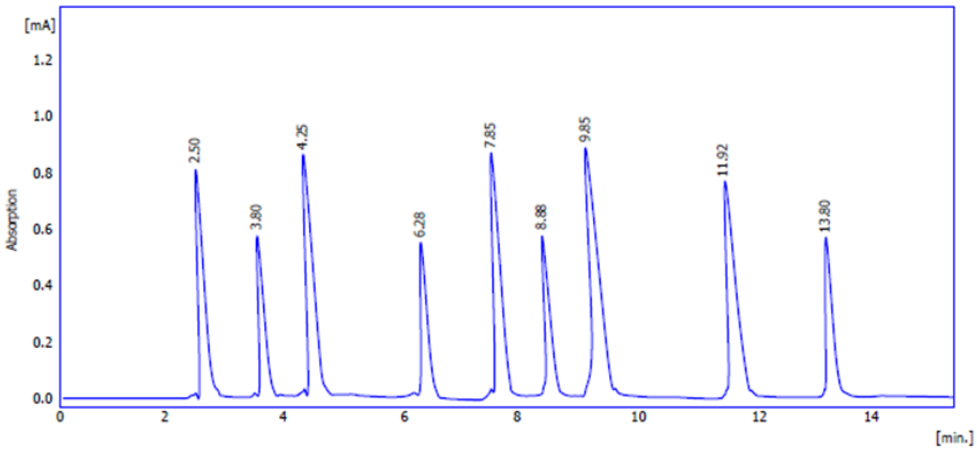
RESULTS AND DISCUSSION

Identification of phenolic compounds isolated from clove buds by HPLC technique

The results of the separation and characterization of IMS extracts are shown in Fig. 1 and Fig.2. After conducting the acid hydrolysis process using the HPLC device, the appearance of phenolic compounds, compared to standard compounds, were Chlorogenic acid, Qurcetine, Gallic acid Apigenin, Caffeic acid, Kaempferol, Ferulic acid, Rutin and Catechine. The diagnosis showed that standard curves drawn for each standard compound, coupled with a specific retention time, it became clear that the highest concentration was at gallic acid, with a retention time of 4.25/min, with a concentration of 31.25 mg/gm1-, followed by Qurcetine, with a retention time of 3.80/min, with a concentration of 20.66 mg/gm-1. And the compound Rutin, with a retention time of 11.92 / min, at a concentration of 19.58 mg/gm-1, while the concentrations of Kaempferol, Ferulic acid, Apigenin, Chlorogenic acid, and Catechine were in the ratios of 18.49, 16.22, 14.56, 12.56, 8.56) mg/gm-1 respectively with varying retention times of 8.88, 7.85, 6.28, 2.50, 13.80/min Caffeic acid appeared at the lowest concentration of 6.25 mg/gm-1 with a retention time of 7.85/min, as shown in Fig. 1. This is consistent with the study carried out by Hashim *et al*., (2013).

The results reached by the study showed that it contains the following compounds: Eugenol, Caffeic acid, Kaempferol, Vallinic acid, Ferulic acid, Ellagic acid, Chlorogenic acid, Borneal. These effective compounds were used to evaluate the inhibitory effect on the growth of two isolates of Gram-positive bacteria, *Bacillus subtilis* and *aureus Staphylococcus*, and three isolates of Gram-negative bacteria, *Escherichia coli, Pseudomonase aeruginosa, and Salmonella typhimurium*. It agrees with Yousif *et al*. (2023) who separated the phenolic compounds (Vanillic Acid, Kaempferol, Eugenol, Gallic Acid), where they used phenolic compounds extracted from cloves at a concentration of 20% to inhibit the pathogenic fungus *Botrytis cinerea* that was causing rotting of apple fruits, *B. cinerea*. It also agrees with Moradi *et al*. (2023), who found phenolic compounds (Ferulic acid, Rutin, Quercetin, Caffeic acid, kaempferol, and Gallic acid) and their use in the treatment of peritoneal adhesion in mice.

