Effect of some plant extracts against bacterial species isolated from urinary tract infection patients

Astabraq A. Mukalaf [1], Amal A. Kareem*[2] and Rabia Ali Aboud[3]

[(1,3)] Department of medical analysis, College of Health and medical Technology, Middle Technical University

[(2)] Research Unit, Collage of Health and medical Technology, Middle Technical University

E-mail (*): amal.aziz68@yahoo.com

Abstract

Antibacterial activity of aqueous plants extracts (*Plantago ovata, Cucurbita moschata and Rosmarinus officinalis*) prepared with different concentrations were investigated against some bacterial genus isolated from urinary tract infection (UTI) patients.

Methods: Urine samples were collected from UTI patients and bacterial genus were identified by biochemical tests. Antibacterial activity of aqueous plants extracts (*Plantago ovata ,Cucurbita moschata and Rosmarinus officinalis*) were determined by agar diffusion method.

Results: Rosemary watery extract were potentially effective against *S.aureus* isolates (diameter of inhibition zone was 28mm) . Plantago watery extract was the most active against *Proteus spp. and Pseudomonas spp* (diameter of inhibition zone 10mm). Cucurbita moschata watery extract exhibited the highest inhibitory effect *on Proteus spp.and E.coli* with (8mm,10mm) inhibition zone. We are of the opinion that aqueous plants extracts (*Plantago ovata. Cucurbita moschata and Rosmarinus officinalis*) could potentially be used for treatment of UTIs especially to the tested microorganisms.

Keywords: *UTI, Rosemary, Plantago ovata, Cucurbita moschata, watery extract, inhibition zone.*

تاثير بعض المستخلصات النباتية على البكتيريا المعزولة من المصابين بالتهاب المجاري البولية

م.د. إستبرق علي مكلف[1] ، أ.م.د. آمال عزيز كريم[2] و أ.م. ربيعة علي عبود [3]

الخلاصة

حضرت تراكيز مختلفة لمستخلصات مائية لنباتات (القرع ,لسان الحمل ,الورد الماوي) وقيست الفعالية الضد بكتيرية لبعض اجناس البكتيريا المعزولة من المرضى المصابين بالتهاب المجاري البولية .طرق العمل: جمعت عينات الادرار من المرضى المصابين بالتهاب المجاري البولية وشخصت اجناس البكتيريا باختبارات الكيمياء الحيوية .حددت الفعالية الضد بكتيرية للمستخلصات النباتية المائية بطريقة انتشار الهلام .النتائج:اظهر المستخلص المائي للورد الماوي تاثيرا ضد عزلات بكتيريا العنقوديات الذهبية (قطر منطقة التثبيط 28 ملم).واظهر المستخلص المائي لنبات لسان لحمل فعالية ضد بكتريا *Proteus spp.* و *Pseudomonas spp.* (قطر منطقة التثبيط 10 ملم) اما المستخلص المائي لنبات القرع اظهر تثبيط على بكتيريا *Proteus spp* و *Escherichia.coli* . (قطر منطقة التثبيط *8* ملم و10 ملم). ولهذا نحن مع مبدا استخدام المستخلصات النباتية المذكوة انفا لعلاج التهاب المجاري البولية خصوصا الاحياء المجهرية المشخصة.

الكلمات المفتاحية : نبات اكليل الجبل ، نبات لسان الحمل ، نبات القرع ، الخلاصة المائية ، مناطق التثبيط

Introduction

Urinary tract infections characterizes one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today [1]. The incidence of UTI is greater in women as compared to men which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors [2]. It is estimated that about 35% of healthy women suffer symptoms of Urinary tract infection at some stages in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency [3].

Many types of bacteria causing (UTIs) inducing inflammation within the urinary tract. Nearly 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder.Organisms causing UTI are derived primarily from the aerobic members of the fecal flora . [4]

The use of medicinal plants has become increasingly widespread and has been enriched by the vast biodiversity and the mixing of indigenous, African, and European cultures [5]. The antimicrobial properties of plant extracts and isolated compounds have been investigated by a number of researchers worldwide [6]. In Brazil, the consumption of herbal medicines is growing at a rate of 20% a year, following the re-evaluation of the global use of medicinal plants for the treatment of several diseases [7].

