Antimicrobial Activity of Plant Extracts of Artemisia , Citrullus and Capparis

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ABSTRACT
The current study shows the effectiveness of three plant extracts of Artemisia, Citrullus fruits and Capparis roots against the microbes of some common and important pathogens, which have become resistant to many antibiotics due to genetic mutations that occur in them, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia .The sensitivity of the studied isolates varied according to the type of extract and the type of solvent used, Alcoholic or Aqueous, as well as on the type of bacteria. While the effect of the effectiveness of the extracts of the fruits of the Citrullus plant and the roots of the capers plant showed a variation from one bacteria to another and from one extract to another.

1. Introduction

The frequent use of antibiotics is a growing concern because of the harmful effects on human health and the environment (Soares, *et al*., 2021) . Nowadays, doctors and pharmacists often prescribe drugs, and these drugs have been discovered and manufactured industrially, which facilitate rapid recovery from diseases and the elimination of some pathogens, but with excessive use, the side effects of the host or patient increase due to the resistance, and for this reason, it is currently preferable to use medicines derived from herbal plants to avoid excessive reliance on chemical medicines manufactured in recent times. Therefore, many medicinal herbs were discovered and studied and their ability to inhibit and prevent the growth of microorganisms that cause these diseases, infections and anti-cancer. And viruses and therefore it is important to shed light on some medicinal plants available in our environment (Mandalari,*et al*., 2007) . (Gull, *et al*., 2015), especially during the present time as a result of the emergence of many strains resistant to antibiotics, and these plants.

Artemisia : a wild perennial shrub with large branches and perennial leaves, of which there are 400 species (Al-Shaye'a, *et al*., 2009). It is classified from the family of plants Asteraceae (Lachenmeier, *et al*., 2010) (Turuspekov, *et al*., 2018). The medicinal importance of the Artemisia plant is due to the fact that it contains active substances such as Volatile oils, Flavonoids, Alkaloids, Saponins, Glycosides, Phenols and Resins (Pawar, *et al*., 2015). and (Al-Snafi, *et al*., 2015). Most types of Artemisia contain Artemisinin, the main component of the plant, and other bioactive compounds that have unique pharmacological activities against Gram-positive and Gram-negative bacteria (Soares, *et al*., 2021) .

Citrullus: It is a medicinal plant belongs to the Cucurbitaceae family. It contains many active

substances, the most important of which is Colocynthin, which is a very bitter biochemical from glycosides and is highly toxic (Marvi, *et al*., 2017)). There are about 120 genera and 825 species, and it also contains resinous substances, Pectin and Saponins. The fruits of this plant have been used as a treatment for asthma, bronchitis and sore throat (Kannan, *et al*., 2020)). Citrullus seeds contain a large group of fatty acids such as Citric, Myrstic, Palmitic, Oleic, Linoleic and Linolenic (Pravin, *et al*., 2013).

Capparis : It is a perennial evergreen plant with a bluish-green color, of which there are 350 species and known by several names. It is classified in the family Capparidaceae. It is a future source of invaluable nutrients for human food and has been used in traditional medicine to treat many human infections. Phytochemical analysis showed that this plant contains high amounts of many bioactive components and molecules, which are responsible for various pharmacological activities. These activities include antioxidant effect, antibacterial effect, and anti-mutagenic effect (Adwan, G.M. and Omar, G.I., 2021).

2. Materials and Method

1/ Collection of used plants

Medicinal plants were obtained from Citrullus and Artemisia from local markets in Thi- Qar governorate, and capers plant roots from local agricultural lands in the governorate. They were classified in the Department of Life Sciences / College of Education for Pure Sciences at Thi-Qar University.

The plant extracts were prepared, taking 200 g of each plant, cleaned and washed before use, left to dry, then ground by an electric mill, kept in bags, and placed in the refrigerator at a temperature of - 4 ° C until use.

2/ Aqueous and alcoholic plant extracts

The alcoholic extract was prepared according to the slightly modified method from (Bandar., 2013) and (Messaoudi., 2020) by soaking 60 g of previously preserved plants in 300 ml of ethanol alcohol at a concentration of 70 % for 72 hours with stirring from time to time. The solution was filtered using Six layers of gauze (Ahmeethunisa, AR and Hopper, W., 2010) and the filtrate was collected by sterilized dishes and left to dry. The dried material was collected and crushed. The same method was used before for the process of preparing the water extract of plants, but using water to prepare the extracts.

3/ Bacterial isolates

Bacterial isolates were obtained from the Microbiology Laboratory of the College of Education for

Pure Sciences at Thi - Qar University. Four isolates of bacteria that have different characteristics of resistance to antimicrobial drugs were studied after being grown on Nutrient Agar medium, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*, which are among the causes important pathology in hospitals.