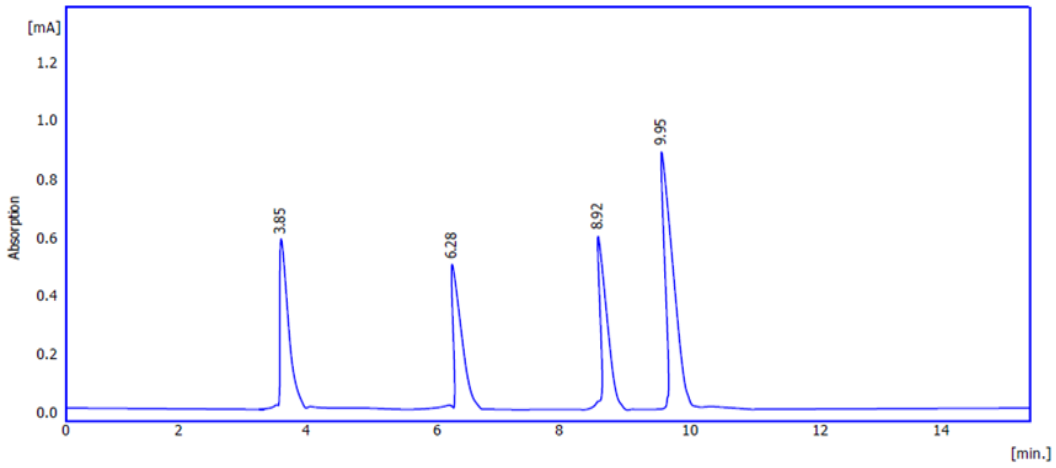
The other phenolic compounds were identified in the



Result chromatography Table (Uncal - F:\ IMS))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.50	3214.55	803.56	12.05	12.11	0.20	
2	3.80	2145.98	611.25	10.25	10.15	0.15	
3	4.25	4126.98	804.22	12.56	12.33	0.20	
4	6.28	2365.44	594.12	9.55	9.65	0.13	
5	7.85	4288.74	804.48	12.58	12.34	0.20	
6	8.88	5269.78	600.14	9.42	9.44	0.15	
7	9.85	6320.15	804.98	12.66	12.32	0.20	
8	11.92	3652.90	785.64	10.58	10.33	0.17	
9	13.80	2874.57	608.44	9.66	9.41	0.13	
	Total	34259.56	6416.59	100.00	100.00		

Fig. 1. *Curves of phenolic compounds extracted after the acid hydrolysis process IMS extract of flower buds of clove plant using HPLC technology*



Result chromatography Table (Uncal - F:\ Acetone))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.85	3524.49	601.25	24.56	24.88	0.15	
2	6.28	2644.89	580.46	22.15	22.23	0.15	
3	8.92	3652.16	603.56	23.25	23.65	0.15	
4	9.95	6521.08	800.14	29.99	29.85	0.25	
	Total	16342.15	2585.41	100.00	100.00		

Fig. 2. *Curves of phenolic compounds extracted after the acid hydrolysis process of acetone extract from the flower buds of cloves using HPLC technology*

extract of acetone phenols, namely: Qurcetine, Apigenin, Kaempferol, Ferulic acid. The result of the diagnosis showed that standard curves drawn for each standard compound, coupled with a specific retention time, it became clear that the highest concentration was at Qurcetine, with a retention time of 3.85/min, at a concentration of 12.58 mg/gm-1. It was followed by Ferulic acid, with a retention time of 9.95/min at a concentration of 11.25 mg/min. Grief 1-. And the compound Kaempferol with a retention time of 8.92 / min at a concentration of 10.44 mg / g-1. Apigenin appeared at a low concentration of 6.59 mg/gm-1 and a retention time of 6.28/min, as shown in Fig. 2 .The results of Jimoh *et al*. (2017) showed similar phenolic compounds (Kaempferol, Chlorogenic, Ferulic acid, Apigenin, Rutin, Gallic acid) to the current study. It also agrees with El-Saber *et al*. (2020), who found phenolic compounds viz. Gallic acid, Vanillin, Oleanolic acid, Quercetin, Crategolic acid, Kaempferol.

Isolation and identification of pathogenic fungi of strawberry plant:

The results of isolation and identification of the roots of strawberry plants, which showed symptoms of yellowing and rotting, showed the presence of the fungus *M. phaseolina*, as shown in Fig. 3. These results agreed with previous studies in terms of isolation and diagnosis (Al–Handel *et al*.,2021; Al-Ameri *et al*.,2018).

