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RESEARCH ARTICLE

The inhibitory effect of Calendula Officinalis and Salvia Officinalis on growth of some bacterial isolates from urinary tract infections

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Abstract

This study was carried out to evaluate Calendula Officinalis and Salvia Officinalis. The antibacterial activity of alcoholic and watery extracts of these plants was performed on bacteria isolated from human urinary tract infections including (*E.coli, Klebsiella , Proteus and pseudomonas*).we studied the bacterial sensitivity to (9) antibiotics Rifampin (RA) ,Trimethoprim (TMP),Ceftriaxone (CRO), Azithromycine (AZM),Doxycycline(DO), Eerthromycin (ER), Ampcilline (AM)Sulfatmethoxazole(SXT) Cloxacillin (CX).
Distilled water Calendula officinalis extract gave the highest zone of inhibition *Escherichia coli O157*. The ethanol and water extract indicated significant antibacterial activity (growth inhibition zone diameters ranging from 9 to 30 mm) against *E.coli O 157* Similar results have been followed by *pseudomonas* and followed by *Proteus* and followed by *klebsiella*.

Distilled water Salvia Officinalis extract gave the highest zone of inhibition on *Proteus* as the ethanol and water extract indicated significant antibacterial activity (growth inhibition zone diameters ranging from 15 to 29 mm) against protease , Similar results have been followed by *Escherichia coli O157* (18 to 28) and followed by , *Klebsiella* (17- 28) and followed by *Psudomonous*.
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Introduction:

Urinary tract infection (UTI) is the second most common infectious presentation in community medical practice.
Worldwide about 150 million people are diagnosed with UTI each year, and UTI are classified as uncomplicated or complicated [1].Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency, and occasionally suprapubic tenderness. Acute pyelonephritis describes the clinical syndrome characterized by flank pain or tenderness, or both, and fever, often associated with dysuria, urgency, and frequency [2]. More than (95%) of urinary tract infections are caused by a single bacterial species. *E. coli* is the most frequent infecting organism in acute infection [3, 4].
Klebsiella, *Staphylococci*, *Enterobacter*, *Proteus*, *Pseudomonas*, and *Enterococci* species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population [5].

Medicinal plants are usually used for Ayurvedic, Unani and other treatments in rural areas. Recent discovery shows that these plants have fewer side effects than the Allopathic medicine. The medicinal importance of these plants is due to presence of chemical substances in them. Some of the important bioactive compounds are Alkaloids, Glycosides, Triterpenoids, Terpenoids Flavonoids, Phenols, Reducing sugars, Saponins, Steroids and Tannins [6].

Calendula has antibacterial and antifungal activity [7,8] , and it has been used for the treatment of burns, abrasions, skin inflammations, ulcers, wounds and eczema[9]. It has been used internally for the treatment of gastiritis , bleeding of duodenal ulcers and colitis [10] Researchers from Venezuela examined extracts of dried flowers from Calendula officinalis for its inhibitory effects on the human immunodeficiency virus type1(HIV 1) [11] Calendula officinalis extracts show anti-cancer effects in vitro studies on tumor cell lines, derive from Leukemias, melanomas, fibrosacomas, breast, prostate, cervix, lung and pancreas [12]

In some studies, Calendula Potential In vitro data, was inactive against *Aerobacter aerogenes, Bacillus subtilis, E. coli, Klebsiella pneumonia, Proteus morganii, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Strep faecalis, Staphylococcus aureus*[13,14,15,16].

Salvia officinalis L. called sage is a popular plant belongs to the family of Labiatae. [17, 18] More recent studies on the biological activity of sage showed that the plant possesses some antimicrobial and antioxidant properties. It has tonic stimulant properties and it is also used in perfumery, in cosmetics [19, 20].

The essential oil extracted from S. officinalis, L., used as a medicinal herb, has antibacterial activity due to the presence of 1, 8-cineoland of an antifungal substance [21].

S. officinalis has many different uses; essentially, it has been used as herbal remedy for a wide range of disorders and illnesses by applying it either internally or externally. It is employed as diuretic, tonic, menstruation's promoter, local styptic, antiseptic, anti inflammatory, antifungal and spasmodic pain relief [22]. It is also used as treatment for dysentery, coughing, indigestion, ulcer, varicose veins, insect bites [23; 24]. On the other hand, it is used for treating nervous conditions, trembling, should be used carefully since large doses can be toxic [25].

