Effect of Alcoholic Extract from Three Plants that is Rich of Flavonoids on two Virulence Factors in E. coli

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Abstract
In this study, the flavonoid content was estimated by HPLC for Vitis vinifera, zingiber officinale and date palm phoenix dactylifera after alcoholic extracts (Ethanolic extracts) and exposure of Escherichia coli that were isolated in 30 Covid-19 patients. From the medical isolation unit in Salah al-Din Hospital, Tikrit city, Republic of Iraq, bacteria were exposed to plant extracts containing flavonoids at different concentrations and the minimum inhibitory concentrations (MIC) were estimated. Inhibition zones were observed at all concentrations, and then the polymerase chain reaction (PCR) was performed. On two of the most important genes that encode two of the virulence factors in E.coli bacteria, the fimA gene and the PgaD gene, and this procedure showed clear effects on the level of the mentioned genes through PCR technology, which opens the door to the possibility of wider utilization and the development of effective compounds And its exploitation from various plants with a medical reputation known historically "in light of the health difficulties we are going through due to a pandemic Corona virus.

Keywords: Zingiber officinale, Vitis vinifera, phoenix dactylifera flavonoids, E.coli, PCr, PgaD, FimA

Introduction

Escherichia coli, or the so-called colon bacteria, is one of the most effective types of gram-positive bacteria and the most widespread. It is found in different foods, with humans, animals, and various places in the environment. In most cases, these bacteria are harmless, but in some cases they are harmless. The strains of them can cause diseases either directly or opportunistically accompanying a specific disease (Wolfensberger et al., 2019).

E coi bacteria can cause pneumonia or urinary tract infection and other types that may cause diarrhea, especially in children (Bin et al., 2018). Often these bacteria are one of the types involved in infection associated with many diseases that directly affect human health, which worsens the situation Pathogenesis and its exacerbation (Wu et al., 2020; Zhu et al., 2020) The basic symptoms of SARS-COV-2 can be accompanied by a group of non-specific symptoms, which causes many cases of direct misdiagnosis of the disease, which leads to the wrong course of treatment, not to mention the additional effects on the patient's health. Due to the treatments and their interactions, suspicion and doubt in identifying clinical cases leads many doctors to believe in the necessity of conducting laboratory microbial isolation tests and diagnosis for the possibility of the presence of types of accompanying infections in order to cut doubt with certainty and make confirmation as fully as possible (Moore Parma et al., 2020) Many studies have been clearly established and approved It is necessary to study plants with potential medicinal effect as multiple anti-factors for many pathogens due to the effective content they have of secondary metabolites and to highlight their true value in this field and The need to identify their content of natural active compounds and the investigations and concentrations necessary for their appropriate use (Rios & Recio, 2005) Antibiotic resistance is a major global problem that opened up prospects for seeking to develop alternative or new therapeutic agents due to the urgent need for it (Makabenta et al., 2021) Flavonoids are among the most important active active compounds Towards many pathogens, and it is one of the polyphenolic compounds that are produced as a result of secondary metabolism in most plants and herbs, fruits, vegetables and large trees (Maleki et al., 2019) The effective activity of flavonoids compounds has been recorded in more than one study that it can be exploited as an antidote to the activity of wide types of bacteria from Through its virulence factors and that it can interfere with the inhibitory action in a synergistic manner with each other or with other chemical compounds or with antibiotics (Cushnie & Lamb, 2011) the presence of different concentrations of flavonoid compounds has been recorded in a very large group of plants such as Zingiber officinale (Ghasemzadeh et al., 2010) and grapes vitis vinfera (Braidot et al., 2008) and date palm (phoenix

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dactylifer) (Tahvilzadeh et al., 2016), which have proven their biological efficacy in many diseases such as Cancer, diabetes mellitus, blood pressure, antioxidants, antibacterials, antivirals, nervous tonics and immune enhancers. These uses and developments came throughout history to the present. Successive studies are still trying day after day to discover many components of medicinal and pharmaceutical active substances in these three plants and others tyle-1-fimbriae is considered one of the most important (virulence factors)(Shanmugapriya & Patwardhau, 2012).

in several strains of E.coli, which are thread-like polymer proteins whose presence is closely related to pathogenicity by increasing the adhesion ability of bacteria (Klemm, 1985). The FimA gene is the main adhesion subunit gene of Type 1 fimbriae (Mainil, 2013); While the pgaD gene is one of the main genes involved in the synthesis of Poly-beta-1,6-N-acetyl-D-glucosamine (PEA), the main protein to complete the process of formation and assembly of the protozoans of EColi bacteria on target cells and tissues (Cong et al., 2022).

