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## Antibacterial activity of *Rosmarinus officinalis* and *Dodonaea viscosa* leaves extracts against *Escherichia coli* and *Staphylococcus aureus*

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### Abstract

The isolates from urine and synovial fluid samples of *Escherichia coli* and *Staphylococcus aureus* bacteria were isolated and identified, in order to study alternative treatment or antibacterial agents of plant extracts from *Rosmarinus officinalis* and *Dodonaea viscosa* plants. The isolates were identified by using cultural and biochemical tests, in addition to API 20E kit as confirmation test. The results exhibited that Rosemary extract prevents the biofilm formation and causes inhibition to bacterial growth, while the doddonia extract causes antibacterial activity on the tested bacteria.

**Keywords:** Antibacterial activity, plant extract, *Rosmarinus officinalis*, *Dodonaea viscosa*.

## الفعالية المضادة للبكتريا لمستخلصات اوراق نبات ال *Rosmarinus officinalis* و *Staphylococcus aureus* و *Escherichia coli* ضد بكتريا *Dodonaea viscosa*

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### الخلاصة

العزلات من عينات الادرار و السائل الزليلي من بكتريا *Escherichia coli* و *Staphylococcus aureus* عزلت وشخصت، بهدف دراسة علاج بديل او عوامل مضادة للبكتريا من مستخلصات نباتية من نباتات *Rosmarinus officinalis* and *Dodonaea viscosa*. شخصت العزلات بواسطة الفحوصات الزرعية والبايوكيميائية فضلا عن API20E كفحوصات توكيدية. ان مستخلص ال Rosemary منع التصاق البكتريا على السطوح وسبب تثبيط لنمو البكتريا، بينما مستخلص نبات ال doddonia سبب فعالية مضادة للبكتريا على بكتريا الاختبار .

### Introduction

***Rosmarinus officinalis*:** Is an important native and an old cultivated plant of mediterranean origin. It is grown in different places of the world and used as an fragrance plant, also a herbal remedy with a medical benefits for regulating blood pressure, enhancing the circulation, diuretic, treatment of migraines also as antiseptic and antimycotic agent. Rosemary is used in the food industry which

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suffer of food spoilage bacteria that attach and form a biofilm on food contact surfaces and food product, the rosemary effectiveness play role in preventing this problem through the evaluation of antibacterial activity as well as the effect of this extract on biofilm formation against *Staphylococcus aureus* and *Escherichia coli* bacteria [1, 2]. Rosemary contains phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid and the antioxidants carnosic and carnosol [3].

***Dodonaea viscosa***: is a shrub of flowering plant in the soapberry family, sapindaceae, that believed to be habitat in tropics and subtropics region. *D. viscosa* commonly known as hausa and it has medicinal properties. So that it use orally or as poultice to treat a variety of causes [4]. The parts of plant as stems, leaves and roots are use in the treatment of several diseases, they act as antibacterial, antifungal, antiviral, antihelmintic, anti-inflammatory, anti oxidant, antidiabetic, antidiarrheal agent. In addition to some properties like larvicidal, molluscidal and spasmolytic properties. The essential oil from *D. viscosa* has antibacterial activity and effectively inhibit *S. aureus*. The alcoholic extract of *D. viscosa* exhibits antibacterial properties [5]. In addition the essential oil from *D. viscosa* was tested for antimicrobial activity against gram positive and gram negative bacteria by the well of inhibition method. The oil showed effective antibacterial activity against *S. aureus* and was not inhibitory to *Micrococcus flavus* and *Sarcina lutea* [6]. Also the crude extract of *D. viscosa* has inhibitory effects against *Streptococcus pyogenes* and *Corynebacterium diphtheriae*. But has no activity against *E. coli* and *Pseudomonas aeruginosa*. [7]. The aim of the study to get natural substances that use in the treatment of bacterial infections and to prevent their ability to form bacterial biofilm.

## Materials and Methods

### Bacterial isolates:

The bacterial isolates of *E. coli* and *S. aureus* were isolated from the samples that taken from Madinat Altib laboratories and private laboratories. the samples were taken from UTI infections and synovial fluid samples. The isolates were identified by using cultural and biochemical tests and according to [8].

### Collect the leaves of *Dodonaea viscosa*, *Rosmarinus officinalis*:

Leaves of *Dodonaea viscosa* and *Rosmarinus officinalis* were collected from the garden of Biology Department of Baghdad University. They were washed by distilled water (D.W) and dried at 45 °C. Then they were grinded and stored until they used.

**Preparing of plant extracts:** Number of methods were used to preparation of the plant extracts as follows

### *Rosmarinus officinalis* extract:

The essential oil of *R. officinalis* was extracted by using 250 g of dried leaves through Clevenger hydro distillation method. The plant powder was boiled with D.W. for 3 h then the oil kept at 4 °C until it use [9].

### The aqueous extract for leaves of *Dodonaea viscosa* :

#### A- Hot aqueous extraction:

The stock solution of 20% plant extract was prepared by using 100 ml of boiling water plus 10 gm of leaf powder and the mixture was left for 3 hr. after that the mixture was filtered by using whatman no.1 filter paper, then the solution was centrifuged at 10000 rpm for 10 min, the supernatant was placed in oven at 45°C to dry, after that 2 gm of the dried powder was taken and mixed with 100 ml of sterilized distilled water to prepare a stock solution in order to prepare (2.5%,5%,10% and 20%) concentration of the extract [10, 11].