Material and methods:

(50) Urine samples were collected from patients, from both sex and average age (2years- 11years) admitted to hospital Central Teaching Hospital of Pediatric.

All samples were inoculated on Nutrient broth. Culture Media used for bacterial isolation include blood agar, Nutrient agar, MaCconky agar, Eosin methylene blue (E.M.B), Sorbitol MaCconky agar with addition Cefixime tolerate to isolate *E.coli O157* and tested by latex kit ,and the biochemical properties were tested depending on the method of [26] bio-chemical is (Sulfur Indole Motility Test (SIM), Triple Sugar Iron (TSI), Simmon Citrate Test (SC), Urease and methyl red–Voges-Proskauer Test (MR/VP).Then sensitive to antibiotic listed below (Rifampin (RA), Trimethoprim (TMP), Ceftriaxone (CRO), Azithromycine (AZM), Doxycycline(DO), Eerthromycin (ER), Ampcilline (AM)Sulfatmethoxazole(SXT), Cloxacillin (CX) and sensitive to plants .

Plant material:

Salvia officinalis and calendula officinalis were procured from local market in Baghdad city .

Plant extract:

Plants were blended in an electrical blender (sharp, Japan) until obtained final powder , weight 150 gm of plant in flask and added 450 ml of alcohol solution 70% Ethanol, put the flask in freezer (-20ċ) for (9) days after that put it in on a magnetic stirrer for 20 minutes, filter the extract by gauze then filter paper (240mm) in size then put in oven 37ċ for 3days to preparation of dose concentration according to[27] as table (1) .

Preparation of inoculums:

Suspension of organism was prepared as per McFarland nephalometer standard [28].A 24hours old culture was used for the preparation of bacterial suspensions. The suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9%w/v) and the turbidity was adjusted such that it contained approximately 1.5x108cells/ml.

Agar well diffusion method:

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121˚c for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160˚c for (1.5) hour .30ml of sterile nutrient agar was seeded by organisms (about 2ml according to McFarland standard).

Pores were made on the medium using a sterile borer and 0.1ml of the extracts were added to respective pore. The Petri plates seeded with organisms containing extracts were kept in refrigerator at 4˚c for 1hour to facilitate the diffusion of the extracts into the media. After diffusion the Petri plates were incubated at 37˚c for 24 hours in an incubator and zone of inhibition was observed and measured using a scale.

Antimicrobial Susceptibility Testing:

The Kirby-Bauer method is based on the diffusion of antibiotics impregnated in previously dried paper disks, deposited on the surface of Muller-Hinton agar.

Transfer 4 to 5 colonies in an appropriate broth (Tryptone Soy broth: -Place in a 37°C incubator (in general 2 to 5 hours) until an opacity is obtained which is equivalent to the standard opacity of a barium sulfate suspension (density of 0.5 on the McFarland scale).Add a sterile swab to the inoculums adjusted to the opacity standard, and drain excess broth by pressing the swab on the walls of the tube. Inoculate Muller-Hinton agar. The swab should be passed 2 or 3 times over the entire surface in order to obtain homogeneous inoculums. Allow the plates to dry for 10 minutes before depositing the disks. Place the disks using slight pressure to insure good adhesion to the agar. They should be situated at least 15 mm from the edge of the dish and sufficiently far apart so the inhibition zones do not overlap. Measure the inhibition zone with a compass. Refer to the table for interpreting inhibition zones furnished by the suppliers of antibiotic disks in order to establish the correlation between the inhibition zone and the minimal inhibitory concentration (M.I.C.) **[**29**].**

Results:

Bacterial result showed that bacterial isolates (57%) found in 75 urine samples. The current study showed that *E.coli* (22%) was the commonest bacterial isolates followed by *Klebsiella* (15%), *Proteus* (13%), *and pseudomonas* (7%).

Some samples expressed mixed bacterial isolates and other showed pure single bacterial colonies.

All the bacterial pathogens used in this work demonstrated susceptibility to the Ethanol, Distilled water using extracts of Calendula officinalis and of Salvia officinalis are shown in tables (30,31). Distilled water Calendula officinalis extract gave the highest zone of inhibition (30mm) 200 mg\ ml on *Escherichia coli O157* as table(2) The ethanol and water extract indicated significant antibacterial activity (growth inhibition zone diameters ranging from 9 to 30 mm) against *E.coli O 157* Similar results have been followed by *psudomonas* (9 to 27) and followed by protease (17-28) and followed by *klebsiella* (10-24) these results are agreement with some previous studies with the work of Chakraborthy who showed antimicrobial activity of the leaf extracts of Calendula officinalis over *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Candida albicans and Aspergillus niger*[32] and [33],(2011). The antimicrobial activity of plants extracts may be due to tannins,saponins,phenolic compounds ,essential oils and flavonoids [38].

