Research Article

Antimicrobial Effects of Five Essential Oils Plant on Multidrug Resistant Bacteria Responsible for Urinary Infections

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ABSTRACT

Urinary tract infections (UTIs) are ones of the most common infections worldwide. Lesions which are mainly caused by microorganisms that inhabiting in hospitals, known and characterized by their both resistance against antibiotics and high ability of biofilms formation. In this study, we have evaluated the effect of 5 essential oils medicine plant, which are *Allium cepa L*., and *Allium sativum* were from Amaryllidaceae family, *Elettaria cardamomum maton*, *Zingiber officinale* and *Curcuma longa* of Zingiberaceae family against bacterial species most responsible for UTIs. A total of 8 MDR *E. coli* strains. were tested, which varies between reference strains and clinical multidrug resistant. 5 oils (onion, ginger, garlic, cardamom, turmeric) Onion oil was the most antimicrobial activity and compared with garlic oil. The finding of this study indicate that essential oil appears as an excellent solution for treatment of UTIs, especially against failure problems seen in care services, which are over common in the last years.

Keywords: Urinary tract infections, Essential oils, Antimicrobial activity, Multidrug resistance.

INTRODUCTION

Infections of the urinary tract are frequent and sometimes can induce severe threat both in human and veterinary medicine, mostly affecting dogs and cats[1]. Urinary tract infections (UTI) may be localized to the upper tract (kidney and adjacent ureter) or the lower tract (bladder and adjacent urethra) and more than one organ is often involved[2]. These infections are usually caused by bacteria, mainly those of the intestinal micro flora. Escherichia coli and Enterococcus spp. are the most frequent agents encountered in UTI cases[3]. Even though infections by haematogenous route are possible, bacteria usually colonize the genito-urinary tract by ascendant route. In view of the anatomic structure, females are more prone to UTI than males [4]. Most of UTIs are hospital-acquired infections in which they represent up to 40% and 34% of infections acquired in long-stay units[5]. Regarding pathogenic responsible, Enterobacteriaceae were the most common bacteria detected, in which they causes up to 84.3% of UTIs [6]. Others pathogens are also identified especially Enterococcus faecalis [7] and Staphylococcus spp[8, 9]. UTIs present a real problem currently, not only because these types of infections are in increasing in last years, notably in developed countries, but also the pathogens responsible are commonly multidrug resistant bacteria to antibiotics used in routine[10, 11].

The resistance against antibiotics was detected in several genera, including Escherichia, Enterobacter, Klebsiella, Proteus, Salmonella, Serratia and Pseudomona[12]. There is an urgent need thus, for highlighted new antimicrobial agents which will be used against treatment failures seen in UTIs related to multidrug resistance. Plants preparations have long been used in treatment of all kinds of human infectious diseases, including UTIs. Presently, it's has been clear that plants are a promising and wealthy source for safe and effective new antimicrobial agents [13].

Essential oils, also known as volatile oils, are products of the secondary metabolism of aromatic plants. Various essential oils have been reviewed to possess deferent biological properties such as anti-inflammatory, sedative, digestive, antimicrobial, antiviral, or antioxidant activities[14]. Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, parasitical, insecticidal, medicinal, and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural, and food industries[15]. In recent years, direct killings as well as sensitizing activities against microbes have been reported for essential oils[16]. Essential oils of the genus Origanum L. showed promising antibacterial against uropathogenic E. coli including even multi-drug resistant strains [17, 18, 19]. A recent randomized study in patients with lower uncomplicated UTI showed that non-antibiotic herbal therapy was non-inferior to antibiotic therapy in the treatment of the acute phase of UTI [20]. In addition, essential oils of Pelargonium graveolens and Coriandrum sativum were shown to potentiate the activity of ciprofloxacin and gentamycin, respectively, against selected uropathogens [21, 22].

MATERIALS AND METHODS

Plant material

The present study focussed on collection of five different plants for their essential oil yielding *Allium cepa L*., and *Allium sativum* were from Amaryllidaceae family, *Elettaria cardamomum maton*, *Zingiber officinale* and *Curcuma longa* of Zingiberaceae family.

Freshly collected plant material was thoroughly washed with water, air-dried, and directly subjected for oil extraction. However, lengthy leaves (leaves with 10 cm and above size) were chopped into small pieces. About 150 grams of leaves were subjected to hydro-distillation in a modified Clevenger-type apparatus for about 31/2 hours. Collection, quantification and preservation of essential oil Extracted was collected carefully in a vial and dried with anhydrous sodium sulphate (Na2SO4) to remove the residual moisture and then filtered. The yield of oil was quantified in percentage (w/w) and stored in an airtight vial, maintained at 4oC until further use.