Rosmarinus officinalis Linnaeus, commonly known as rosemary, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, belonging to the family Lamiaceae [8]. Properties as a spice, its antibacterial, anti-inflammatory activity, the role of modulator of the nervous system, and hyperglycaemia, OluwatuyI *et al*., 1994, isolated five compounds from the extract ethanolic of R. officinalis: carnosic acid, carnosol, 12-methoxy-transcarnosic acid, 12-methoxy-trans-carnosic acid and 12-methoxy-cis-carnosic acid to test the antibacterial activity against MDR bacterial with efflux pump [9]. Most of the studies have shown synergistic activity of several compounds resulting from the secondary metabolism of the plant as having activity against MDR bacteria. Gram negative bacteria are responsible for most of antibiotic resistant infectious diseases due to the impermeability of external membrane [10].

Plantago ovata has been endowed with diverse pharmaceutical and pharmacological activities. It is widely used in numerous medicines owing to its both pharmaceutical properties such as mucilage, superdisintegrant, gelling agent, suspending agent as well as pharmacological actions like anti-diarrheal, anti-constipation, wound healer, hypocholestrolemic and hypoglycemic [11].*Cucurbita moschata* (Pumpkin) seeds have a high nutritional value, provides good quality oil, and excellent source of protein [12]. In addition to good health benefits, pumpkin seeds are less expensive and are widely distributed.

In the traditional medicine in North America and Mexico, pumpkin seeds have been used as an anthelmintic agent and for supportive treatment in functional disorders of the bladder [12]. The healing powers of plants have been used for hundreds of years; about 80% of the available therapeutic substances are originated from medicinal plants [13,14]. Scientists showed that the plants had medicinal properties for their biological activities ranging from antimicrobial to antitumor. The antimicrobial activity of plants has many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [15].

Methods

Collection urine samples

Midstream urine samples were collected in sterile containers by using clean and sterile catch method recommended by [16]. Then culture on nutrient agar, blood agar and MacConkey agar plates, using sterile standard loop (1ml) then incubated at 37ºC for 24 hours. Gram negative isolates were identified by standard biochemical tests. (Enterobactericeae pathogens) identified by: 1. IMVIC test (indol production, methyl red, vogas-proskauer and citrate utilization). 2. TSI (triple sugar iron). 3. Gelatin liquefaction. Gram positive isolates were identified by: 1. Catalase test. 2. Coagulase test (tube and slide method). 3. Mannitol salt agar (for S. aureus). All the tests above done according to [17]. Collection urine samples and identification of bacterial isolate .

Preparation of plant aqueous extract

One hundred grams of plants powder was dissolved in 1 liter of water and kept on the automatic shaker for 24 hours for extraction of water-soluble compounds. Extraction was allowed to proceed for 48 h. The mixture was let to semi dry yelling thick crude then the concentration were prepared from this crude [18].

Detection of antibacterial activity by agar diffusion assay [19]

After culturing the organisms separately in nutrient broth ,100mg concentration of the leaf and seed mixture of plant extract prepared , the broth was inoculated onto freshly prepared Muller Hinton agar plates to identified the effect of that concentration of plant extracts on various genus of infectious bacteria, then incubated culture at 37°C for 24 h .

The agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. The agar was left to set and in each of plates (10 mm in diameter) was cut using a sterile pasture pipette and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micro liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 h. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated .

Results

About fifty samples of urine collected from patients suffering from urinary tract infection (UTI) from Baghdad hospital midstream urine samples were collected and the results revealed high percentage of UTI infections in age group (adults) 54% then 1day-12 months ,1-3 years and children 24%, 12%, and 10% respectively as showed in table (1).

