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Studying the effect of plant extracts of *Saliva officinalis L.* and *Costus speciosus L.* on bacterial species causing urinary tract infection in Samarra

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ABSTRACT

The current study aimed at the possibility of benefiting from the plant extracts of the *Costus speciosus* and *Salvia officinalis* plants in the treatment of bacterial diseases through knowing the inhibitory effect of these extracts on the isolated bacterial species. The aqueous cold - hot and alcoholic extracts ethanol – methanol were tested with concentrations of 70,50,10 mg / ml and by the method of diffusion in pollen of *Costus speciosus .L* and *Saliva officinalis L.* on the bacterial genera *Pseudomonas areuginosa, Klebsiella pneumonia, Enterobacter spp.* that isolated from patients with urinary tract infection in the city of Samarra, where the results showed that the two types of water extracts, especially the hot ones, are more efficient than both alcoholic extracts of The two plants mentioned above. Where the concentration of 70 mg / ml gave the aqueous extracts a high inhibitory capacity and for both plants compared to the other concentrations, as it was found that the inhibitory capacity increased with increasing concentrations, while the alcohol extracts had a low inhibitory capacity to no compared to the aqueous extracts.

Introduction

The use of plant-based medicines is dated back to ancient times[1], medicinal plants play an important role in protecting humans from many diseases because they contain factors or components that improve the body's immunity against infection and various diseases[2]. These components are found in the form of groups that have a medicinal effect and are effective against many microorganisms, especially viruses and bacteria [3]. Among these active ingredients are Alkaloids, Glycosides, and Flavonoids, all of which are used according to their medicinal effect [4]

Plants and medicinal herbs differ in the active substances they contain, which contribute to the treatment of many diseases such as urinary tract infection, which is one of the common diseases that affect different groups of ages and this inflammation results from the high multiplication of many causes, especially bacteria [5] Creating changes in the ideal function of the urinary tract and the kidneys[6] .

One of the plants used in this study is *Saliva officinalis L.*, which belongs to the Lamiaceae family (see figure 1) , which is one of the oldest medicinal plants because it contains the most effective compounds against various diseases[7]

Kingdom : Plantae
Phylum:- Euphllophytina
Class:- Angiospermae
Order:- Lamiales
Family:- Lamiaceae
Genus:- *Salvia*
Species:- *Salvia Officinalis*

Fig. 1: *Salvia Officinalis* plant [8]

As well as the Indian *Costus speciosus. L* plant, which belongs to the Costaceae family(see figure 2)

Kingdom:- Plantae

Sub kingdom:- Tracheobinota
Superdivision:- spermatophyta
Division:- Mangoliophyta
Class:- Liliopsida
Sub class:- Zingiberidae
Order:- Zingiberales
Family:- Costaceae
Genus:- *Costus*
Species:- *Costus speciosus*

Fig. 2: *Costus speciosus* plant [9]

as well as the Indian *Costus speciosus .L*, as many studies have proven that this plant has Anti-bacterial and anti-fungal properties[10], and the active ingredients differ according to the plant fraction used. Where scientific experiments have shown that the effect of the active substances produced chemically it doesn't lead to the physiological effect of the same active substance extracted from medicinal plants, In addition, the chemical-producing substances have many side effects that are often harmful [11], and the aim of this study is to know the effect of plant extracts, whether alcoholic or cold and hot aqueous extracts, on some species that cause urinary tract infection.

Materials and Methods

- Bacterial species used in study

Three bacterial species negative for Gram stain, *Klebsiella pneumonia*, *Pseudomonas areuginosa*, and *Enterobacter spp*. Were collected from incoming patients at Samarra General Hospital, For age groups from 5 years to 55 years within the period from 15/7/2018 to 30/9/2018 and by 125 samples, samples collected from midstream urine in sterile and sealed containers and from both sexes male and female, and cultured on Nutrient agar medium at one of the laboratories of Samarra University for a period not exceeding one hour after it was collected. So it was incubated at a temperature at 37˚C for a period of 24 hours. The developing colonies were selected, the cultivar characteristics were studied, and they were diagnosed based on biochemical tests to determine the studied species.

