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Comparative Bactericidal Efficacy of Some Plant Essential Oils against Uropathogenic MDR *E.coli*

Archana Kulkarni[1], Seema Nimbarte[2] and Nasreen Jan[3]
[1,]Dharampeth M.P. Deo Memorial science College, Nagpur.
[2,]Sevadal Mahila Mahavidyalaya, Nagpur.
E-mail: nimbarte.seema@gmail.com

Abstract:

Plant essential oils, extracts of medicinal plants, herbs, and spices constitute very potent natural bioactive agents. The antiseptic qualities of plant essential oils have been recognized since antiquity and hence were used in the present study to confirm their antiuropathogenicactivity. Urinary tract infection (UTI) is a serious health problem affecting millions of people worldwide each year. *Escherichia coli, belonging to various serotypes, is one of the important causative agent of UTI. In the present study a total of 70* uropathogenic *Escherichia coli (UPEC) isolates were collected, serotyped and their antibiotic* resilience*pattern was studied. The UPEC isolates belonged to 13 different serotypes. Antibiotic* resilience*pattern* revealed that these UPEC isolates were, resistant to commonly prescribed antibiotics like ampicillin (87.5%), streptomycin (60%), gentamycin (68.5%), kanamycin (72.8%) and erythromycin (71.4%) but showed sensitivity to cefuroxime (72%), nitrofurantoin (72%), norfloxacin (72%), nalidixic acid (69%), tobramycin (69%), tetracycline (65.8%). The results indicate that most UPEC isolates were resistant to 4 – 5 antibiotics. The emergence of such multidrug resistant (MDR) bacteria demands the use of an alternative green source and traditional method to combat drug resistance. In view of this the essential oils of tea tree, thyme, fennel and pine were assessed for their *in-vitro* bactericidal efficacy against *UPEC.* The bactericidal activity of the whole oils was assessed by using disc diffusion method and minimum inhibitory concentration (MIC). Tea tree oil exhibited higher activity than thyme followed by fennel and pine oil against UPEC. The result of the bioassay showed that essential oils possess potent bactericidal property.

Keywords:

UPEC, MIC, tea tree oil, thyme oil, fennel oil and pine oil.

Introduction:

UTIs are one of the most frequently acquired bacterial infections and *E. coli* accounts for asmany as 90% of all community-acquired UTIs (Marjanca Starčič *et al.,*) . Approximately 50% of all women have had a UTI by their late 20s. About 20–30% of women with first UTI will have two or more infections; while 5%, will develop chronic recurring infections which greatly disrupt a woman's life (Marrs *et al.*, 2005). UTI causing *E. coli* commonly belong to serotypes like O1, O2, O4, O6, O7, O18 and O83 (Moreno *et al.*, 2006). However, the prevalence of different serotypes varies in different regions. Antibiotics are largely used to treat UTI's. In majority of UTI cases empirical antimicrobial treatment is given even before lab results of urine culture are available, without knowing the susceptibility pattern of the causative agent. The clinical management of UTI is thus becoming complicated due to increasing incidence of infections caused by multidrug resistant UPEC. The clinical efficacy of many existing antibiotics is thus being threatened by the emergence of multi-drug resistant

(MDR) pathogens (Bandow *et al*., 2003), reduction in the susceptibility pattern of pathogens and the side effects of some antibiotics.

These resistance problem demands that an effort be made to screen various essential oils for their potential antimicrobial compounds synthesized as secondary metabolites of the plant. The most important of these bioactive compounds being, alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids capable of producing definite antibacterial and antifungal activity.

The antimicrobial activity of EOs has long been recognised and they have been extensively tested *in vitro* against a wide range of pathogenic bacteria and fungi (Kalemba and Kunicka, 2003). The mechanism by which the essential oils exert their antimicrobial activity is poorly understood but the main target appears to be the cell membrane of bacterial cells (Burt, 2004; Di Pasqua et al., 2007). Because of this, Gram negative microbes are in general more resistant to the antimicrobial activity of EOs due to the presence of an outer membrane (Kalemba and Kunicka, 2003; Burt, 2004).

Fennel oil is used as flavoring agents in food products, as a constituent of cosmetic and pharmaceutical products. They are also known for their diuretic, anti-inflammatory, hepatoprotective, antispasmodic, analgesic, antioxidant and anticancer property (Anand *et al.,* 2008). Thyme oil has strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities. Tea tree oil is a natural product with strong antibacterial, antifungal, and anti-inflammatory activity. The therapeutic properties of pine oil are antimicrobial, antineuraligic antiseptic, antiviral, bacterialcidal, adrenal cortex stimulant as well stimulant to the circulation and nervous system. Pine oil can be in the treatment of the respiratory tract, for muscular aches and pains, and as a urinary cleanser. Many of the essential oils also find use as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and as resins. The aim of the current study was therefore to investigate the bactericidal efficacy of four plant essential oils against *UPEC isolates.*

Material and methods:

2.1 Essential oils:

The Essential oils of tea tree, thyme, fennel and pine were procured from commercial samples in local stores.