Culture medium and inoculum preparation

The test organisms were sub-cultured onto fresh plates of Mueller–Hinton agar (HiMedia laboratories) for 24 h at 37°C for bacteria respectively. Colonies from these plates were suspended in Mueller–Hinton broth to a turbidity matching 0.5 McFarland standard (10^8 CFU/mL). The media used for antimicrobial assays were Mueller–Hinton agar for bacteria. All were incubated appropriately for a period of 18–24 h

Antimicrobial Resistance of Essential Oils by Well Diffusion Assay

Different assays like disc diffusion assay, well diffusion assay, micro-dilution assay, measurement of minimum inhibitory concentrations are often used for measuring the antimicrobial activity of essential oils and plants based constituents. Generally, agar diffusion method is used when large numbers of oils are tested with large number of test strains. The antibacterial activities of the selected essential oils were determined by Agar well diffusion assay techniques. In this method, 50 µL of standardized inoculum of each test bacterium were spread onto sterile Muller–Hinton Agar. About 8 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 100 µL of the essential oils. The plates were kept at room temperature for 1 h to allow proper diffusion of the oil into agar and then incubated at 37°C for 24 hr. The essential oils having antimicrobial activity inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimetres.

GC-MS Profiling of Essential oils

The essential oil was analysed using GC-MS (Thermo Scientific, Triple quadrupole MS, TSQ 8000) with two fused silica capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm). Injector and detector temperatures were set at 22°C and 25°C, respectively. One micro-litre oil sample diluted with methanol were injected and analysed with the column held initially at 50°C for 1 min and then increased by 5°C/min up to 28°C. Helium was employed as carrier gas (1 mL/min). The identification of the different compounds was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds using library database. Identification of chemical constituents present in essential oils was investigated using gas chromatography.

RESULTS AND DISCUSSION

Studies were conducted on essential oils of plants, to study the antibacterial activity against eight MDR *E. coli* strains. Among the 5 test plants the yield of Essential oils was highest in *Allium cepa L.* (1.18%) while lowest was observed in *Elettaria cardamomum maton* (0.1%). A yield of 0.42% was observed in Curcuma longa and 0.25% was observed in *Zingiber officinale* and *Allium sativum.* (Table 1).

The antimicrobial activity results of *E. coli*, against strains collected from urinary infections show that the most important activity is recorded by *C. cassia*, since this oil showed significant activity against all Gram positive and negative strains including *E. coli* which is known by its resistance to essential oils. In addition, the greater diameters were obtained by this EO with inhibition zone of 39 mm for some strains such as the case of *E. coli*. From the eight strains of *E. coli*, were tested, which varies between reference strains and clinical multidrug resistant. 5 oils (onion, ginger, garlic, cardamom, turmeric) Onion oil was the most antimicrobial activity and compared with garlic oil.

Antimicrobial activity of the five essential oils (onion, ginger, garlic, cardamom, turmeric) was tested against eight *E. coli* isolates. The antibacterial activity of essential oils against each strain is summarized in table given below. Best results were shown by onion oil which was followed by turmeric oil. The maximum zone of inhibition 15mm was shown by onion oil against 4 isolates, while garlic oil showed least antibacterial activity. For most of the isolates garlic showed no antibacterial activity. The antibacterial activity decreased visibly according to decrease in zone size (Table 2).

The results of minimum inhibitory concentrations MICs correlate with those of the zones of inhibition since the smaller MICs were obtained with the larger inhibitions zones and vice versa.

Table 1: Yield of Essential Oil from Selected Plants

Sl. no.	Essential oil from plant	Family	Yield of essential oil (%) in %
1.	*Allium cepa L.*	Amaryllidaceae	1.18
2.	*Curcuma longa*	Zingiberaceae	0.42
3.	*Zingiber officinale*	Zingiberaceae	0.25
4.	*Allium sativum*	Amaryllidaceae	0.25
5.	*Elettaria cardamomum maton*	Zingiberaceae	0.1