With *Proteus* the highest zone of inhibition was (28) mm in diameter. With *klebsil*. Highest zone of inhibition recorded was (24) mm in diameter. Within confirm by previous study of [34]

Distilled water Salvia officinalis extract gave the highest zone of inhibition (29mm) 200 mg\ ml on protease as table (3) The ethanol and water extract indicated significant antibacterial activity (growth inhibition zone diameters ranging from 15 to 29 mm) against protease , Similar results have been followed by Escherichia coli O157 (18 to 28) and followed by , *Klebsiella* (17- 28) and followed by *Psudomonous*(11-28) these results are agreement with some previous studies with the work of [35]et al.,(2004)showed the greater efficiency of Salvia officinalis L. The different species analyzed were over (96%) effective against *Escherichia coli*, (100%) against *Klebsiella pneumoniae*, over (83%) against *Proteus mirabilis*, (75%) against *Morganella morganii*, (100%) against *Enterobacter aerogenes*, and (100%) against *Klebsiella oxytoca.* There was no antimicrobial activity on *Pseudomonas aeruginosa*.

The higher resistance among Gram-negative bacteria might be due to the presence of phospholipidic membrane which limits the effect of oil on the cell membrane [36]

While [37] et al., 2011 found the essential oil showed a temporary bacteriostatic effect on *Escherichia coli*, *Salmonella typhi*, as well as *Pseudomonas aeruoginosa*. In comparison with most known antibiotics, the efficiency of S. officinalis essential oil was much better, especially against bacteria resistant to antibiotic**.**

Table (1): preparation of dose concentration:

Volume of solvent ml (distilled water &ethanol)	Weight of dried extracts	Concentration of extract
10	0.5	50%
10	1	100%
10	1.5	150%
10	2	200%

Table (2): In vitro antibacterial activity of part of Calendula officinalis flower:

Bacteria	Concentration of extract	Zone of inhibition (mm diameter)	
		ethanol	water
E .coli O157	50 mg\ ml	9	19
	100 mg\ ml	10	26
	150 mg\ ml	11	28
	200 mg\ ml	12	30
E .coli spp.	50 mg\ ml	8	14
	100 mg\ ml	11	16
	150 mg\ ml	14	21
	200 mg\ ml	18	24
Proteus	50 mg\ ml	21	17
	100 mg\ ml	23	19
	150 mg\ ml	25	22
	200 mg\ ml	28	23
Klebselia	50 mg\ ml	10	18
	100 mg\ ml	14	20
	150 mg\ ml	17	23
	200 mg\ ml	18	24
Psudomonous	50 mg\ ml	9	16
	100 mg\ ml	12	21
	150 mg\ ml	19	24
	200 mg\ ml	23	27

Table (3) : In vitro antibacterial activity of part of Salvia officinalis flower:

Bacteria	Concentration of extract	Zone of inhibition (mm diameter)	
		ethanol	water
E .coli O157	50 mg\ ml	R	20
	100 mg\ ml	18	23
	150 mg\ ml	20	25
	200 mg\ ml	24	28
E .coli spp.	50 mg\ ml	R	16
	100 mg\ ml	R	19
	150 mg\ ml	22	24
	200 mg\ ml	26	27

Protease	50 mg\ ml	15	20
	100 mg\ ml	18	22
	150 mg\ ml	22	25
	200 mg\ ml	25	29
Klebselia	50 mg\ ml	17	20
	100 mg\ ml	18	23
	150 mg\ ml	21	25
	200 mg\ ml	24	28
Psudomonous	50 mg\ ml	11	13
	100 mg\ ml	14	22
	150 mg\ ml	20	25
	200 mg\ ml	26	28

Table (4) Mean zone of inhibition of antibiotics:

Mean zone of inhibition of antibiotics(mm)					
Antibiotic	*E.coli O157*	*E.coli spp*	*Protease*	*Klebselia*	*Psudomonous*
RA	-R		-R	5	-R
TMP	-R		-R	20	-R
CRO	-R		10	-R	-R
AZM	28		-R	30	18
DO	9		-R	6	10
ER	8		10	-R	-R
AM	-R		-R	-R	-RA
SXT	23		-R	-R	-R
CX	-R		-R	-R	-R

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