4/ Measuring the effectiveness of plant extracts

Concentrations (1 g , 2 g , 3 g) of the previously prepared plant extracts were dissolved in 10 ml of distilled water and kept in the refrigerator at a temperature of - 4 °C.

Bacterial isolates were activated before detecting the effectiveness of plant extracts using Nutrient Agar medium and incubated at (37°C) for a period of (18-24) hours after which (2 - 3) colonies were transferred by a sterile loop and a series of dilutions were made in test tubes according to McFarland method. The dilution was ($1 \times 10^{3-}$) is suitable for bacterial growth, as a smear was taken by means of a swab and spread it on the prepared Muller-Hinton Agar medium and left at laboratory temperature for(15-20) minutes to absorb the inoculum. Wells to add the plant extracts in the amount of(100 ul) from each of the prepared concentrations, then it was left at the laboratory temperature for (1 – 2) hours in order to imbibe the extracts (Al-Akidi and Kawkab Idris, 2012).

3. Results and Discussion

The results shown in Tables (1 , 2 , 3) Which shows the significant differences between samples as well as in Figure (1 - 1) clarified the role of plant extracts in influencing the microbial activity of some pathogenic bacterial isolates. The aqueous and alcoholic extract of Artemisia plant was more effective in inhibiting the growth of all bacterial isolates, and this result was identical to the results of (Ćavar, et al., 2012) for most of the studied isolates, and it was identical to the study of (Ahameethunisa and Hopper, 2010), regarding *Escherichia coli* and *Pseudomonas aeruginosa* for the Ethanolic extract, while the result of *Staphylococcus aureus* for the extract The Ethanolic Alcohol was identical (Hassanshahian, M. and Khosravi, F., 2016), add to the bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. As for the results of *Klebsiella pneumoniae* bacteria, they are identical to what was stated by (Pawar, *et al*., 2015) who used the Methanol Alcohol extract. As for the Aqueous extract, its results were also positive of inhibition of isolates. The result of inhibition of *Staphylococcus aureus* isolates was identical to the results of (Askary, *et al*., 2021), and the results of the extract were positive of inhibition on isolates, and this result was different from the study of (Furtado and Lewis., 2021). The reason for this may be due to the different Aqueous extraction method, the plant parts used, or the type of isolates used.

As for the results of the effectiveness of the fruits of the Citrullus plant used, whether they were Alcoholic or Aqueous extracts, their results varied in effect on the inhibition of the studied bacterial

isolates. As for *Staphylococcus aureus* , the result was negative, which may be back to the a little different method and conditions of extraction. The bacteria isolates of Pseudomonas aeruginosa, *Klebsiella pneumonia* , and *Escherichia coli* showed similar positive results to those of (Kannan, and Rajan., 2020) and (Guitar, *et al*., 2005), except for *Staphylococcus aureus*, which are negative for the Aqueous and Alcoholic extract of Citrullus fruits and may be Because of the time factor and the solvent used in preparing the extracts, the effect on the inhibitory activity of the extracts, especially Aqueous extracts, as stated in the study of(Messaoudi, *et al*,. 2020).

The results of Alcoholic Ethanolic extract of Capparis plant roots showed positive through its effect on the bacterial growth of isolates *Escherichia coli* and *Staphylococcus aureus*, and this result was in agreement with the study of Gull, *et al*., 2015)) at a concentration of 200 ml/mg and 300 ml/mg, as well as came in agreement with the study (Vyas, *et al*., 2020). As for the effect of the extract on the isolates *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, it was little or no, and this result was in agreement with the study (Al-Akidi and Kawkab Idris, 2012).

Figure (1-1) Effect of the effectiveness extracts on the growth of bacterial isolates

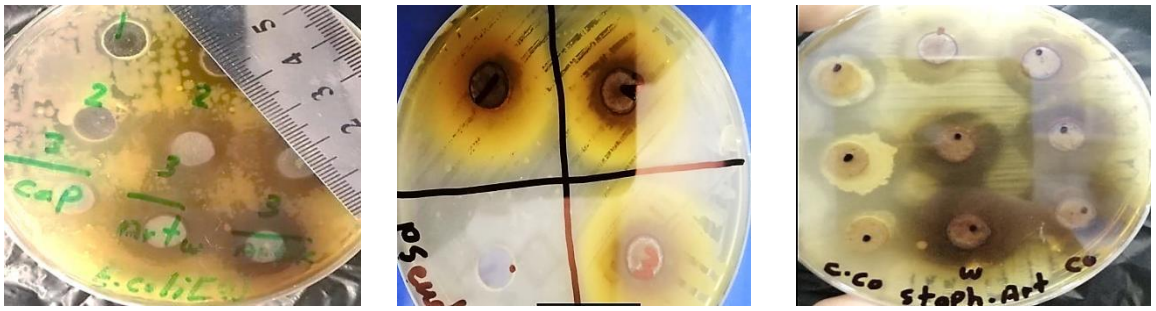

Table 1: Activity of Artemisia Alcoholic and Aqueous Extract on growth of isolated