2.2 Bacterial cultures and growth conditions:

Bacterial cultures of uropathogenic *Escherichia coli* were obtained from various pathological laboratories. All the test cultures were maintained on Trypticase Soya Agar (M990) and stored at 4°C and subcultured on nutrient broth for 24 h prior to testing. These bacteria served as test pathogens for antibacterial activity assay. *E.coli* ATCC 25922 (beta-lactamase negative), procured from MTCC, Chandigarh served as reference culture.

2.3 Antibacterial activity assay:

In vitro antibacterial activity of the essential oils was evaluated by disc diffusion method using Mueller-Hinton Agar with determination of inhibition zones (IZ) in mm. For this, sterilized blank Whatman's filter paper discs of size 6mm were used. These discs were impregnated with essential oils under study for 20 minutes and kept in slanted position so as to drain off excess oil. These discs were later weighed and amount of oil per disc was fixed at 15mg.A lawn culture of test strain(having density equivalent to McFarland 0.5 standard) on Muller Hinton Agar was exposed to the disc of oils. The incubation conditions used were 24 h at 37^0C. After incubation, the diameters of the growth inhibition zones were measured in mm. Fifteendifferent regularly prescribed antibiotics were used as positive control.

2.4 Serology:

Serotyping of *E. coli* (UPEC) isolates was performed at National Salmonella and Escherichia Center, Central Research Institute, Kasuali (H.P).

2.5 Broth microdilution assay (Minimum inhibitory concentration):

In micro-dilution broth assay, all tests were performed in Mueller Hinton Broth. A stock solution of 5% (v/v) of each EO was prepared with Tween 80. A series of two-fold dilutions of each oil were carried out in 96-well microtitre plates over the range of 5% to 0.00976563%.The inocula (5μl) were then added to all the wells (except negative control) of plates, which were incubated at 37^oC for 24 h. MIC was determined visually with the aid of a reading mirror. MIC was determined as the lowest concentration resulting in no growth.

Result and discussion:

3.1 Antibiotic R*esilience* Pattern of UPEC:

The antibiotic resilience pattern of UPECserotypes (Table-1,Fig-1) revealed resistance to different antibiotics. Out of 70 clinical isolates more than 50% isolates showed resistance to different antibiotics. 85.7% *E. coli* were resistant to Ampicillin, 60% to Streptomycin, 68.5% to Gentamycin, 72.8% to Kanamycin and 71.4% to Erythromycin. The lowest resistance of *E. coli* was recorded for antibiotics like Cefuroxime, Nitrofurantoin and Norfloxacin. The results were confirmed with the reference culture of *E. coli* (ATCC 25922).

These observations are well supported by the previous studies of Oteo *et al.,* who reported that the *E. coli* strains show 68% resistance to Ciprofloxacin, 70% to Gentamycin, 55% to tobramycin, 63% to Amoxicillin and 54% to Trimethoprim - Sulphamethoxazole.

3.2 Serotyping :

In the present study the UPEC isolates belonged to 13different O serotypes: O1 (17.1%), O60 (11.4%), O138 (8.6%), O20 (8.6%), O22 (8.6%), O6

(8.6%), O8 (8.6%), O19 (5.7%), O102 (2.9%),O86 (2.9%),O54 (2.9%),O166 (2.9%), however, 2.9% were Non Viable and 8.6% were Rough (Table 2).UPEC strains usually belonged to a limited number of O serogroups, mainly O1, O2, O6 O18, and O75 (Johnson, 1991).However, in this study, the most frequent was O1 (17.1%) which is well-established as associated with UTIs. Similar findings were reported by other researchers (Emamghorashi *et al.*, 2011, Blanco *et al.,* 1995).

3.3 Antiuropathogenic pattern of plant essential oils:

The antiuropathogenic activity of tea tree, thyme, fennel and pine oil UPEC isolates was determined by measuring the zone of inhibition (ZOI). The results (Table 3) revealed that **maximum ZOI of 20±3 mm** was recorded with the tea tree oil indicating that the oil was very promising for microbial control. These results are similar to those found by Carson and Riley (1995).