Molecular identification of *M. Phaseolina*

Extraction of DNA: The fungal isolate was grown using a single spore technique on the medium water agar. DNA of *M. Phaseolina* extracted by Genomic DNA mini-Kit (Geneaid) and the isolated genomic DNA of *M. Phaseolina* examined by running it through an agarose gel electrophoresis revealed well separated chromosomal DNA bands.

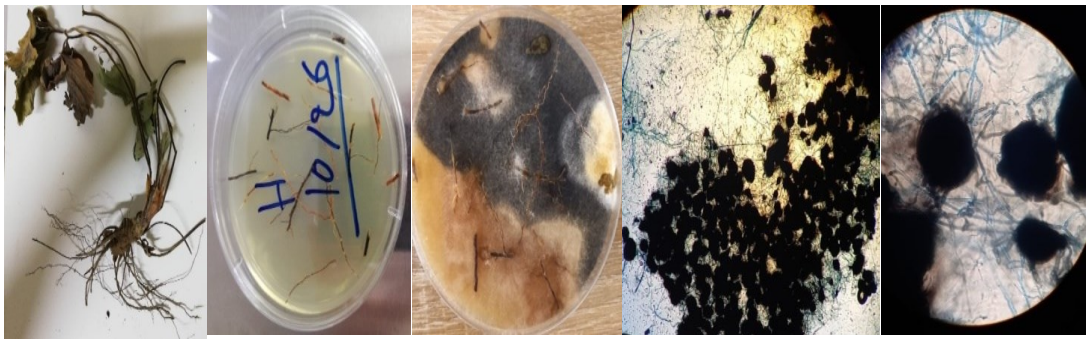
Amplifying the ITS region using PCR

Primers ITS1(5- TCCGTAGGTGA ACCTG CGG-3) and ITS4 (5- TCCTCCGCTTATTGATATGC-3) were used to symmetrically amplify the ITS1, ITS2, and 5.8S gene target regions in purified DNA. The resulting PCR products separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and viewed under UV light. 615 bp are shown by bands (Fig. 4). These findings are in complete agreement with those of a morphologically based, preliminary identification of Macrophomina isolates. In a similar study, Sánchez *et al*. (2017) diagnosed the *M. phaseolina* molecularly that was isolated from strawberry crown and roots

Nucleotide sequence of the selected fungal isolate

After PCR reaction was completed and amplifying the desired target was amplified, using a certain extraction kit, from the agarose gel the DNA bundle was cut and put in an Eppendorf tube, and the forward primer and deionized water were added to it. It was sent to ZYMO company in America to detect its nucleotide sequence. The results came as a fasta file after four weeks, as shown in Fig. 5.

The nucleotide sequences were deposited directly in the Gene Bank, which includes a comprehensive and free publicly available Database, which is provided with footnotes of references and vital information, which continues to grow exponentially, and the size of its da-



(A) Symptoms of root rot in strawberry plants caused by *M.phaseolina* infection

(B) Roots of infected strawberry plant *M.phaseolina* on potato and dextrose agar

(C) Appearance of *M.phaseolina* on infected roots

(D) *M. phaseolina* isolated from roots of infected strawberry plants at 10X magnification.

(F) *M. phaseolina* isolated from roots of infected strawberry plants at 40X magnification

Fig. 3. *Isolation and identification of M. phaseolina*

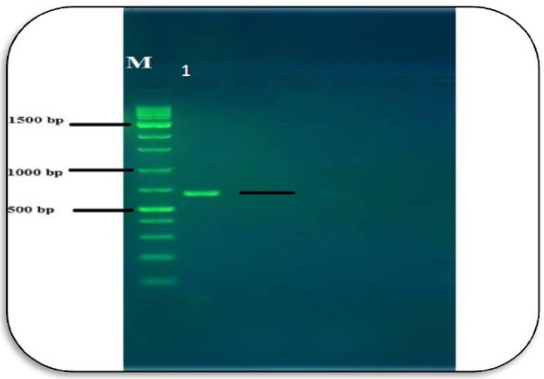


Fig. 4. *DNA amplification product of selected local fungal isolates on agarose gel, if M represents: volumetric marker pathway, 1: DNA bundle*

tabase increases with new species every day. A group of programs available on the NCBI website http://www.ncbi.nlm.nih.gov/blast provide searches for areas of identity or similarity between the entered sequences and the sequences deposited in the gene bank and then compare them to infer the identity of the living organisms as shown in Fig. 6 .. The outputs of the line-up process showed that the nucleotide sequence of the