Bacteria Concentration	***E. coli***	***S. aureus***	***S. aeruginosa***	***K. pneumonia***
	Inhibition Zone of Artemisia Alcoholic Extract Mean ± SD			
100	12.6 ± 0.57^{b}	23.5 ± 0.86	9.00 ± 1.00^{c}	10.6 ± 1.52^{b}
200	11.7 ± 0.57^{b}	21.7 ± 1.52	14.6 ± 0.57^{a}	11.8 ± 1.60^{b}
300	14.7 ± 0.58a	22.6 ± 1.50	11.7 ± 1.52^{b}	15.0 ± 0.87^{a}
p. value	**0.002**	**0.317**	**0.002**	**0.020**
LSD	**1.15**	**Non-Sig**	**2.20**	**2.74**
Bacteria Concentration	***E. coli***	***S. aureus***	***S. aeruginosa***	***K. pneumonia***
	Inhibition Zone of Artemisia Aqueous Extract Mean ± SD			

100	10.6 ± 0.57	15.8 ± 0.28^{c}	11.0 ± 1.00^{b}	9.66 ± 0.57^{b}
200	10.8 ± 0.28	18.1 ± 1.25^{b}	9.66 ± 0.57^{b}	14.7 ± 1.52^{a}
300	11.0 ± 1.00	21.8 ± 0.76^{a}	13.7 ± 0.57^{a}	10.3 ± 0.57^{b}
p. value	**0.842**	**< 0.001**	**0.002**	**0.002**
LSD	**Non-Sig**	**1.73**	**1.48**	**1.99**

Table 2: Activity of Citrullus Alcoholic and Aqueous Extract on growth of isolated bacteria

Bacteria Concentration	***E. coli***	***S. aureus***	***S. aeruginosa***	***K. pneumonia***
	Inhibition Zone of Citrullus Alcoholic Extract Mean ± SD			
100	8.33 ± 0.57^{b}	0.00 ± 0.00	9.00 ± 1.00^{c}	10.8 ± 1.75^{b}
200	9.00 ± 1.00^{ab}	0.00 ± 0.00	15.3 ± 0.57^{a}	11.7 ± 1.52^{ab}
300	12.0 ± 2.64^{a}	0.00 ± 0.00	11.7 ± 0.57^{b}	14.5 ± 1.80^{a}
p. value	**0.048**	**1.00**	**< 0.001**	**0.045**
LSD	**3.33**	**Non-Sig**	**1.48**	**3.39**
Bacteria Concentration	***E. coli***	***S. aureus***	***S. aeruginosa***	***K. pneumonia***
	Inhibition Zone of Citrullus Aqueous Extract Mean ± SD			
100	11.3 ± 0.57	0.00 ± 0.00	11.0 ± 1.00^{a}	14.8 ± 0.76^{b}
200	10.6 ± 0.57	0.00 ± 0.00	9.66 ± 0.57^{ab}	20.3 ± 0.57^{a}
300	11.0 ± 1.00	0.00 ± 0.00	9.33 ± 0.58^{b}	13.8 ± 0.76^{b}
p. value	**0.576**	**1.00**	**0.072**	**< 0.001**
LSD	**Non-Sig**	**Non-Sig**	**1.48**	**1.4**

Table 3: Activity of Capparis Alcoholic Extract on growth of isolated bacteria

Bacteria Concentration	***E. coli***	***S. aureus***	***S. aeruginosa***	***K. pneumonia***
	Inhibition Zone of Capparis Alcoholic Extract Mean ± SD			

100	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00
200	11.3 ± 0.57^{b}	10.3 ± 0.57^{a}	11.7 ± 0.57^{a}	0.00 ± 0.00
300	14.7 ± 0.57^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00
p. value	**< 0.001**	**< 0.001**	**< 0.001**	**1.00**
LSD	**0.94**	**0.66**	**0.66**	**Non-Sig**

4. Conclusions and Recommendations

1- The aqueous and alcoholic extract of Artemisia showed a clear effect on all studied isolates. As for the effectiveness of the aqueous and alcoholic extract of the fruits of the bitter melon plant and the alcoholic extract of the roots of the capers plant, it had a different effect on the isolates.

2- It is recommended to conduct further studies to identify the main compounds in these plants that are responsible for inhibiting the growth of bacterial isolates, and thus it can be a potential means through which the effect of transmitted pathogens can be reduced.

3- It is preferable to use other solvents in the process of extracting active substances in plants such as Hexane, Chlorform, Diethyl Ether alcohol or other solvents.

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