Table (1): Samples distribution of UTI infected patients and age groups

Age group	No.of patients	% of Patients no.
1 day-12 months	12	24
1-3 years	6	12
Children	5	10
Adults	27	54
Total	50	100

Table (2) represented the percentage of bacterial causing UTI infection and different age groups that showen infected with many types of bacteria at age group (1day-12 months) specially with *P.aeuroginosa* 50% . *Proteus spp,* 55.5% infected age groups (1-10 years) , *P.aeuroginosa* 50% more infected age groups 10-20 years than other types of bacteria , *S.aureus* 100% highly causing UTI in age groups 20-30 years ,while *E.coli* in30-40 years was less infected 12%, 40-50 years revealed *Klebseilla sp* with 16.6% and *E.coli* 16% ,50-60 years revealed not infected with any type of bacteria while 60-70 years infected with *Enterobacter spp*. 14.2% and *E.coli* only.

Table (2): The percentage of bacterial infection of UTI and different age groups

Age group	**%. of *E.coli***	**%. of *Proteus spp.***	**%. of *Enterobacter spp.***	**%. of *Klebseilla spp.***	**%. of *P.aeurogin osa***	**%. of *S.aureus***
1day-12 months	20	44.4	42.8	0	50	0
1-10years	16	55.5	0	50	0	0
10-20 years	16	0	0	33.3	50	0
20-30 years	16	0	42.8	0	0	100
30-40 years	12	0	0	0	0	0
40-50 years	16	0	0	16.6	0	0
50-60 years	0	0	0	0	0	0
60-70 years	4	0	14.2	0	0	0
Total	100	100	100	100	100	100

The diameter of inhibition zones by (mm.) according to watery extract of some plant (*Rosmarinu officinalis* , *Plantago ovat*, *Cucurbita moschata*) at concentration (100mg/ml) as appeared in table (3) and figures (1 to 6) which give goal to *Rosmarinu officinalis* inhibition zone to all types of bacteria in this study compared with other kinds of watery plant extracts.

Table (3): Diameter of inhibition zones by (mm.) according to watery extract of some plant extract (*Rosmarinu officinalis*, *Plantago ovat*, *Cucurbita moschata*) at concentration (100mg/ml)

Type of bacteria	Watery plant extract inhibition zone (mm)		
	Rosmarinu officinalis	***Plantago ovat***	***Cucurbita moschata***
Staphylococcus aureus	28	8	7
Klebsiella spp.	12	7	8
Escherichia coli	12	10	10
Pseudomonas spp.	10	7	6
Proteus spp.	10	10	10
Enterobacter spp.	11	7	0

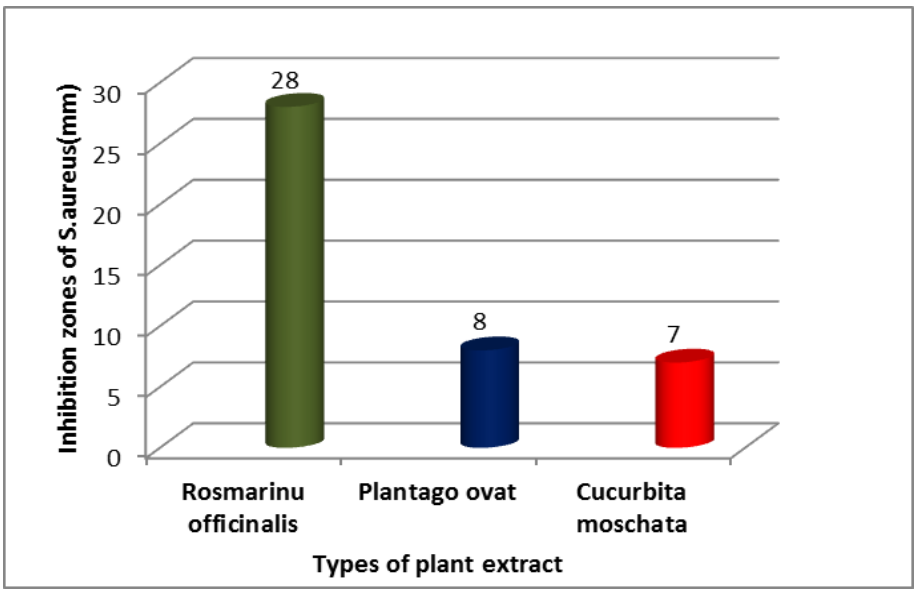

Figure (1): Effect of three aqueous plant extract in *Staphylococcus aureus* bacteria.