Table 2: Antibacterial Activity of Essential Oils

Zone of Inhibition in diameter (mm)					
Isolates	**Onion Essential oil**	**Garlic Essential oil**	**Turmeric Essential oil**	**Ginger Essential oil**	**Cardamom Essential oil**
U1	20	20	NZ	NZ	NZ
U5	18	NZ	19	NZ	NZ
U15	15	NZ	NZ	NZ	NZ
U25	19	20	NZ	NZ	NZ
U26	26	NZ	28	NZ	NZ
U51	22	NZ	NZ	NZ	NZ
U58	25	NZ	NZ	25	NZ
U65	NZ	NZ	NZ	NZ	NZ

∗NZ – No Zone

Table 3: GC-MS Profiling of Essential Oils

S No	Compound Name	% of Probability	S. No	Compound Name	% of Probability
1	Methyl propyl disulfide	78.21	30	à-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-,cyclic methylboronate	12.54
2	Methyl isopropyl disulphide	20.89	31	W-18	10.11
3	1,2-Dithiolane-1-oxide	0.20	32	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	9.71
4	2-Propanesulfinic acid, methyl ester	0.13	33	á-N-Acetylneuraminic acid, methyl ester-2-methyl-7,9-methyl-boronate-3,8-di(trim ethylsilyl)-	6.86
5	2-[N-Aziridyl]methylpyrrole	0.09	34	Tetrasulfide, dimethyl	77.89
6	Dimethyl trisulfide	98.48	35	Pentaulfide dimethyl	18.95
7	Bis-(methylthio)-phosphine	0.98	36	Bicyclo[2.2.2]octan-2-one, 5-chloro-, exo-	0.78
8	S-Methyl methanethio sulphonate	0.18	37	1,2, Ethanediol dimethane sulphonate	0.15
9	Thiophosphordiamide, S-methyl ester	0.09	38	Acetamide,N-methyl-N-[4-[4-fluoro-1-hyxahydropyridyl]-2-butynyl]	0.15
10	Dimethyltetrahydrospiro[cyclopentane-1,1'- pyrrolizin]-7'(7a'H)-one O-methyl oxime	0.05	39	Trisulphide Dipropyl	94.87
11	t-Butyl-(2-[3-(2,2-dimethyl-6-methylene-cyclohexyl) propyl]-[1,3]dithian-2-yl)-dimethyl-silane	9.26	40	Di-n-propyldithiophosphinic acid	1.97
12	6-Dimethyl(chloromethyl)silyl oxypentadecane	5.98	41	S-Isopropyl 2-propanesulphonothioate	0.72
13	N-(O-Nitrophenylthio)-l-leucine	5.98	42	1-(tert-butyldimethylsilyl)-3-ethyl-1H-pyrrole-22,5-dione	0.52
14	N,N'-Pentamethylenebis[s-3- aminopropyl thiosulfuric acid	4.34	43	Methanimidamide,N-(4-chlorophenyl)-N-N-dimethyl	0.11
15	2-Dimethyl(prop-2- enyl)silyloxydodecane	3.67	44	(E)-1-(Prop-1-en-1-yl)-3-propyltrisulfane	29.69
16	Disulfide, dipropyl	51.42	45	1,2,4-Trithiolane, 3,5-diethyl-	19.18
17	Disulfide, 1-methylethyl propyl	29.61	46	trans-3,5-Diethyl-1,2,4-trithiolane	4.04
18	Disulfide, bis(1-methylethyl)	18.53	47	1,4-Dithiane-2,5-diol, 2TMS derivative	3.02
19	Propyl	0.18	48	(Z)-1-(prop-1-en-1-yl)3--propyltrisulfance	2.90
20	Methyl pentyl disulphide	0.02	49	Tetrasulfide, dipropyl	67.70
21	Pyrazole[4,5-b]imidazole, 1-formyl-3-ethyl-6-á-d- ribofuranosyl-	12.55	50	2,3, Dihydro-4H-benzo(h)thiochromen-4-one	2.99
22	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	12.54	51	Naphtho[2,3-b]thiophene-4,9-dione	2.88
23	l-Gala-l-ido-octose Octose	4.40	52	3,4-Dichloroisocoumarin	2.15
24	Trisulfide, methyl propyl	94.40	53	Disulfide, methyl propyl	1.69
25	dipropyl methylphosphonodithioite	0.94	54	a-D-Glucopyranoside,Methyl-2-(acetylamino)-2-deoxy-3-o-(trimethylsilyl)-0cyclic methylboronate	31.18
26	3,6-Dibutyl-1,2-dihydro-1,2,4,5-tetrazine	0.34	55	5 Methyl-1,2,3,4-tetrathiane	42.32
27	Benzoicacid 4-[1-hydroxy-2-(-oxopyrrolidino) hexyl]- ethylester	0.24	56	Cyclic octaatomic Sulphur	98.61
28	2Thiatricyclo[3.3.1.1(3,7)]decane	0.24	57	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	37.78
29	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4- azabutane-1,4-diyl)]-, diacetate	8.57	58	Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13, tetradecamethyl	26.67