Plant Extracts Preparation

- Cold and Hot Aqueous Extract

The used plant extracts were prepared with a weight of 10 g of dried plant powder in 500 ml of cold distilled water and left for 24 hours at room temperature, after which the infusion is filtered to get rid of the large parts of the plant by filter paper, after which the filtrate is collected in glass containers and placed in the oven to dry plant extracts at a temperature at 40˚ C until dry, then collect the powder and It is placed in sterilized and opaque containers in the refrigerator at a temperature at 10°C until use [12] .

- As for the hot aqueous extract, we follow the same steps mentioned, with replacing the cold distilled water with hot distilled water at 70˚ C, and also preserving the extract until it is used[12] .

- Preparation of alcoholic extracts

This was done by following the same steps in preparing the aqueous extracts, except for the use of ethyl alcohol and methyl, at a concentration of 95% and 80%, respectively, instead of water[12] .

-Test of the inhibitory activity of plant extracts against isolate bacterial species

The aqueous and alcoholic extracts of the studied plants were studied at concentrations70,50,10mg/ml in the growth of bacterial species using the agar well method, where 10 ml of sterile Nutrient broth were inoculated for all the studied bacterial isolates and incubated at 37°C for 16 - 18 hours using a sterile cotton swab, the surface of the dishes was inoculated from the Nutrient agar medium, then 6 ml pits were made using a Cork borer to contain the plant solutions at a rate of 0.5 ml of different concentrations for each pit and three replications for each concentration. Then the dishes were incubated at 37°C for 24 hours. The results were calculated by measuring the diameter of the inhibition zone [13].

Results and Discussion

The results of the experiment, which are shown in Table 1,2,3,4 showed that there was a large variation in the ability of plant extracts to inhibit the bacterial species used in the study, and this depends on the type of extract cold aqueous extracts - hot, alcoholic ethanolic- methanolic extracts and its concentration and species of microorganisms. It was found from the tables mentioned that the cold and hot aqueous extracts had a high inhibitory activity at the two concentrations 50 and 70 mg/ml, with significant differences at the level $p \leq 0.05$ between the rates of

inhibition diameters and the concentrations used. Compared with the alcoholic extracts of both plants *Saliva officinalis.L*, and *Costus speciosus. L.* Where the results showed that the inhibitory activity increases with the increasing of the concentration used, as the concentration of 70 mg / ml gave the highest effectiveness in inhibiting the bacterial genera used in the study. The microbial inhibition can be explained by the presence of active substances in both plants such as flavonoids, alkaloids, tannins and glycosides, which have a higher solubility in water than in alcohol, made the aqueous plant extracts effective in inhibiting bacterial isolates [14] ,Which affected the growth of microbial cells through the means of eliminating microorganisms, especially bacteria in terms of preventing the formation of the cell wall, or a defect in the physical-chemical structure of proteins and nucleic acids, or a defect in the permeability of the cytoplasmic membranes, which affects the osmotic pressure of the cells, which leads to the penetration of bacterial cells by the components of plant extracts [15] and this is consistent with the study conducted by [16] on *Salvia Officinalis* plant and researchers [17]on *Costus speciosus* . As for alcoholic extracts, the methanolic extract showed weak to medium inhibitory ability for *Costus speciosus L.* with a significant difference at concentration 70 mg/ml for *Klebsiella pneumonia* and *Enterobacter spp* isolates, while *Salvia Officinalis* showed a good inhibitory effect against bacterial isolates which under study increases with increasing concentration with significant differences at the level $p \leq 0.05$ and for all used concentrations 10, 50, 70 mg/ml as shown in Table 3. As the difference in the effect is due to the difference in the active substances and their quantities and the type of extract [18], and the ethanolic extract did not have any inhibitory activity for all the concentrations used. This can be attributed to the fact that the percentage of active substances present in both plants may be affected during the extraction process, when the alcohol was not efficient in precipitating the active substances that would cause bacterial growth. These results for *Costus speciosus L.* plant agree with researcher[19]. As for the plant *Salvia Officinalis*, the results completely agreed with the researcher [16] in showing the inhibitory effect in the methanolic extract within the results we obtained, and not showing any activity in the ethanolic extract. As for Table No. 5, it shows the effect of some antibiotics on the growth of bacterial isolates, namely: Nitrofurantion, Gentamycin, Ciprofloxacin, Amoxicillin In comparison with the plant extracts, it was found through the results that the aqueous extracts of both plants at concentrations 50 and 70 mg/ml were superior in giving them a high inhibitory activity compared to the antibiotics under study. As for the alcoholic extracts, the methanol extract showed The *Costus speciosus L* plant has a close inhibitory effect with antibiotics and a good effect for *Saliva officinalis.L*, compared to the antibiotics, while the antibiotics were superior in causing the inhibitory effect compared to the ethanolic extract and for both plants, which had no inhibitory activity. The importance of the results of the current research comes in response to the recommendations and various scientific articles, especially those issued by the World Health Organization, which call for intensifying the efforts of researchers in order to increase the areas of using medicinal plants as sources of alternative medicine due to their high content of bioactive compounds, which scientific experiments confirm their pharmaceutical importance.