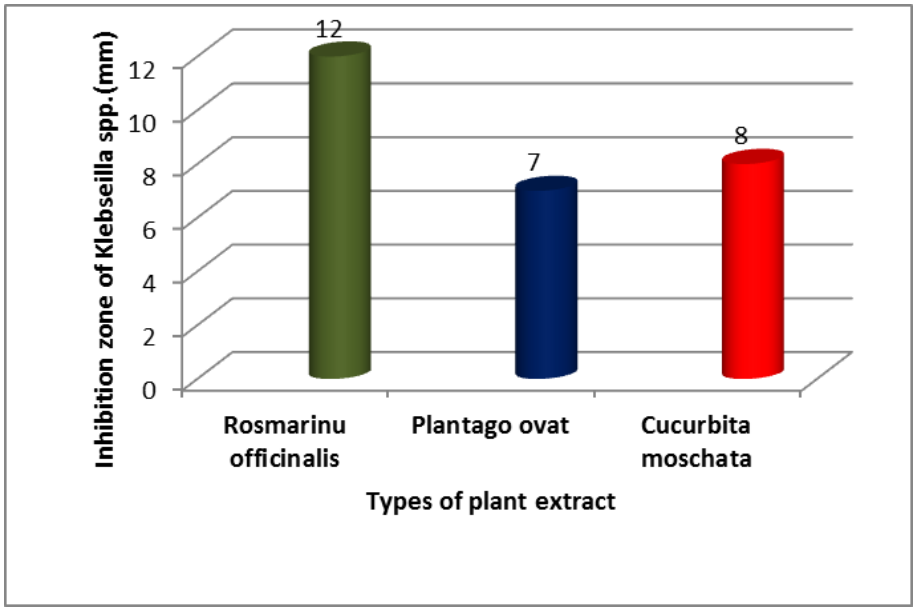

Figure (2): Effect of three aqueous plant extract in *Klebseilla* spp bacteria

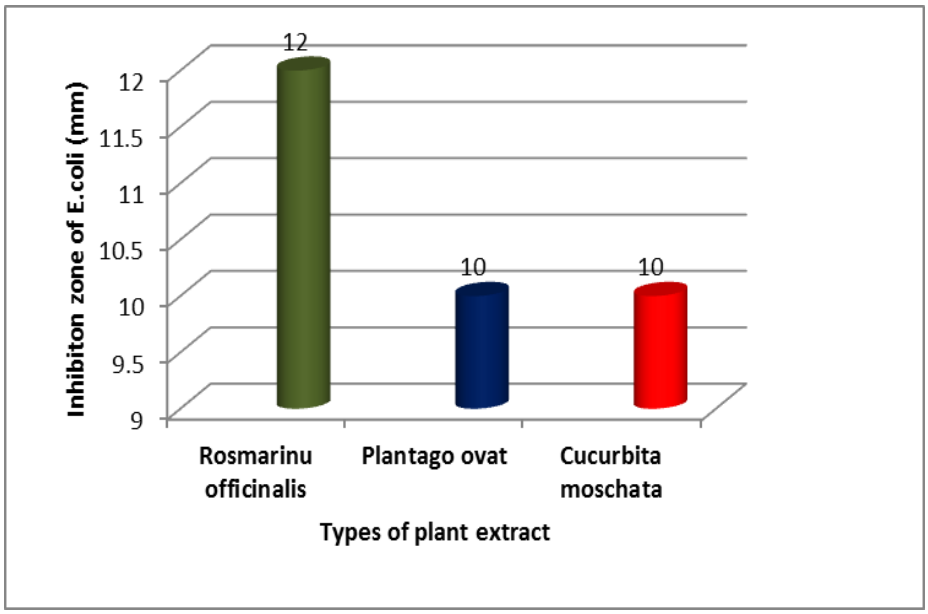

Figure (3): Effect of three aqeous plant extract in *Escherichia coli* bacteria.

