

## Effect of Flavonoid Extracts of Three Famous Plants on Two Virulence Genes *rmpA* and *iucA* of *Klebsiella pneumonia* Isolated as Co-infection from COVID-19 Patients

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www.jrasb.com || Vol. 1 No. 4 (2022): October Issue

Received: 10-09-2022

Revised: 01-10-2022

Accepted: 11-10-2022

### ABSTRACT

In the current study, three plants were identified, *Zingiber officinale*, *Vitis vinifera* and *Phoenix dactylifera*, to extract and isolate flavonoids from them by alcohol extraction from the leaves of these plants, then confirm the extraction of flavonoids alone from the alcoholic extract using Ethyl acetate by Fluid-Fluid Extraction method and expose the isolated *Klebsiella pneumonia* samples. From the respiratory tracts of 25 patients infected with Coronavirus 2019 in Salah al-Din General Hospital in the city of Tikrit - Iraq to find out the extent of the possible genetic influence on the level of DNA, specifically the *rmpA*, *iucA* genes, which have a co-influence in increasing the severity of the infection on the patient.

**Keywords-** Flavonoid Extracts, *Zingiber officinale*, *Vitis vinifera*, *Phoenix dactylifera*, *rmpA*, *iucA*.

## I. INTRODUCTION

After the spread of epidemic Covid 19 worldwide, it increased at a pace parallel to the spread of the accompanying infection, or the so-called super-infection, especially those infections that affect the respiratory system of patients with Covid 19, which caused many cases of poor initial diagnosis of the disease in addition to the multiplier effect on the patient's health (1). *Klebsiella pneumoniae* is one of the strongest and most Gram-negative bacteria in terms of its susceptibility to cause acute and chronic respiratory infections and can thus take an "opportunistic" approach that is highly lethal to immunocompromised individuals (2). (3) It was found that these bacteria are the most isolated among the three bacterial types present in the respiratory tracts of patients with Covid 19, which increase the severity of infection through their virulence factors. Maximum use of them in treating bacterial infections (4), after it was proven that many bacterial strains do not respond to treatment with synthetic antibiotics due to bad and irregular personal use

and failure to follow the instructions of the treatment protocol cycle (5), which naturally led to the emergence of bacterial strains Resistance to treatment with antibiotics (6) Flavonoids are one of the most important active compounds resulting from secondary metabolism that occurs in members of the plant kingdom (7) Flavonoids have many health and medical benefits either in the form of treatments or in the form of nutritional supplements (8). *officinale*, *V. vinifera* and *P. dactylifera* are of great medical importance in addition to their well-known economic importance, as they are considered anti-cancers, bacterial and viral infections, and for the treatment of diabetes and blood pressure, and as essential anti-inflammatory oxidation (9, 10, 11).

## II. MATERIALS AND METHODOLOGY

The fresh leaves of the three plants for this study, *Zingiber Officinale*, *Vitis vinifera* and *Phoenix dactylifera*, were collected from separate areas of Tikrit city - Iraq and were thoroughly washed, dried and ground.

**Flavonoid Extraction :**

The flavonoids were extracted from the leaves of these three plants in two stages: the first was by making an alcoholic extract using ethanol alcohol by the extraction method of the Soxhlet device. This was done by grinding 300 g of powdered leaves and extracting each 100 gm of the powder with 500 ml of ethanol alcohol. The second stage included the extraction of flavonoids from the alcoholic extract using the Fluid-Fluid Extraction method using ethyl acetate with chloroform and distilled water in order to obtain the extracts containing flavonoids alone and to get rid of fats, waxes, terpenes, impurities and other compounds.

**Bacteria Sample Collection :**

*K. pneumoniae* was isolated from the respiratory tracts of 25 patients with Covid 19 lying in the health isolation unit of Salah al-Din General Hospital located in Tikrit city - Iraq for the period from 11-2021 until 3-2022 by Swabs from the laryngeal and pharynx area In addition to samples of Sputum after cultivation, purification and isolation on differential diagnostic media.

Preparation of dilutions of flavonoid extracts and exposing bacterial isolates:

The flavonoid extracts for each plant were prepared at a concentration of 200 mg/ml as a stock solution, after which the sterilization process was carried out by pasteurization method, 100% - 75% -50% - 25% dilutions were prepared, and the minimum inhibitory concentration (MIC) was determined, which was at the 100% concentration for the three extracts. The Agar - Well Diffusion Method was used to test the effectiveness and inhibitory ability of flavonoid extracts after inoculation of the bacterial isolates on Muller-Hinton Agar Media. Five holes were made in the inoculated medium, 50 microliters of the four concentrations were added to each hole, in addition to a control sample of distilled water. "Synergistic concentrations of 100% and 75% worked for the three extracts together mixed with some of them to see the synergistic effect".