ATAACTCGTAATTATGTCGGTGCTCGCGCTGAGGGATCGATTCCTGAGGTTACCTACTCATCGAGCCTGT
GAACATACCTATCATGTTGCCTTGGCGGGAACATTCGGCCCCGTAACACGGGCCGCCCCCGCCATTTTGG
GGGGTGGGTAGTGCCCGCCAGGGGTCTACTCATGTTTCAGTCAGTAAATGTTTCAGTTCTGAGTAAACAT
ATTAAATAAATTATATCTTTCATCAGCGGATGTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGACCGCACATTGCGCCCGCTTGGTA
TTCTGGGGGGCATGCCTGTTCGAGCGTCATTTCACCCCTCAGGCCCCTGTGCTTGGTGTTGGGGATCGGC
GGAAGCCGTCCTCTGCGGGCACAACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGC
GTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCAACTTCTGAATGTTG
ACCTCGAATCAGGTAGGAATGCCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

Fig. 5. *Nucleotide sequences of the selected local isolate M. Phaseolina*

selected local fungal isolate belonged to the type *M. phaseolina*, which showed a 100% match with the submitted data of the nucleotide sequences of the reference strain from Spain under registration number OQ672364. under the registration number OQ152623.

Genetic affinity tree

Based on the nucleotide sequence data, the Mega program version 7, available free of charge on the NCBI website, was used to draw the dendogram ,In this regard, the genetic affinity tree was divided based on the results of the nucleotide sequence of the local isolate of the fungus *M. phaseolina,* as shown in Fig. 7. The genetic tree was like what the researcher did for *M. phaseolina* (Sánchez *et al.*,2017).

The percentage of correspondence between *M. Phaseolina* isolates and isolates registered in NCBI

Table 3. Percentage of correspondence between *M. Phaseolina* isolates and isolates registered in NCBI

NO	Accession No.	Country	Source	Compatibility
1	LC764442	Iraq	Macrophomina phaseolina	100 %
2	OM760785	India	Macrophomina phaseolina	99 %
3	OQ672364	Spain	Macrophomina phaseolina	100 %
4	OQ324917	Hungary	Macrophomina phaseolina	98 %
5	OQ152623	Ghana	Macrophomina phaseolina	100 %
6	OP955944	Turkey	Macrophomina phaseolina	98 %
7	ON428214	China	Macrophomina phaseolina	99 %

Fig. 6. *Nucleotide sequences of the reference isolate installed in the GenBank with serial number LC764442.1 Which shows the sites of covariance with the selected local isolate Macrophomina phaseolina*

mentioned in Table 3. Not much is known about the evolutionary relationships of fungi due to several factors, perhaps the most prominent of which is the lack of an adequate record of fossils and fungal diversity. Fungi also include a complex diversity and differ in their ways of living, in addition to the presence of groups of organisms that worked as fungi for a long time due to the similarity of 106 their reproductive overlays and their environmental behavior, so The completion of drawing the evolutionary tree of fungi still needs in-depth research efforts that parallel the expanded taxonomic data.

Previous research (Al-Tarjuman *et al*.,2020; Sánchez *et al.*,2017) indicated that the PCR technique and the analysis of DNA amplification products play a major role in diagnosing different types of fungi with high accuracy, especially those with close phenotypic affinity, in addition to their effective contribution to detecting fungi of medical and industrial importance.

Testing the inhibitory effect of *Eugenia caryophyllus* phenolic extracts against *Macrophomina phaseolina* LC764442

The results shown in Table 4 and Fig. 8 indicated that the acetone extract had a significant effect on the growth rate of the fungus, where the inhibition percentage was 100% for all concentrations used (10, 15, 5) mg/ml .Also, the extract of acetone phenols had a significant effect on the growth of the fungus at all concentrations used, where the percentage of inhibition was 25% at the concentration of 5 mg/ml, while it reached 100% at the concentrations of 10 and 15 mg/ml. The reason is likely that Qurcetine, Apigenin, Kaempferol, and Ferulic acid worked together in their inhibitory action against the fungus *M.phaseolina* under our study compared to the control treatment.