Many experiments based on the reaction and sequencing (PCR) delights have been developed in the detection of many different pathogens from water, food or the surrounding environment and clinical samples. Especially that the quick and direct diagnosis of clinically isolated samples from patients is necessary and important in determining the appropriate therapeutic mechanism (Masuda et al.m 1995). The Polymerase chain reaction (PCR) technology is an important technique in detecting the genetic sequence of pathogens to determine the types of microbes as quickly as possible (Khan & Cerniglia, 1994).

Materials and Methods

Plant samples

The desired purpose of the plant sample is to determine what is required and which plant part is optimal, for example: Pathological samples for HPLC examination:

The fruits of Vitis vinfera, zingiber officinale and phoenix dactylifera were obtained from the local markets in the city of Tikrit in Iraq. They were cut and dried in a room with low light at a temperature ranging between 25 - 35 degrees Celsius, after which they were finely ground and their alcoholic extracts were made. According to the methods used in the source (Azwanida, 2015) and then the Hblc technique to examine the content of flavonoid compounds according to (Jone, 1995) for each sample separately and compare it with the standard method. Isolation and identification of *Escherichia coli.*

For the period from December 2021 to March 2022 and among 30 clinical samples collected from the disease infected with Covid 19 lying in the isolation unit / Salah Al-Din General Hospital in Tikrit - Iraq by Swabs from the region of the pharynx and sputum and incubated at a temperature of 37 C for a period of 24 hours The bacteria were identified according to their microscopic and biochemical examinations, in addition to confirming the diagnosis on the vitek2 compact system.

Isolation of Genomic DNA

The (DNA) of E. coli bacteria was isolated before and after exposure to the plant extracts according to the method (Chan & Kuo, 1993) with some modifications. After the extraction process was completed, we determined the integrity and purity of the DNA by using agarose gel using electrophoresis technique using Red safe dye.