Table 1: Inhibition diameters for cold aqueous extracts under study for isolated genera from urine samples. inhibition zone (mm)

Plant extracts	concentration (Mg / mL)	*Psedomons. aeruginosa*	*Klebsiella. pneumonia*	*Enterobacter. spp*
Costus speciosus .L	10	n.s 0±0	n.s 0±0	n.s 0±0
	50	*5.4±8	*3.4±8	*6.5±9
	70	*1.8±16	*3.5±13	*2.1±16
Saliva officinalis .L	10	n.s 0±0	n.s 0±0	n.s 0±0
	50	*2.6±10	*2.9±5	*4.5±9
	70	*5.9±12	*4.3±9	*3.5±12

n.s: no significant differences at $p \leq 0.05$.
* There is a significant differences at $p \leq 0.05$

Table 2: Inhibition diameters for the hot aqueous extracts under study for the isolated genera from urine samples. inhibition zone (mm)

Plant extracts	concentration (Mg / mL)	***Psedomons. aeruginosa***	***Klebsiella. pneumonia***	***Enterobacter. spp***
Costus speciosus .L	10	n.s 0±0	n.s0±0	n.s0±0
	50	*2.6±10	*1.9±11	* 1.9±9
	70	*1.8±15	*0.48±16	*1.7±15
Saliva officinalis .L	10	n.s 3.1±1	n.s 0±0	n.s 0±0
	50	*1.01±9	*4.8±7	*4.4±5
	70	*4.09±10	*2.8±10	* 4.6±8

n.s: no significant differences at $p \leq 0.05$.

* There is a significant differences at $p \leq 0.05$

Table 3: Diameters of inhibition of the methanolic extract under study for the isolated genera from urine samples. inhibition zone (mm)

Plant extracts	concentration (Mg / mL)	***Psedomons. aeruginosa***	***Klebsiella. pneumonia***	***Enterobacter. spp***
Costus speciosus .L	10	n.s 0±0	n.s 0.6±1	n.s 0±0
	50	n.s 5.03±2	ns2.2±3.5	n.s 3.5±2
	70	n.s 6 ±3	5.7±4	6.2±4
Saliva officinalis .L	10	6.4±5	5.6±6	4.3±6
	50	7.9±7	7.9±7	5±8
	70	6.8±5	11.5±10	9.8±9

n.s: no significant differences at $p \leq 0.05$.

* There is a significant differences at $p \leq 0.05$

Table 4: Diameters of inhibition of the ethanolic extract under study for the isolated genera from urine samples. inhibition zone (mm)

Plant extracts	concentration (Mg / mL)	***Psedomons. aeruginosa***	***Klebsiella. pneumonia***	***Enterobacter. spp***
Costus speciosus .L	10	n.s 0±0	n.s 0±0	n.s 0±0
	50	n.s 0±0	n.s 0±0	n.s 0±0
	70	n.s 0±0	n.s 0±0	n.s 0±0
Saliva officinalis .L	10	n.s 0±0	n.s 0±0	n.s 0±0
	50	n.s 0±0	n.s 0±0	n.s 0±0
	70	n.s 0±0	n.s 0±0	n.s 0±0

n.s: no significant differences at $p \leq 0.05$.