As for the IMS extract significantly affected the fungus growth at all the concentrations used, but with different inhibition rates, reaching (62.5, 87.5, and 100 %)at concentrations (5, 10, and 15 mg/ml). As for the extract of IMS phenols, it did not significantly affect the growth of the fungus at a concentration of 5 mg/ml, where the percentage of inhibition of fungal growth was 12.5%, but in the two concentrations of 10 and 15 mg/ml, the effect was significant with inhibition rates of 62.5 and 100%, respectively. This may be due to the action of the separated phenols, which are Chlorogenic acid, Quercetin, Gallic acid, Caffeic acid, Kaempferol, Ferulic acid, and Rutin, which work together with a higher inhibitory effect than if they were with other effective

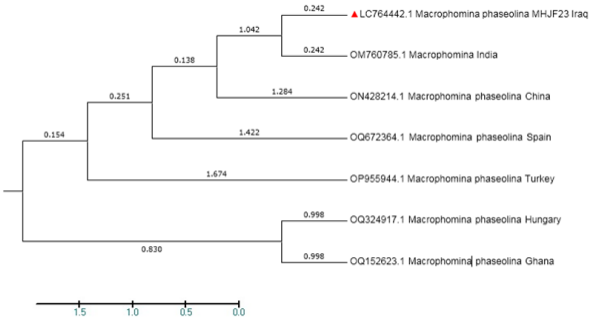


Fig. 7. *Genetic tree showing the genetic relationship between M. phaseolina isolate in this study (marked in red) and global isolates belonging to the same genus*