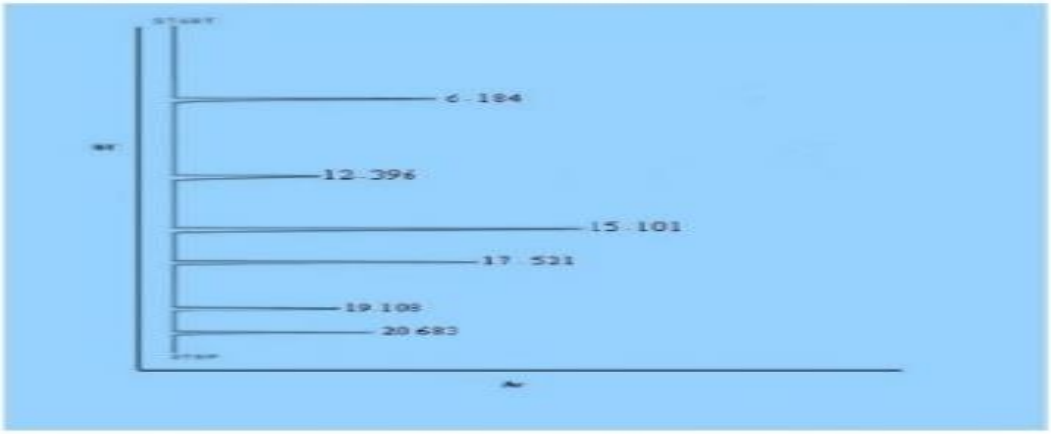
PCR analysis

The (PCR) technique was done using two specialized primers as shown in (accupower PCRremix) supplied by the Korean company Bioneer was used according to the accompanying instructions to complete the PCR reaction, where 50 ng (ng) of Forword and reverse primers were added to each hole and using the PCR device from Applied Biosystems by the following program Which was used in the amplification process: for the first denaturation process, a temperature of 95 was used for a period of (4) minutes, followed by (35) cycles, each with a temperature of 95 for a period of (30) seconds, and (58) degrees for a period of (45) seconds for the (annealing) process) and (72) degrees of heat for (45) seconds for the extension process and for the process of Final extension temperature was (72) at (72) for (5) minutes. The PCR output was analyzed after completion of the reaction to know the results by 2% agarose in electrophoresis technique immersed in 1X solution of SB (SB buffer) at 5 V Filmed with a Samsung mobile camera due to a malfunction of the gel documentation system in our lab, and as a molecular marker for measurement and then using a DNA ladder from (Biolabs) of 1500bp, which was measured by the packets that appeared or disappeared compared to the standard package control package.

No.	Primer	Sequence	Size pb
1.	FimA	F: AACAGCGATGATTTCCAGTTTG R: ATTGCGTACCAGCATTAGCAAT	465b
2.	PgaD	F: ATTACGACCCGACAATCACC R: AGTGTACGCTCATCCTGTGG	340bp

Results

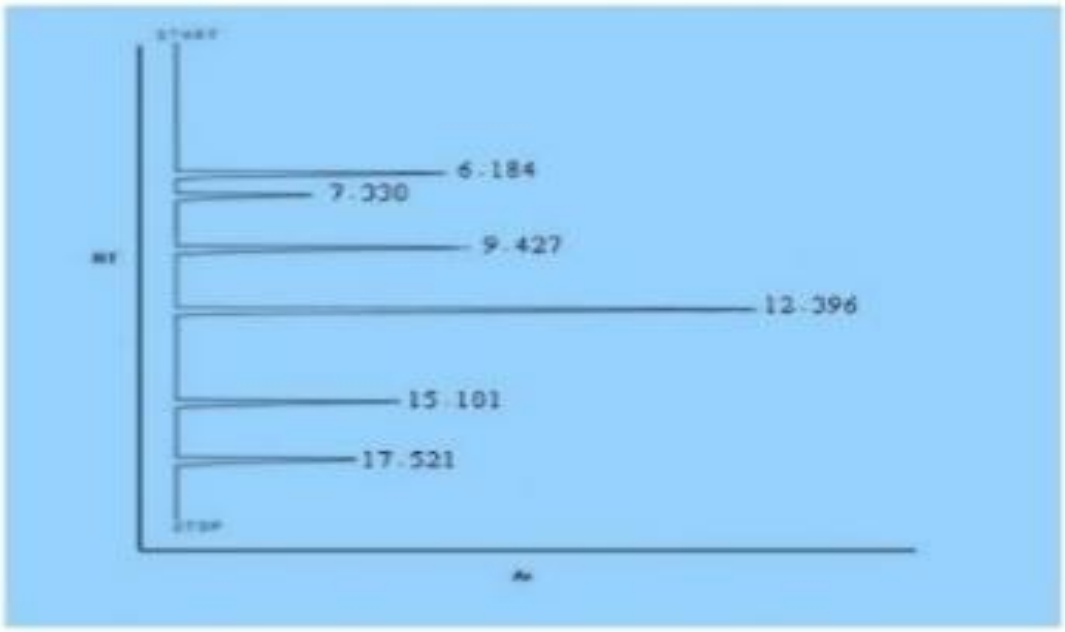
The results of HPLC for alcoholic extracts of Z.officinale, V.vinifera, and P.dactylifera showed a diversity in the concentrations of the content of flavonoids in each of them, according to what is shown with the image of each examination for the analysis of samples separately:



