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Effect of plant extracts of *Salvia officinalis L.* and *Costus speciosus L.* in growth of some bacteria species isolated from urinary tract infections in Samarra city

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ABSTRACT

The present study aimed to investigate the inhibitory effect of alcoholic extract (methanol - ethanol) and flavonoids by diffusion methods of wells of *Salvia officinalis* L. and *Costus speciosus* L. plants on *Escherichia . coli* and *Proteus spp.* isolated from patients with urinary tract infection Samarra city, the samples were collected during the period from 15/7/2018 to 30/9/2018, It was found through the results that the extracts plant that used in this study showed various inhibitory effect against, The bacteria species study imitation , where on differ this effect by difference the concentration that used and type the plant extract , showing *E.coli* significant difference at significant ($p< 0.05$) in inhibition diameter of alcoholic (methanol) extract for *Salvia officinalis* L. (10.61±7.5) at higher concentration used, while these bacteria showed on significant difference in the level of inhibition diameter of alcoholic (ethanolic) extract for *Salvia officinalis* L., whereas the flavonoids Netted significant difference of these plant in some of concentration that used compared with the antibiotic that used in this study. Either regarding *proteus spp.* bacteria notted no significant difference of both the *Costus speciosus L.* and *Salvia officinalis* L. by using the alcoholic (ethanolic) extract, while the flavonoids extract showed no significant difference in *Costus speciosus L.*, whereas showed high significant difference in flavonoids extract of *Salvia officinalis* L., due to the fact that the flavonoids extract is one of the active substance that done laboratory extraction for both the plants where that higher inhibitory effect of microbial growth from the alcoholic (ethanolic – methanolic) extract of plants .

Introduction

pathogenic bacteria is one of the most microorganisms that cause many diseases for both humans and animals [1]and it is one of the most common of urinary tract infections. The urinary system is susceptible to various infections, especially bacterial ones[2]. This inflammation results from the high multiplication of many etiologies, causing changes in the ideal function of the urinary and kidneys [3]. These infections have been classified according the harmful to complicated, uncomplicated or simple depending on the presence or absence of pathogenic cases associated with UTI cases, simple cases are cystitis, while complicated cases include urinary embolism resulting from kidney stones function, or kidney function disorder[4] Despite the widespread prevalence of antibacterial drugs in recent decades, resistance to these treatments by microorganisms have expanded in a way that have led to the emergence of the need for alternative medicines, especially medicinal plants[5] such as *Salvia officinalis* L. and *Costus speciosus* L .The active ingredients vary depending on the plant part used (such as roots or air parts such as leaves and flowers).

Salvia officinalis L. leaves are rich in flavonoids, which are of vital importance[6] produced by the plant to protect its parts from damage to bacterial and fungal diseases. For the same reason can be

considered as natural biocides produced in the plant in response to exposure to pathogens and other active substances phenols, and essential oil as well as Tannins and soap[7]. The roots of *Costus speciosus* L. it is a good source of active ingredients, especially saponin and alkaloids, as it contains gummy substances and fatty acids as well as flavonoids and dyes anthocyanin[8].

Specimen collection and preparation

The specimen were collected from patient that incoming to Samarra Hospital and Healthy Al-Rasheed Center and both of male and female , these samples were transported to the laboratory for identification for period less than 2houres and caltured on culture media that used for primary isolation represented with Nutrient agar media and Incubate in 37^0c for period (24-48) houre , and diagnostic afterward by using microscopical identification and biochemical tests .

Materials and Methods

Preparation of plant extracts

In this study, accomplish the agar diffusion method by action the well for test of of bacterial sensitivity of plant extracts on concentrations (5-10-50-70mg\ml) on culture media represented with muller Hinton agar . The diameter of these wells were 6ml by using cork borer for contain able plant extract with magnitude o.5 ml for each well after diffusion the bacterial supernatant and incubate in 37^0c for 24 houre ,and the result was read with measure the diameter of Inhibition Zone [9] alcoholic (methanol-ethanol) extracts were prepared by weighing 100 g of dried plant powder in 500 ml of methanol and ethanol at a concentration of 95% and 80% [10], and left soaked for 24 hours at room temperature then filtered by gauze to get rid of large portions. The filter was then dried by the oven to obtain the extract of the plant, weight of the powder and collected in sterile containers until use, while the flavonoid extract was prepared according to the method[11].By using a device Soxhlet extractor weighing 15 g of dry and ground sample, well wrapped in a filter paper and placed in a Thimble in the soxhlet extractor apparatus and placed in the beaker a sufficient amount of ethanol (200 ml), the extraction process is carried out for 6-8 hours using a water bath until the extract is obtained and filled in special tubes until used .