**DNA extraction after exposure to flavonoid extracts:**

After the inhibition zones appeared, bacterial colonies were taken from the edges of the halos and cultured on Nutrient broth medium in order to grow the second generation of bacteria after exposure to know the possible effect on the level of DNA. DNA was extracted using the presto Mini gDNA Bacteria Kit supplied by Geneaid.

**Polymerase Chain Reaction Procedure :**

PCR reaction was performed using the Accupower PCR premix kit supplied by Bioneer, following the instructions and additives.

**rmpA Primers:**

No	Primer name	Primer sequence (5'→3')	Size
1	rmpA	Forward: ACTGGGCTACCTCTGCTT CA	536

	Reverse: CTTGCATGAGCCATCTTTC A	
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**iucA Primers:**

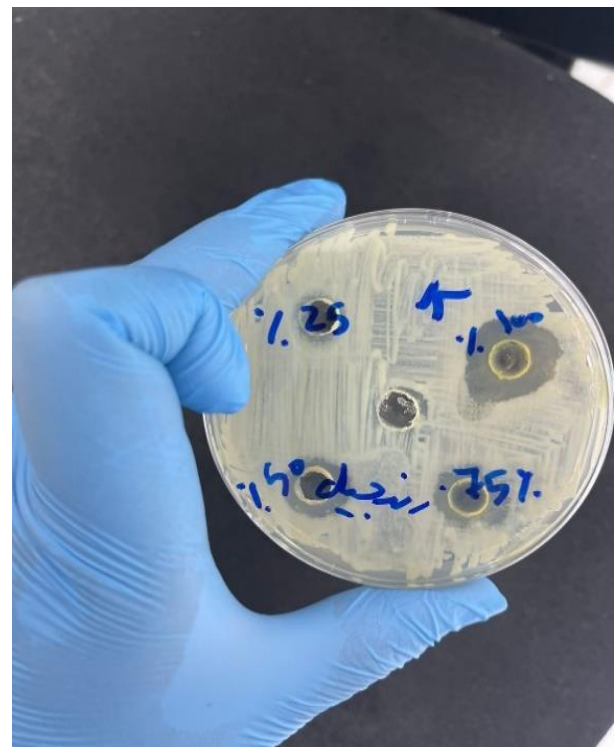
	Primer name	Primer sequence (5'→3')	Size
1	iucA	Forward: AATCAATGGCTATTCCCGCTG Reverse:CGCTTCACTTCTTTCA CTGACAGG	230

**Program:**

Stage	Temperature	Time
First denaturation	95 C	4 min
Denaturation	95 C	30sec
Annealing	58 C	45sec
Extension	72 C	45sec
Final Extension	72 C	5 min

**III. RESULTS**

**Results of exposing *K. pneumoniae* isolates to flavonoid extracts:**



**Figure 1: Inhibition zone of the effect of *Z. officinale* flavonoid extract on *K. pneumoniae*.**



Figure 2: Inhibition zone of the effect of *P. dactylifera* flavonoid extract on *K. pneumoniae*.

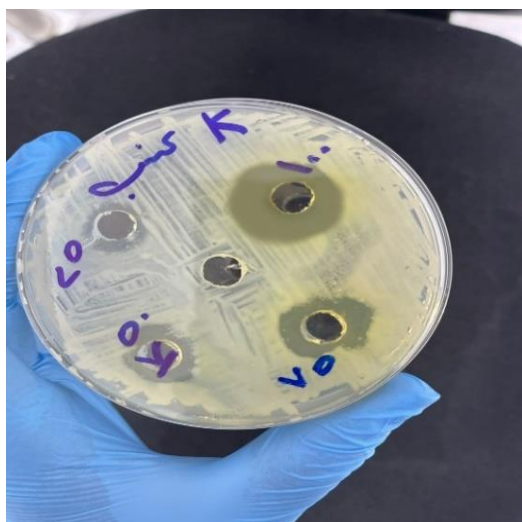


Figure 3: Inhibition zone of the effect of *V. vinifera* flavonoid extract on *K. pneumoniae*.