phoenix dactylifera

SAMPLE 3
CHROMATOPAC C-R10A
SAMPLE NO 3 FILE AWS A.A.
REPORT NO 4 METHOD 8

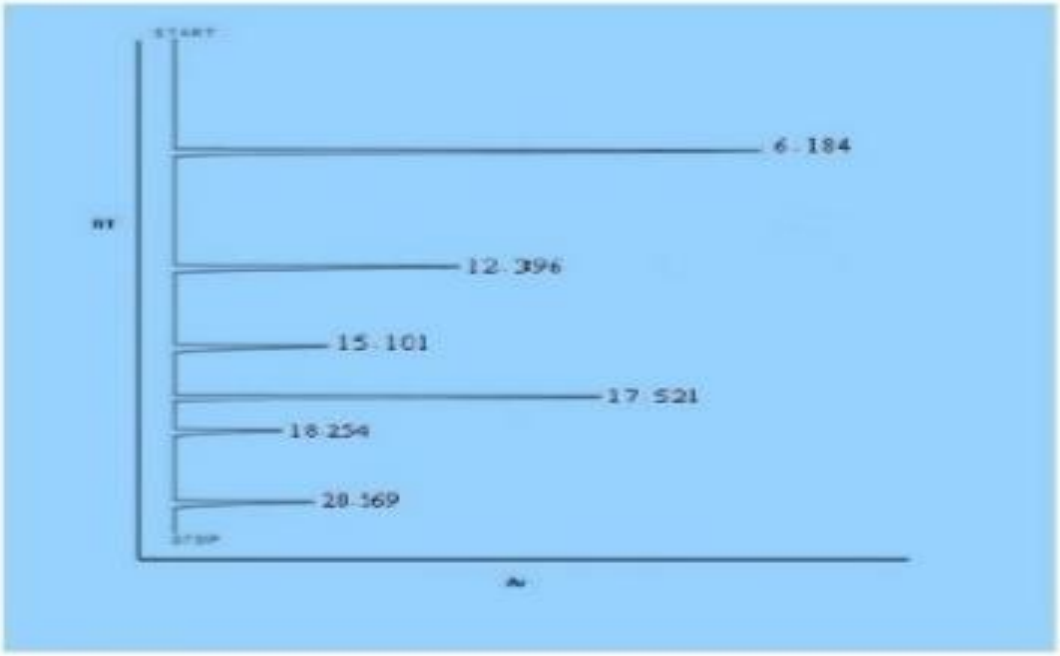
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	6.184	2644			16.7713	QUERCETIN
2	12.396	1279			13.5430	RUTIN
3	15.101	4533			22.9211	NARINGENIN
4	17.521	3576			19.1456	KAEMPFEROL
5	19.108	1354			13.7293	UNKNOWN
6	20.683	1802			13.9202	UNKNOWN
TOTAL		15188			100	



vitis vinifera

SAMPLE 1
CHROMATOPAC C-R10A
SAMPLE NO 1 FILE AWS.A.A
REPORT NO 2 METHOD 8

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	6.184	4298			17.3412	QUERCETIN
2	7.330	2581			10.2032	CATECHIN
3	9.427	4753			17.9320	EPICATECHIN
4	12.396	7201			29.8324	RUTIN
5	15.101	3636			14.0121	NARINGENIN
6	17.521	2990			10.7698	KAEMPFEROL
TOTAL		25463			100	



zingiber officinale

SAMPLE 2
CHROMATOPAC C-R10A
SAMPLE NO 2 FILE AWS.A.A
REPORT NO 3 METHOD 8

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	6.184	6951			25.9720	QUERCETIN
2	12.396	3276			16.3829	RUTIN
3	15.101	1599			12.8936	NARINGENIN
4	17.521	4784			19.9827	KAEMPFEROL
5	18.254	1034			12.3623	UNKNOWN
6	20.569	1238			12.5007	UNKNOWN
TOTAL		18882			100	

(HPLC results of V. vinifera plant). (Results of HPLC assay for P.dactylifrea

Flavonoid Extraction Procedure:

In order to obtain flavonoids from the plant extracts, we took (5) grams of the dried powder of the plant, which was extracted by isohexane, with a volume of (80) ml, initially to remove the oils, terpenes, and waxes.... Then (30) ml of ethanol is added, which was evaporated by the rotary evaporator to reach a volume of (2) ml, where this will be the Crude Flavonoid Mixture, as mentioned in detail in (24b).