#### B- Cold aqueous extraction:

The stock solution of 20% of *Dodonaea viscosa* leaves was prepared by adding 10 gm of leaf powder to 100ml of distilled water and mixed it well for 15 min and left for 24 after covered it, then it filtrated by using whatman no.1 filter paper, after that the solution was placed in test tube to be centrifuged at 10000rpm for 10 min, after that the solution placed in oven at 40 °C to dry. 2 gm of the dried powder was added to 100 ml of sterilized distilled water to prepare stock solution in order to prepare the concentrations (2.5%,5%,10% and 20%) of it [10, 11].

### Preparation of inoculums:

A loopful of pure bacterial colonies from overnight solid media culture was inoculated into sterile nutrient broth. Broth inoculums were incubated at 37°C for 18 hours, after that they used in next steps of the study.

### The antibacterial activity for *D. viscosa* and *Rosmarinus officinalis* extracts:

The antibacterial activity was measured by placing 20 ml of muller-hinton agar in each pre-sterilized petri plates. After solidifying, the isolate were cultivated on this media, and hole was made in the center by using cork borer (8 mm). The central hole was filled with 100  $\mu$ l of plant extract, the control was DMSO solvent. Then the plate was incubate at 37 °C for 24 hours. Appearance clear zone around the well is due antibacterial activity of the plant extract indicating a positive result [12].

### Results and discussion:

#### Identification of isolates:

The isolates that isolated from urine and synovial fluid samples of *S. aureus* and *E. coli* bacteria (6 isolates 3 of each species) were identified by using cultural and biochemical tests, then the identification was confirmed by using API 20E. The results showed that these isolates of *S. aureus* were positive for coagulase, catalase. Results of carbohydrate fermentation test showed that these isolates were able to ferment manitol, results of growth appeared that these isolates were able to grow on staph 110, blood agar and milk agar media with golden pigmented colonies on milk agar. Also the results indicate that these isolates of *E. coli* were positive for methyl red, indol production test, urease and EMB tests, results of carbohydrate fermentation test showed that these isolates were able to ferment lactose. While they were negative for voges proskauer and Utilize of citrate tests. The results were accordance with [8], those indicate that these isolates belong to *S. aureus* and *E. coli*.

#### Antibacterial activity assay :-

The cold and hot aqueous extracts of *D. viscosa* were tested for bacterial activity against *E. coli* and *S. aureus* bacteria by well diffusion method, The results in Tables- (1, 2) showed effective antibacterial activity against *S. aureus* and these results are similar to that reported by [8] who found that the *Dodonea viscosa* extract has bacterial activity against *S. aureus* bacteria. Also the results showed effective antibacterial activity against *E. coli*, and these results are not agrees with [3] and [4] those found there are no effective bacterial activity against gram negative bacteria, thereby suggesting potential against notable Gram positive organisms. Also the results indicate that the isolates of *E. coli* and *S. aureus* bacteria more sensetive for cold extract of *D. viscosa* than hot extract may be belong to evaborate the active materials of volatile oils of the extracts or for efficiency of extraction by using cold water. While the results of the extract of *Rosmarinus officinalis*

**Table 1-** The antibacterial activity of aqueous extraction from *Dedonea viscosa* against *E.coli*.

Concentration (%)	Sample	Inhibition zone for <i>E. coli</i> 1 (mm)	<i>E.coli</i> 2 (mm)	<i>E.coli</i> 3 (mm)
2.5	Cold aqueous extraction	11	12	13
	Hot aqueous extraction	NIZ	NIZ	NIZ
5	Cold aqueous extraction	17	14	14
	Hot aqueous extraction	11	11	11
10	Cold aqueous extraction	16	17	15
	Hot aqueous extraction	13	15	12

\*DMSO = Control, NIZ= No Inhibition Zone.

**Table 2-** The antibacterial activity of aqueous extraction from *Dedonea viscosa* against *S.aureus*.

Concentration (%)	Sample	Inhibition zone for <i>S.aureus</i> 1 (mm)	<i>S. aureus</i> 2 (mm)	<i>S. aureus</i> 1 (mm)
2.5	Cold aqueous Extraction	12	10	11
	Hot aqueous Extraction	10	NIZ	NIZ
5	Cold aqueous Extraction	13	10	11
	Hot aqueous Extraction	11	NIZ	NIZ
10	Cold aqueous Extraction	13	13	12
	Hot aqueous Extraction	11	11	10

\*DMSO = Control, NIZ= No Inhibition Zone.

showed effective antibacterial agent on *E. coli* and *S. aureus* bacteria, similar finding was proposed by [13] they mentioned that the extract of *Rosmarinus officinalis* has bacterial activity against gram negative ranged between 12-14 mm for *E. coli* and ranged between 11-15 mm against gram positive bacteria for *S. aureus*.

### Conclusion

The study confirms using the alternative drugs that characterized without side effects, like aqueous extraction of *Dedonia viscosa* plant of hot and cold aqueous extraction, has shown a wide range of influence on bacteria, the result indicate that the antibacterial activity for these extracts was appear the cold aqueous activity against *S.aureus* and *E.coli*, was more than hot extract. The results represent a good antibacterial compound compare with other antibiotics in the same time the extract have not any side effect compare with the antibiotics. In addition to the extract of *Rosmarinus officinalis* that appeared effective bacterial activity on gram negative and gram positive of bacteria that used in the study.

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