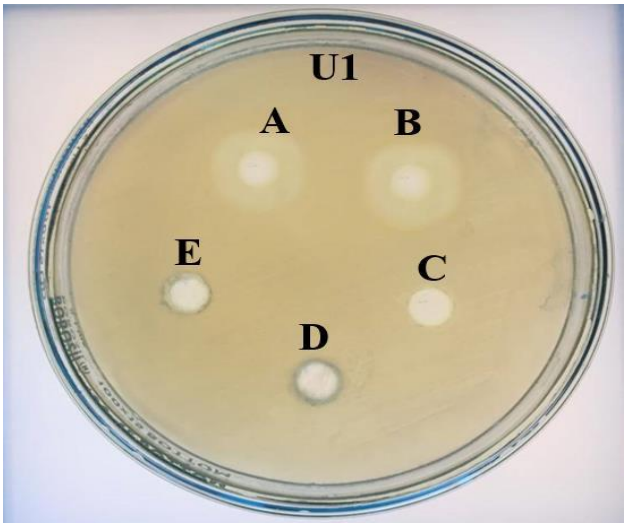

Figure 1: **Antimicrobial Capacity by Well Diffusion Assay**

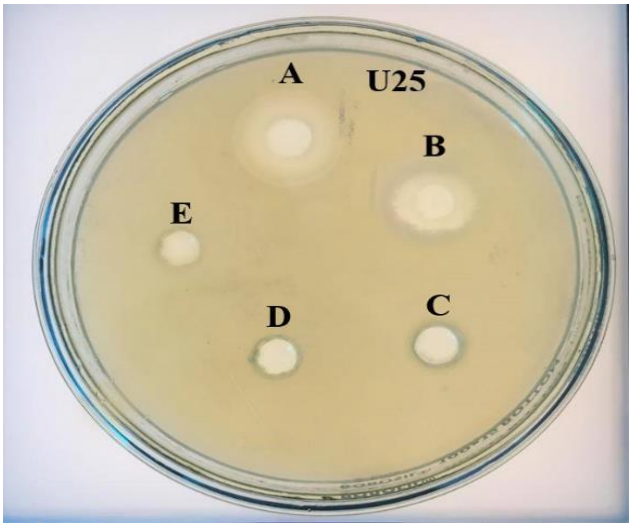

Figure 2: Antimicrobial Capacity by Well Diffusion Assay

A-Onion oil, B- Garlic oil, C-Turmeric oil, D-Ginger oil, E- Cardamom oil

UTIs are the second most common type of infections in human body, which are one of the most serious health problem affecting millions of people each year. UTIs involve infection in the kidneys, ureters, bladder or urethra[23]. For minimized risks of mortality, morbidity, and any renal damage which can be caused by UTIs, clinicians should use the appropriate antibiotic in treatment. Choosing the specific antimicrobial agents appear so difficult, especially because of resistance to antimicrobial agents remarked in bacteria responsible for nosocomial UTIs.

In this study, results obtained are corresponding to literature, in which we have found that almost of bacteria responsible for UTIs is especially *E. coli*, and at less degrees *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* [24, 25]. Also, it's been clear that pathogens responsible for UTIs are commonly from hospital origin, species which are multi-resistant to antibiotics and possess a high ability to biofilm formation [26, 27]. Therefore, the resolution of UTIs problem depend especially in hygiene in hospitals and most important in highlight of new antimicrobial agents in case of treatment failures. Among selected essential oils for this study, the oil of (U1, U25) onion, garlic has shown an interesting antimicrobial activity against all studied bacterial species (Fig.1 &2).

The Onion oil which showed highest antimicrobial activity against MDR strains was subjected to GC-MS Profiling. The percentages of probability of chemical compounds are listed in table.4.

CONCLUSION

In conclusion, UTIs in human are considered as the most serious health problems facing the world. The present study has revealed the importance of natural products to control antibiotic resistant in bacteria which are being a threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective medicines.

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