3.4 Minimum Inhibitory Concentration (MIC) of Plant Oils

MICs encountered in the essential oil literature often vary with regard to contact time between oil and test organism and may not always specify inoculum densities (C.M. Mann and J.L. Markham, 1998). The result of MIC determination using TTO indicted that minimum concentration of **0.075%** (v/v equivalent), was effective against UPEC which were similar to the findings of Gustafson *et al.,* Cosentino *et al.*, Faleiro *et al.* The MIC of the essential oil reported here agree very closely with those found by other researchers. MICs of 0.03% and 0.08% (v/v equivalent) working with *E. coli* strains was reported by Rota *et al.,* 2004 and Carson *et al.*, 2006. The range of values in the literature reflects the differences in media composition, methodology and strains of bacteria used. The MIC values of different plant oils is indicated in Table—4.

Table 1: Antibiotic resistance shown by Uropathogenic ***E. coli*** (UPEC) (N=70)

Sr. No	Antibiotics	No. of susceptible Samples	No. of resistant Samples	% of resistant samples
1	**Ampicillin**	**10**	**60**	**85.7**
2	**Streptomycin**	**28**	**42**	**60.0**
3	Chloramphenicol	40	30	42.8
4	Tetracycline	46	24	34.2
5	Tobramycin	48	22	31.0
6	**Gentamycin**	**22**	**48**	**68.5**
7	**Kanamycin**	**19**	**51**	**72.8**
8	Nitrofurantoin	50	20	28.0
9	Norfloxacin	50	20	28.0
10	Co-trimethoprim	37	33	47.1
11	Nalidixic	48	22	31.0
12	**Erythromycin**	**20**	**50**	**71.4**
13	Colistin	40	30	42.8
14	Sulphamethoxazole	38	32	45.7
15	Cefuroxime	50	20	28.0

Table 2: Percentage of samples with respective serotype

Sr. No.	Serotype	%
1	Non Viable	2.9
2	**O1**	**17.1**
3	O102	2.9
4	O138	8.6
5	O166	2.9
6	O19	5.7
7	O20	8.6
8	O22	8.6
9	O54	2.9
10	O6	8.6
11	**O60**	**11.4**
12	O8	8.6
13	O86	2.9
14	Rough	8.6

Table 3: Zone of inhibition recorded withplant oils against UPEC isolates

Sr. No.	Plant Essential oil	ZOI	Remark
1	Tea tree oil	**20±3**	S
2	Thyme oil	**19±3**	S
3	Fennel oil	**7±2**	R
4	Pine oil	**5±2**	R

S: Susceptible; **R**: Resistant

Table 4: Determination of MinimumInhibitory Concentration (MIC) of*Plant oils* against UPEC isolates

Sr. No.	Plant Essential oil	MIC against UPEC
1	Tea tree oil	0.031%
2	Thyme oil	0.08%
3	Fennel oil	0.37%
4	Pine oil	Resistant

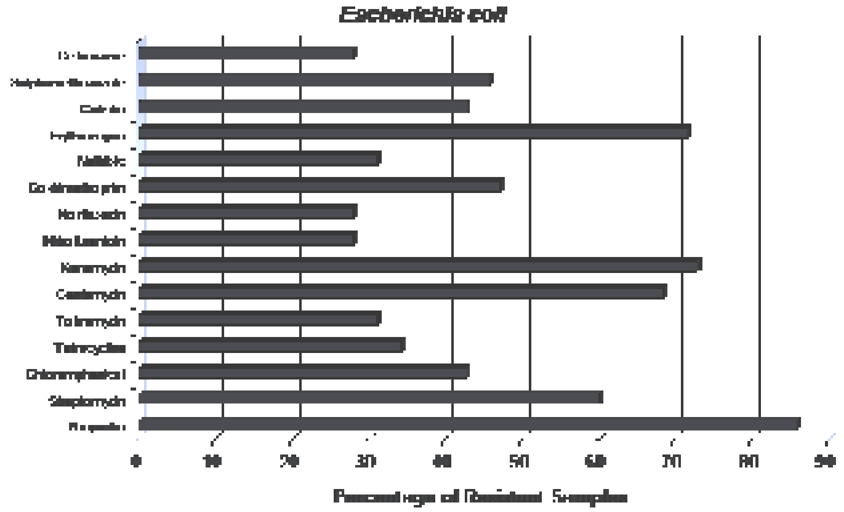

Figure 1: Antibiotic resistant pattern of UPEC

Conclusion:

It was found in the present study that there is an increase in the prevalence of resistance to a number of third generation antibiotics which is a matter of global concern. Hence a careful monitoring of the antibiotic resilience *pattern before giving empirical treatment is a need.*

In light of this an alternative treatment of uropathogens is necessary to prevent antibiotics becoming obsolete, and where appropriate, alternative to antibiotic ought to be considered. The data presented confirm the bactericidal effect of tea tree and thyme oil. The oils represent an inexpensive natural antibacterial agent against UPEC and hence further studies on these oils needs to be investigated as natural antibiotics.

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