Results and discussion

The results showed that the alcoholic (ethanolic) extracts of *Salvia officinalis* .L and *Costus speciosus* L. no significant difference as inhibition effect between the concentration of these extract that used to *Proteus spp* . and *E.coli* that isolated from urin samples at significant level ($p<0.05$) compared with antibiotics, while the alcoholic (methanolic) extract of *Costus speciosus* L. demonstrated no inhibition zone in concentration(50) at mean (1.5±2.12) of these extract with no significant difference of all concentration that used to the bacteria . The methanolic extract of *Salvia officinalis* .L showed increase significant difference at high concentration of this extract with mean inhibition zone that reached to(7.5 ±10.61) in high concentration of this study (70), while these extract showed no significant difference in *Costus speciosus* L. in *Proteus spp* .bacteria but *E . coli* demonstrated significant difference in concentration (10, 70) of these bacteria that isolated from urin samples . The flavonoid extracts of *Costus speciosus* L. explained no significant inhibitory effect of these plants extracts of all concentration that used in this study , but the same extract showed the extend of significant inhibitory in concentration (10, 50,70) adverb apparentiy in tabe (1) and (2) , These results corresponding with the research Al- Shekhany [12], where this may be attributed to synergistic action of active substance in that plant that inhibit microbial growth in case of use the alcoholic extract (ethanol , methanol) of these plants represented with inhibitory activity against gram positive and negative bacteria especially *proteus spp* . and *E. coli* this result agreed with the researchers [13] and [14] that explained the combination of inhibitory activity with the fact that the majority of active substance that may have been presented in most plant extracts that studied ,where this effect led to the deposition of many active substance including alkaloid , flavonoid and phenols, The combined effect was shown to inhibit microbial growth in this study .

Table (1): Inhibitory effect of alcoholic extract (ethanol / methanol). and flavonoids extract in *Proteus mirabilis* isolated from urine inhibition zone (mm)

Type of treatment	Concentration Mg/ml	Mean ± SD	Type of treatment	Concentration Mg/ ml	Mean ± SD
ethanolic extracts of *Costus speciosus*. L	5	0.0 ±0.0 n.s	Ethanolic extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	0.0 ±0.0 n.s		10	0.0 ± 0.0 n.s
	50	0.0 ±0.0 n.s		50	0.0 ± 0.0 n.s
	70	0.0 ±0.0 n.s		70	0.0 ± 0.0 n.s
Methanolic extracts of *Costus speciosus*. L	5	0.0 ±0.0 n.s	methanolic extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	0.0 ±0.0 n.s		10	6.54±7*
	50	2.12±1.5 n.s		50	9.01±6.37*
	70	0.0 ± 0.0 n.s		70	10.61±7.5*
Flavonoid extracts of *Costus speciosus*. L	5	0.0 ± 0.0 n.s	Flavonoid extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	0.0 ± 0.0 n.s		10	6.89±12.62*
	50	1.23±0.87 n.s		50	2.29±5.37*
	70	2.59±1.83 n.s		70	4.24±7.5*
n.s: no significant differences at $p \leq 0.05$. * There is a significant differences at $p \leq 0.05$			Nitrofurantoin		0.60±0.85
			Levofloxacin		0.96±3.51
			Amikacin		1.11±2.9
			Trimethoprim		0.54±2.05
			Gentamycin		0.38±1.98
			Ciprofloxacin		0.68±1.65
			Amoxicillin		0.67±0.4
			Norfloxacin		0.50±2.25

Table (2): Inhibitory effect of aquatic plant extracts (hot / cold), alcoholic extract (ethanol/ methanol). and flavonoids extract in *E. coli* isolated from urine inhibition zone (mm)