Exposing bacteria to plant extracts:

Initially, the three extracts from each plant were prepared at a concentration of 200 mg/ml for the stock solution, and after sterilization was done by the pasteurization process, the dilutions were prepared: 100%, 75%, 50%, 25%, and the minimum inhibitory concentration was determined, which was Up to 100% of all extracts compared to Media Negative Control.

The Agar-well diffusion method was used to test the inhibitory activity after inoculation of Muller-Hinton Agar medium with bacteria by means of a cotton swab and spread it evenly in all the plates and holes were added 50 microliters of each of the four concentrations in addition to a control sample of Water as a standard control sample, after which it was incubated for 24 hours at a temperature of 37, and after it was taken out, the bacteria were taken from the tips of the inhibitory diameters that appeared in the wells of the concentration of 100% and 75% and were planted and grown again on Nutrient Agar medium with the aim of growing The second generation of E.coli bacteria after exposure, from which we will know the extent of the change in the level of DNA at these two concentrations.

Synergistic inhibitory activity test

In addition to the mentioned concentrations for each extract, two synergistic concentrations were made by adding 17 microliters of all 100% concentrations of the three extracts together and the same work was done for the 75% concentration of the three extracts of ginger, grapes and dates, and then growing the second generation of bacteria in a new medium After exposure to the synergistic extract to know the potential damage at the genome level after performing the process of (DNA) extraction after exposure and doing the (PCR) reaction.

PCR reaction results:

The use of alcoholic plant extracts of Zingiber officinale, Vitis vinifera and phoenix dactylifera showed a clear effect on the level of DNA of E.Coli bacteria, specifically on the activity of the genes under the current study, FimA, PgaD that encode two of the most important virulence factors and are directly related to the pathogenicity of this bacteria. And in varying proportions according to the concentrations used from the extracts and at the level of the two genes, as shown in figures and tables 2 and 1 -.

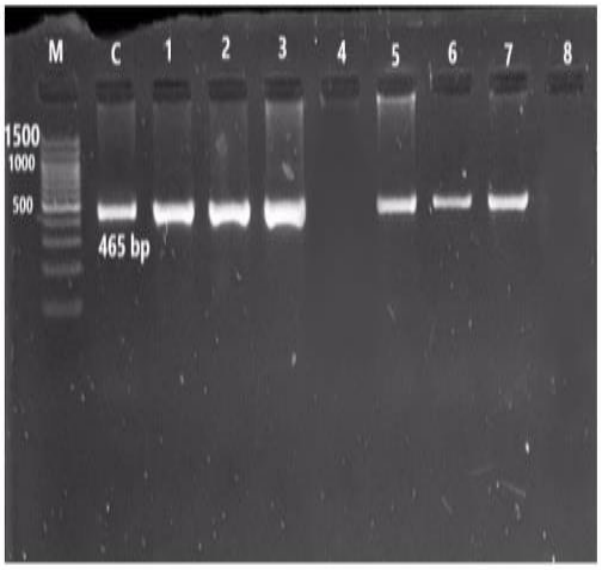


Figure 4 - The result of the PCR reaction for the fimA . gene

No.	Table (1): - Effect of the alcoholic extracts of the three plants at a concentration of 100,75% on the fimA gene.Control	+
1.	75 % Conc- Z. officinale	+
2.	75 % conc. V. vinifer	+
3.	75 % Conc. P. dactylifer	+
4.	75 % conc synergistic extract	-
5.	100 % Conc Z. officinal	+
6.	100 % conc V. vinifer	+
7.	100 % Conc . P. dactylifer	+
8.	100 % conc synergistic extract	-
∑ Polymorphism=	25 %	
Sample: E-Coli isolated from Covid - 19 patients. Gene: fimA (+) sign is the presence of the package. The sign (-) is the absence of the package.		