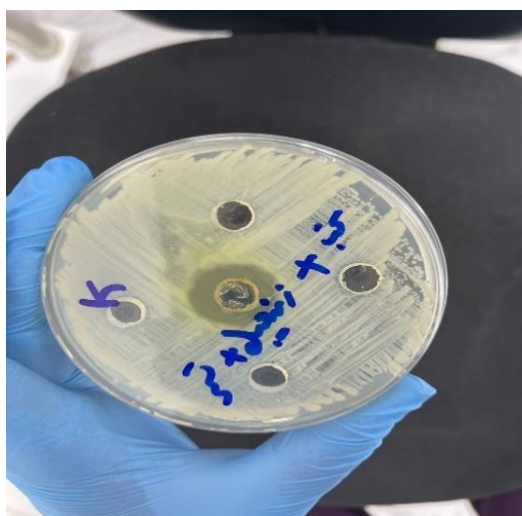


Figure (4) Inhibition zone of the effect of Synergistic mixture flavonoid extract on *K. pneumoniae*

All treatments with flavonoid extracts showed a clear effect on *K. pneumoniae* bacteria except for the synergistic mixture that did not show any clear effect through the absence of the Inhibition Zone.

The results of the PCR reaction to find out the polymorphism at the level of the *rmpA* and *iucA* genes:  
**1\_ Polymorphism level in of *rmpA* gene primers:**

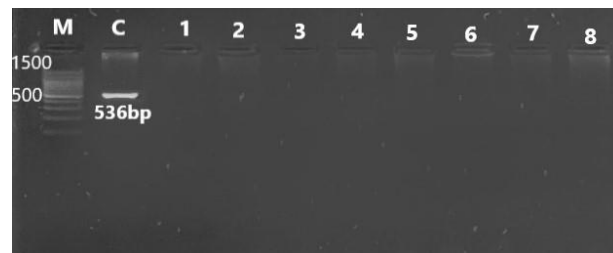


Figure 5: PCR result for the extent of Polymorphism of the *rmpA* gene on agarose.

Table 1: Shows polymorphism ratios for each extract for *rmpA* gene primers

gene		<i>K. pneumoniae rmpA</i> gene
M	Size bp	536
C	control	+
1	<i>Z. officinale</i> 75%	-
2	<i>V. vinifera</i> 75%	-
3	<i>P. dactylifera</i> 75%	-
4	Syn.extract 75%	-
5	<i>Z. officinale</i> 100%	-
6	<i>V. vinifera</i> 100%	-
7	<i>P. dactylifera</i> 100%	-
8	Syn.extract 100%	-
Polymorphism %		100%

**2\_ Polymorphism level in *iucA* gene primers :**

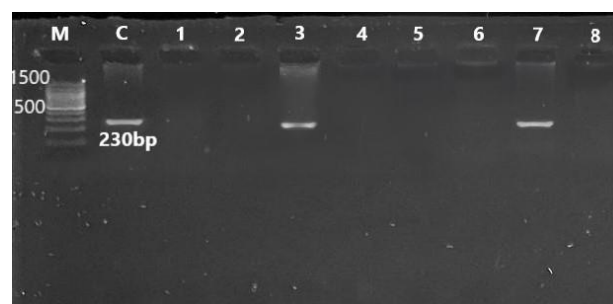


Figure 6: PCR result for the extent of Polymorphism of the *iucA* gene on agarose.

Table 2: Shows polymorphism ratios for each extract for *iucA* gene primers

gene		<i>K. pneumoniae iucA</i> gene
M	Size bp	230
C	control	+

1	Z. officinale 75%	-
2	V.vinifera 75%	-
3	P. dactylifera 75%	+
4	Syn.extract 75%	-
5	Z. officinale 100%	-
6	V.vinifera 100%	-
7	P. dactylifera 100%	+
8	Syn.extract 100%	-
Polymorphism %		75%

#### IV. DISCUSSION

The exacerbation of the problem of the component shown by bacterial strains towards a wide range of antibiotics motivated many researchers to find suitable alternatives by making use of the plant kingdom and its vast by-products and how to exploit them for this purpose (13). The World Health Organization (WHO) has directed the need to expand the use of plant-based remedies as an essential alternative to manufactured chemicals (14). The effect of flavonoids as one of the most important active compounds present in different concentrations in different plant hosts lies in their inhibition of the bacterial cytoplasmic membrane or as inhibitors that prevent or disrupt the formation of the bacterial cell wall and inhibitors of disruption of bacterial DNA synthesis as flavonoids act as disruptors of DNA gyrase and helicase (15) All this implies, based on the prior knowledge that these active compounds are primarily inhibitors of topoisomerase enzymes (16)

#### V. CONCLUSIONS

From the above results obtained, we find that the rmpA gene was significantly affected by all treatments in all single and synergistic concentrations by %100, while the iucA gene was not affected by date palm treatments at all, while it was affected by ginger and grape extracts as well as by the synergistic mixture extract and this gives us A perception "on the possibility of exploiting flavonoids in these three plants or others and developing them in the therapeutic field or as nutritional supplements after conducting more clinical tests, especially" in light of the Covid 19pandemic.

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