Type of treatment	Concentration Mg/ml	Mean±SD	Type of treatment	Concentration Mg/ml	Mean±SD
ethanolic extracts of *Costus speciosus*. L	5	0.0 ± 0.0 n.s	Ethanolic extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	0.0 ± 0.0 n.s		10	0.0 ± 0.0 n.s
	50	0.0 ± 0.0 n.s		50	0.0 ± 0.0 n.s
	70	0.0 ± 0.0 n.s		70	0.0 ± 0.0 n.s
Methanolic extracts of *Costus speciosus*. L	5	0.0 ± 0.0 n.s	methanolic extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	1.94±1.18 n.s		10	3.47±3.15 n.s
	50	5.46±4.37		50	6.08±6.06*
	70	7.09±4.93*		70	6.84±5.12*
Flavonoid extracts of *Costus speciosus*. L	5	0.0 ± 0.0 n.s	Flavonoid extracts of *Salvia officinalis* .L	5	0.0 ± 0.0 n.s
	10	0.0 ± 0.0 n.s		10	8.26±13.12*
	50	0.0 ± 0.0 n.s		50	6.24±3.90 n.s
	70	3.44±9.03*		70	7.05±4.75*
n.s: no significant differences at $p \leq 0.05$. * There is a significant differences at $p \leq 0.05$			Nitrofurantoin		0.73±2.22
			Levofloxacin		1.23±3.58
			Amikacin		0.59±1.15
			Trimethoprim		2.07±1.35
			Gentamycin		0.78±1.75
			Ciprofloxacin		1.02±1.63
			Amoxicillin		0.79±0.7
			Norfloxacin		1.13±2.63

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تأثير بعض مستخلصات نباتي الميرمية *Salvia officinalis* L. والقسط الهندي *Costus speciosus* L. في نمو بعض انواع البكتريا المعزولة من التهابات المسالك البولية في مدينة سامراء

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الملخص

هدفت الدراسة الحالية الى معرفة التأثير التثبيطي للمستخلصات النباتيةالكحولية (الميثانولي – الايثانولي) ومستخلص الفلافونيد بطريقة الانتشار عبر الحفر لنباتي الميرمية *Salvia officinalis* L. والقسط الهندي *Costus speciosus* L. على الانواع البكتيرية *Escherichia.coli* و *Proteus spp.* المعزولة من المرضى المصابين بالتهابات المسالك البولية في مدينة سامراء، حيث جمعت العينات ضمن المدة من 2018/7/15 ولغاية 2018/9/30 إذ تبين من خلال النتائج ان المستخلصات النباتية المستعملة في الدراسة أظهرت فعلاً تثبيطيا متباينا ضد الانواع البكتيرية قيد الدراسة فقد اختلف هذا التأثير بأختلاف التراكيز المستعملة ونوع المستخلص النباتي، أذ أظهرت بكتريا *E.coli* ارتفاعا معنويا عند مستوى معنوي ($p<0.05$) في قطر التثبيط للمستخلص الكحولي (الايثانولي) لنبات الميرمية (10.61±7.5) عند أعلى تركيز مستعمل ، بينما أظهرت تلك البكتريا عدم وجود فرق معنوي في مستوى قطر التثبيط لمستخلص نبات الميرمية الكحولي (الايثانولي)، فيما سجل مستخلص الفلافونيد لنبات الميرمية فرقا معنويا لمعظم التراكيز المستعملة مقارنة مع المضادات الحياتية المستعملة في الدراسة. أما فيما يخص بكتريا *Proteus spp.* فقد لوحظ عدم وجود فروقات معنوية لكل من نباتي القسط الهندي والميرمية بأستخدام المستخلص الكحولي الايثانولي، بينما أظهر نباتي القسط الهندي والميرمية وجود فروقات معنوية في معظم التراكيز المستعملة للمستخلص الميثانولي، أما بالنسبة لمستخلص الفلافونيد فقد أظهر عدم وجود فرق معنوي في نبات القسط الهندي، فينما أظهر ذلك المستخلص وجود أرتفاع معنوي في مستخلص الفلافونيد لنبات الميرمية نظرا لكون مستخلص الفلافونيد من المواد الفعالة التي تم أستخلاصها مختبريا لكلا النباتين أكثر تأثير تثبيطي في النمو الميكروبي من المستخلصات الكحولية (الايثانولية – الميثانولية) للنباتين.