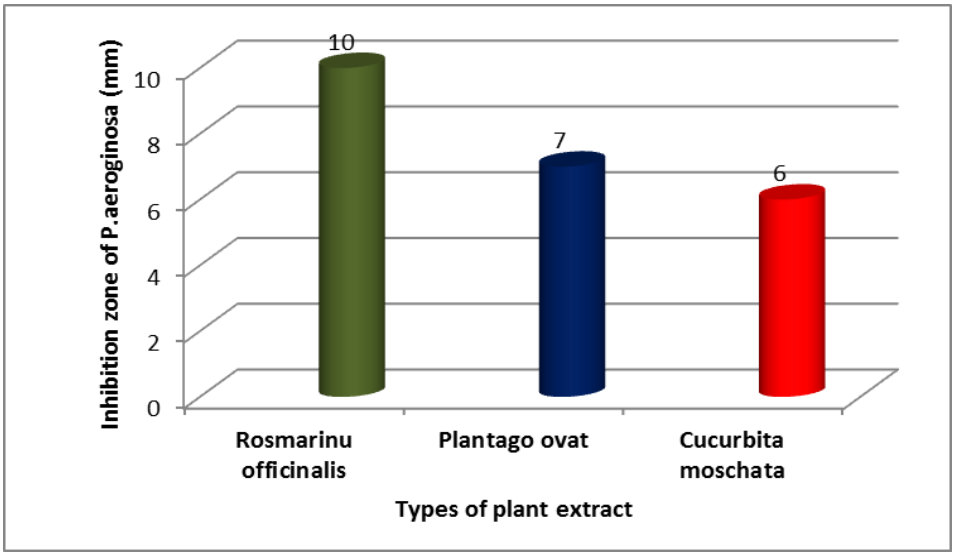

Figure (4): Effect of three aqueous plant extract in *Pseudomonas aeruginosa* bacteria

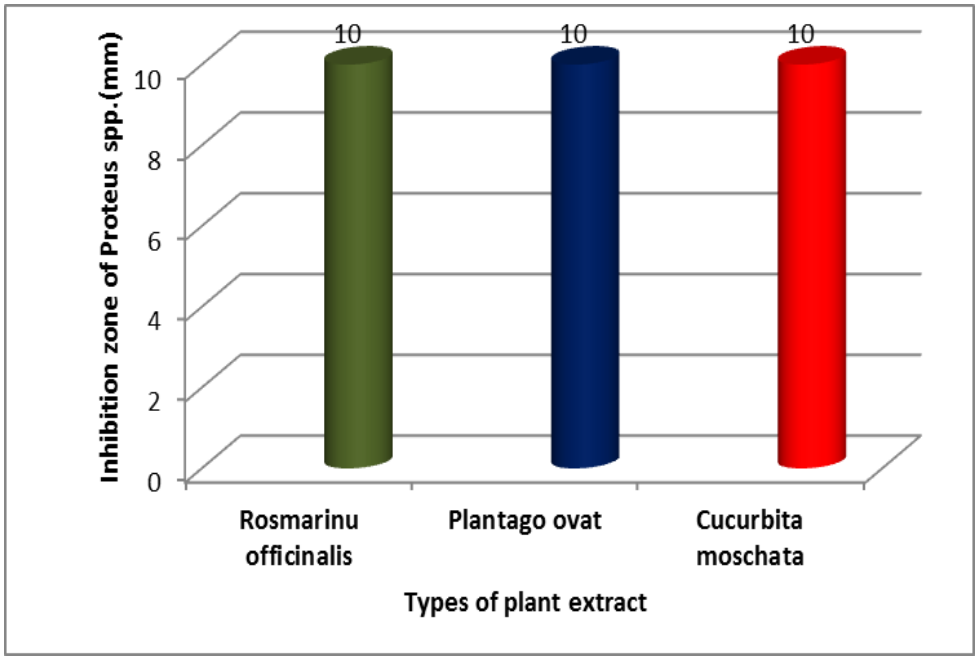

Figure (5): Effect of three aqueous plant extract in *Proteus spp* bacteria.