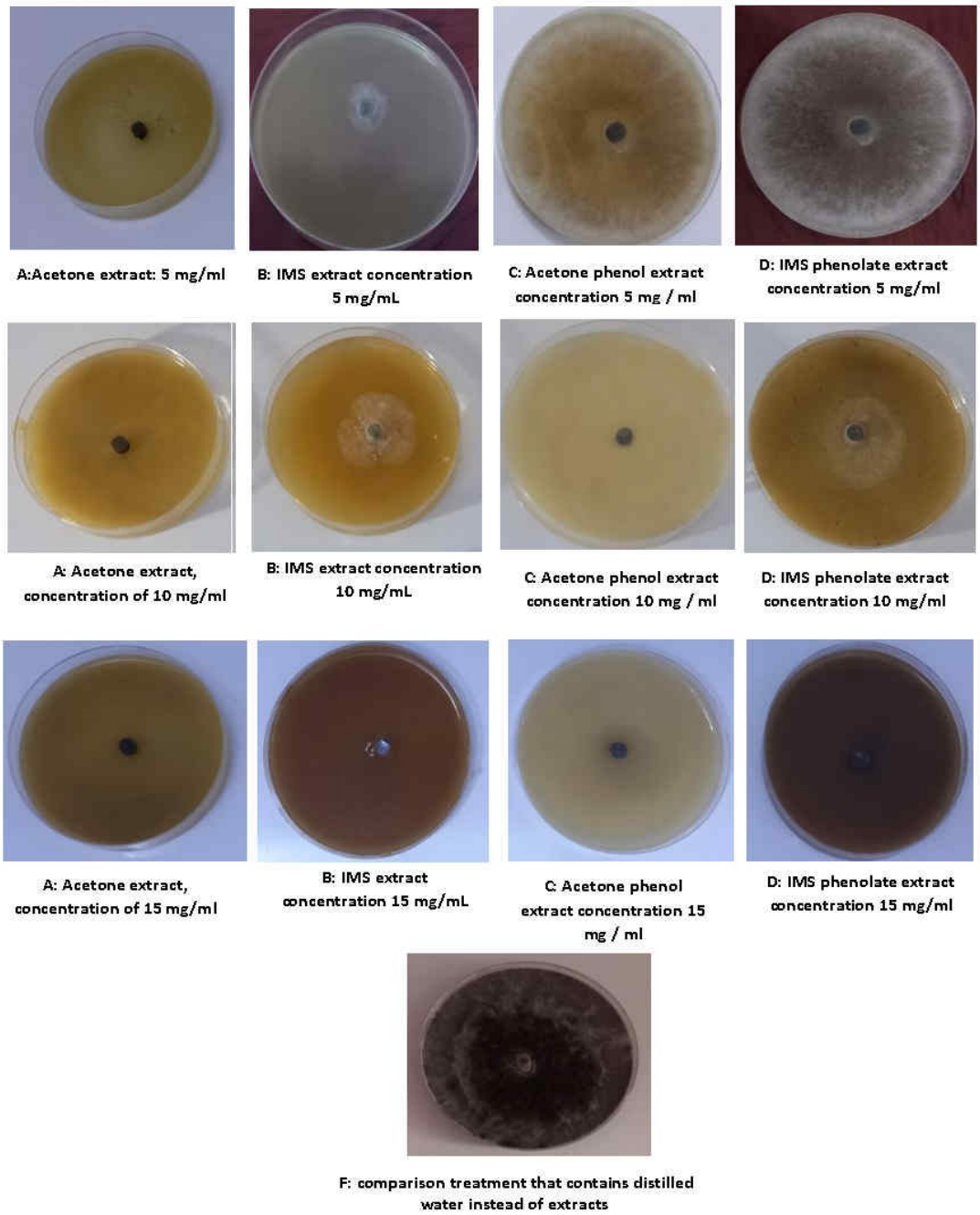


Fig.8. A-F. *Effect of types of clove extracts on the growth of Macrophomina phaseolina*

compounds in the same extract, which may inhibit growth by inhibiting the effectiveness of the protease enzyme by destroying the cell wall and cytoplasm (Andrade *et al*.,2006). This result agreed with the researcher (Abid,2009) where the alcoholic extract was used to inhibit types of pathogenic bacteria**.**

The wide range of effects of acetone and IMS extracts of cloves may be attributed to their phenolic contents, as Shoko *et al*. (1999) explained that phenols are the most important compounds that inhibit fungi, including Gallic acid and Apigenin, which is known to be the most inhibitive towards fungi and microorganisms .Phenols have an inhibitory ability towards fungi, as they can interact with cell membrane proteins, which causes a change in the membrane's permeability and, thus a defect in the respiratory activities within the fungal hyphae. For this reason, the high inhibitory activity appeared towards the fungi under our study compared to the comparison treatment (Jafeer *et al*., 2016).

The phenol extract of the clove plant contained Gallic acid, which had anti-activity through its effect on DNA replication. It showed the growth of *M.phaseolina* fungus by stopping the DNA strand synthesis process by inhibiting the activity of the DNA gyrase enzyme of the

Table 4. Effect of Eugenia caryophyllus phenolic extracts on the growth of *M. phaseolina*

Concentrations (mg/ml)	Comparison (water sterile distilled)	IMS phenolate extract	Acetone phenol extract	IMS extract	Acetone extract	Concentration effect
	Average diameter of fungal colonies in the treatment (cm)					
5	8a	7b	6c	3d	0f	5a
10	8ab	3d	0f	1e	0f	2b
15	8a	0f	0f	0f	0f	2c
extract effect	8a	3b	2c	1d	0 *e	

*Values are the average of 3 replicates. in each column the same letter mean the values are not significantly different in Least Significant Difference between the means at the required level of probability (L.S.D. at (P ≤.25)).

fungus, which inhibits the supercoiling of the fungal DNA strands(Takó *et al*., 2020). The phenolic extract *of flower buds of clove plant* also contains Caffeic acid, Kfaempferol, which has anti-fungal activity, *M.phaseolina* (Hashim *et al*., 2013)**.**

Conclusion

The results of this investigation showed that *E. caryophyllus* contains a lot of phenolic chemicals such as Chlorogenic acid, Qurcetine, Gallic acid Apigenin, Caffeic acid, Kaempferol, Ferulic acid, Rutin and Catechine , also The acetone solvent was better at withdrawing phenolic compounds from the plant than the ethanol solvent because it is a semi-polar solvent and it gave a higher inhibitory effect against the fungus *M. phaseolina* at all the concentrations used, but with different inhibition rates, as the inhibitory ability of these extracts increased directly with the increase in the concentration of the extract .Among the extracted phenols, Ellagic acid had an antimicrobial activity through its effect on DNA replication, like the action of broad-spectrum anticonvulsants

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