* There is a significant differences at $p \leq 0.05$

Table 5: Sensitivity of isolated bacterial species to the antibiotics used

species of bacteria / Antibiotic	*Psedomons . Aeruginosa*	*Klebsiella. pneumonia*	*Enterobacter. spp*
Nitrofurantoin	0.61±1.4	0.84±2.48	0.96 ±1.6
Amoxicillin	0.72 ±0.7	0.72±0.52	0.60±1.18
Ciprofloxacin	0.86 ±1.86	1.11±1.74	0.94±1.98
Gentamycin	0.64 ±1.42	0.66±2.14	0.82±1.8

Illustrations of the sensitivity test of plant extracts against bacterial species

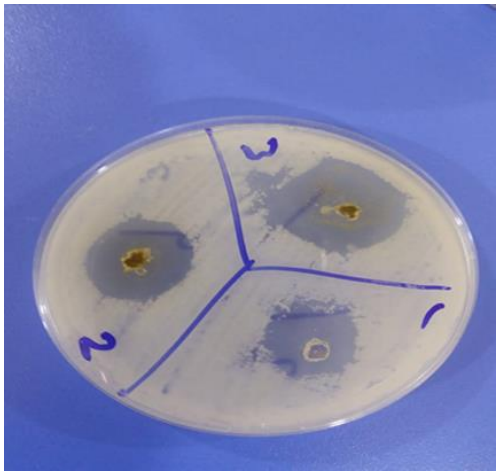

Fig. 3: *Salvia Officinalis* Extract

No. 1 :- 10 mg/ml concentration,
No. 2:- 50 mg/ml concentration,
No. 3 :- 70 mg/ml concentration

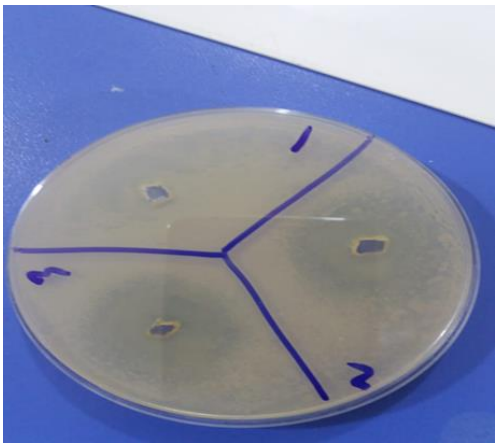

Fig. 4: *Costus speciosus* Extract

No. 1:- 10 mg/ml concentration,
No. 2:- 50 mg/ml concentration,
No. 3:- 70 mg/ml concentration

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دراسة تأثير مستخلصات نباتي الميرمية *Saliva officinalis .L* والقسط الهندي *Costus speciosus .L* على الانواع البكتيرية المسببة لالتهاب المسالك البولية في سامراء

هدى علي هادي

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الملخص

هدفت الدراسة الحالية الى امكانية الاستفادة من المستخلصات النباتية لنباتي القسط الهندي والميرمية في معالجة الامراض البكتيرية من خلال معرفة التأثير التثبيطي لهذه المستخلصات اتجاه الانواع البكتيرية المعزولة. حيث تم اختبار المستخلصات المائية الباردة – الحارة والكحولية الايثانولية – الميثانولية وبالتراكيز 70,50,10 ملغم / مل وبطريقة الانتشار في الاكار لنباتي القسط الهندي *Costus speciosus .L* والميرمية *Saliva officinalis .L* على الاجناس البكتيرية *Pseudomonas areuginosa* , *Klebsiella pneumonia* , *Enterobacter spp* التي عزلت من المرضى المصابين بالتهاب المسالك البولية في مدينة سامراء , حيث اظهرت النتائج ان المستخلصات المائية بنوعيها سيما الحارة أكفأ من المستخلصات الكحولية للنباتين انفي الذكر. حيث اعطى التركيز 70 ملغم /مل للمستخلصات المائية قدرة تثبيطية عالية ولكلا النباتين مقارنة بالتراكيز الاخرى , حيث وجد ان القدرة التثبيطية تزداد بزيادة التراكيز أما المستخلصات الكحولية فقد كانت ذات قدرة تثبيطية واطئة الى معدومة بالمقارنة مع المستخلصات المائية .