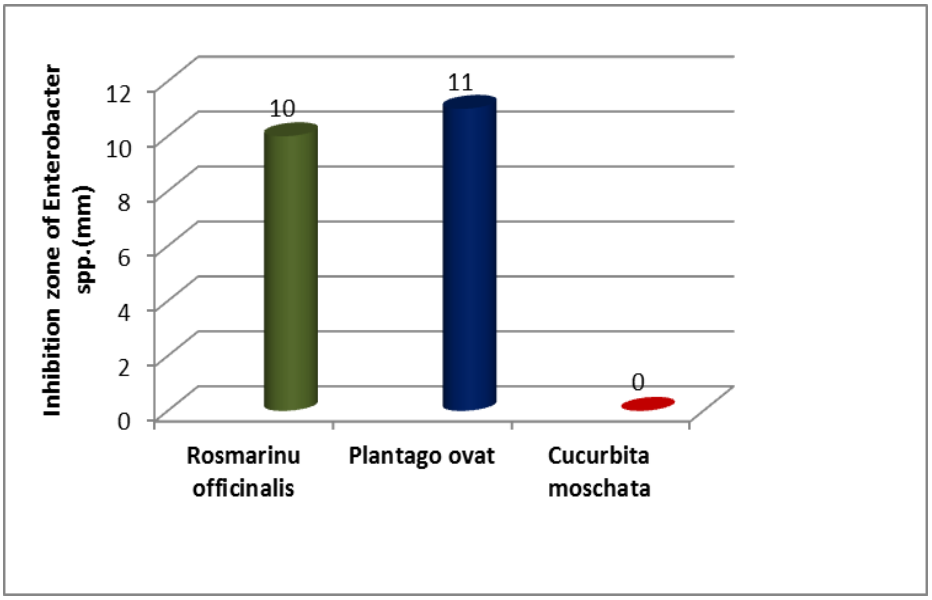

Figure (6): Effect of three aqueous plant extract in *Enterobacter* spp. Bacteria.

Discussion

Plants have always been a common source of medicaments, which makes it reasonable for decision-makers to identify locally available plants that could usefully use in therapy. This study determined the inhibitory activity of different extracted crude proteins, (extracted from seeds and leaves) for growth of different microorganisms .

The effect of some plant extracts on some pathogenic bacteria isolated from patients with urinary tract showed *E.coli* was the highest bacteria seen in different age groups that was agree with may researchers because it come from feces [20] .

UTI noticed in adult patients 54% more than new born (1 day -12 month) 24%. All inection was at age group (1day-12 month) because of newborns and young infants present an immature immune system, which makes them more susceptible to infectious agents present during this period . It is known that newborns are more vulnerable to infections than children and adults. Observed differences in the innate and adaptive immunity are responsible for decreased

neonate's defenses [21], the higher inhibition zone was 28 mm by *Rosmarinu officinalis* watery extract on *S.aureus*, Moreno et al. (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol [22].

While *Plantago ovat* watery extract give higher inhibition zone 10mm effected on *Proteus* spp. And *Pseudomonas* spp. *P. ovata* is one of the most important medicinal plants that have been used since ancient times for various reasons in Iran and have high antibacterial properties. Its effect on various bacteria, including Staphylococcus aureus, S. pyogenes and Bordetella bronchiseptica has been determined [23] and *Cucurbita moschata* watery extract affected on *Proteus* spp.and *E.coli* (8mm,10mm) inhibition zone respectively . *Cucurbita moschata* could be a potential and safe antimicrobial agent in future. The present study revealed that all the microbes tested were sensitive to different extracts from pumpkin .

However, dichloromethane extract of pumpkin showed remarkable antimicrobial activity against most of the bacterial strains and could be classified as a good source for potent natural antimicrobial agent against microbes taken into account in this study [24].

Conclusion

These plants are available and cheap because they grow wildly in nature or cultivated and helpful to treat UTI in patients.

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