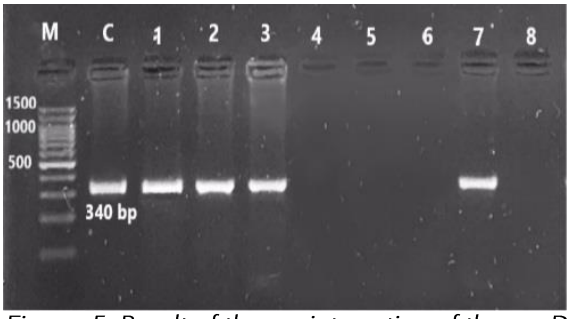


Figure -5- Result of the pcr interaction of the pgaD gene

Table (2): - Effect of the alcoholic extracts of the three plants at a concentration of 100,75% on the pgaD gene		
No.	Control	+
1.	75 % Conc- Z. officinal	+
2.	75 % conc. V. vinifer	+
3.	75 % Conc. P. dactylifer	+
4.	75 % conc synergistic extract	-
5.	100 % Conc Z. officinal	-
6.	100 % conc V. vinifera	-
7.	100 % Conc . P. dactylifer	+
8.	100 % conc synergistic extract	-
∑ 〖Polymorphism=〗	50%	
Sample: E-Coli isolated from Covid – 19 patients Gene: PgaD (+) sign is the presence of the package. The sign (-) is the absence of the package.		

Through the results of the PCR reaction, clear effects of the three plant extracts were found on the DNA level of E.coli bacteria, especially on the PgaD gene, to a greater degree than their effect on theThe fimA gene where the missing group (Bands) in the PgaD gene was (4) (Bands), while the number of missing bands for the fimA gene was (2) bands only for the non-associative concentrations exclusively.

Discussion

In the last four years, the world has witnessed the outbreak and exacerbation of one of the respiratory diseases resulting from infection with the emerging corona virus, and due to the lack of a real direct treatment for it and the delay in the production of effective vaccines against it, this led to a high speed in the production of the disease and its overlap with many accompanying causes. For many types of bacterial and fungal infections (Zhu et al., 2020). E.coli, which is a member of the Enterobacteriaceae family, is considered one of the most common causes of human and animal diseases, as its presence is associated with many diseases, and the large number of molecular studies carried out on it comes from the availability of its complete genome, as it had a large presence in studies and tests of DNA cloning DNA (Mohamedin et al., 2018) that many strains of E.coli confirmed a clear

resistance to antibiotics, which may reach 6 times in some strains (WHO, 2007). Day after day, towards a huge spectrum of antibiotics and the side effects that have a direct impact on human health by weakening the immune system and damaging the stomach and intestines by killing the normal medical flora and contributing to damaging the liver and kidneys and other disadvantages. All of these things directed those in the medical field And researchers have to find alternatives and solutions from natural extracts (Reinthaler et al., 2003) and because of these and other reasons, the World Health Organization (WHO) has directed The importance of expanding the use of plant-based medicines, as an alternative to their chemical-source counterparts (Islam et al., 1994). Flavonoids are yellow-colored compounds belonging to the group of polyphenols present in different concentrations in various plants in the form of secondary metabolites whose function is to protect plants against many unfavorable conditions surrounding them. Cancerous, immune tonics, neurotonics (Kumar & Pandey, 2013). The effect of flavonoids on bacteria, whether they are gram-negative or gram-positive, can be in several ways to inhibit, including inhibition of cytoplasmic membrane formation, inhibition of cell wall formation, inhibition of DNA synthesis by disrupting the work of DNA gyarse, (helicase) (Ahmed et al., 2015). In addition, flavonoids are known through many previous research to be topoisomerase inhibitors (Plaper et al., 2003).

Conclusion

We conclude through this study that we conducted the clear effect of Z.officinale, V.vinifera, and P.dactylifera on its flavonoid extracts after purification from alcoholic extracts that flavonoids have a clear effect on the genome level of E.coli bacteria isolated from the respiratory infection associated with coronavirus covid. 19 By linking with the nitrogenous bases of DNA and disrupting two of the most important factors of virulence for these bacteria at certain concentrations of these extracts, which opens the door to the possibility of benefiting and developing these effective compounds in treating many other pathogens and expanding clinical tests to serve modern treatment protocols And testing their safety in terms of potential toxicity as alternatives to antibiotic treatment.

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