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ANTI-BACTERIAL STUDIES OF ESSENTIAL OILS AGAINST MDR *ESCHERICHIA COLI* ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

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ABSTRACT
Drug resistance to common bacterial pathogens has been increased to a great extent and causes treatment failure for management of number of infections in the community. For controlling the resistant pathogen a strong and safer anti-bacterial agent is needed in urgency. In present study, a common bacterial pathogen *E. coli* was isolated from different clinical specimens and their drug resistance profile was investigated, similarly selected five essential oils was tested against the isolates for its anti-bacterial activity using disc diffusion method. The MDR pathogenic *E. coli* were found to be susceptible to the essential oils in the order of activity as cumin seed > clove > allspice > coriander, while cardamom oil failed to show the anti-bacterial activity. From the study, it was concluded that the essential oils from cumin seed, clove, allspice and coriander had a strong antibacterial activity for the MDR pathogenic *E. coli*. These oils could be used to control the infections caused by the resistant *E. coli* and be a good source of an effective anti- *E. coli* agents.

KEYWORDS: antimicrobial agents, clinical specimen, *E. coli*, essential oils, MDR.

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases and growing problem of antimicrobial resistance has become a significant public health concern worldwide and especially in developing countries as a result of overuse and misuse of antibiotics (Ruifang *et al.*, 2006). According to the Centers for Disease Control and Prevention, healthcare-related infections led to 98,987 deaths in the USA in 2002, almost five times higher than a decade ago (Klevens and Monina, 2007). This problem was more complicated through the emergence of multidrug resistant (MDR) strains. MDR clinical isolates of *E. coli* pathogenic strains are commonly seen today in clinics representing a major healthcare problem with increased morbidity and mortality worldwide (Lockhart *et al.,*2007).

Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.*, 2004). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2004; Kordali *et al.*, 2005).

In recent years there has been an increasing interest in the use of natural substances and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds from plant. They possess a wide spectrum of pharmacological activities. The antimicrobial effects of essential oils have been documented and used in herbal medicine in many countries (Longbottom *et al*., 2004; Sonboli *et al*., 2005). The main advantage of natural agents is that they do not enhance the "antibiotic resistance", a phenomenon commonly encountered with the long-term use of synthetic antibiotics.

Despite the fact that *Escherichia coli* as a commensal bacteria can be found in intestinal microflora of a variety of animals including man, not all the strains are harmless, and some can cause debilitating and sometimes fatal diseases in humans. Pathogenic strains are divided into intestinal pathogens causing diarrhea and extraintestinal *E. coli* (ExPEC) causing a variety of infections in both humans and animals including urinary tract infections (UTI), meningitis and septicemia. Urosepsis caused by Uropathogenic *E. coli* (UPEC) are the cause of approximately 80% human UTIs. ExPEC are the pathogens associated with neonatal meningitis, nosocomial bloodstream infections, respiratory infections, UTI or bacterimia in long-term hospitalized patients (Jafari *et al*., 2012). Resistance to antimicrobials has made combating these infections a major problem worldwide (French, 2010). Hence, the present study was mainly implicated for controlling the infections caused multidrug resistant *E. coli* by the use of essential oils.

MATERIALS AND METHODS

Essential Oils

Five samples of essential oils viz., cumin seed (*Cuminum cyminum*), Allspice (*Pimenta dioica*), Clove (*Syzygium aromaticum*), Coriander (*Coriandrum sativum*), Cardamom (*Elettaria cardamom*) were procured from Rakesh Sandal industries Kanpur (UP) and tested against eight isolates for determination of antibiogram. Essential oils were checked for sterility by observing no growth after spreading essential oil sample on nutrient agar (Hi-Media, Mumbai) and incubating at 37ºC.

Isolation and identification of bacterial pathogens-

Different 62 clinical specimens were collected from the hospitalized patients and streak onto the selective media viz. Hi- chrome agar veg. media and EMB agar (Hi-media, Mumbai), also characterized by their morphological and biochemical reactions i.e. Gram stain, motility, IMViC tests. Upon isolation from the clinical specimen a more common isolates were *E. coli* among different the specimens, no doubt along with the *E. coli* the other isolates were also present like *Streptococcus pyogenes* (pus), *Proteus vulgaris* (urine), *Styphylococcus aureus* (urine), *Pseudomonas aeruginosa* (blood), and *Klebsiella spp* (oral thrush). But among the all isolates *E. coli* was found to have prevalence in a large number of specimens. The 8 pathogenic and multi-drug resistant E. *coli* isolates from ear swab, pus, vaginal fluid, urine, catheter tip, blood, stool and oral thrush were selected for the study.

Determination of drug resistance profile of *E. coli* isolates:

The eight isolates were tested against the eight commercial antibiotics viz. Streptomycin 10µg (S10), Ofloxacin 5µg (OF5), Cefprozil 30µg (CP30), Tetracycline 10µg (T10), Ciprofloxacin 5µg (CF5), Ampicillin10µg(A10), Erythromycin10µg (E10), and Norfloxacin 10µg (NX10), by Kirby-Bauer disc diffusion method on Muller Hinton agar (Hi- Media, Pvt. Lab., Mumbai). The zone size to the corresponding antibiotic was interpreted in accordance to performance standards for antimicrobial disc susceptibility tests, CLSI, 2009 guidelines (CLSI, 2009).

Determination antibiogram of clinical isolates against essential oils:

Six mm sterile disc were loaded with 20 µL of oil. The pre-loaded disc then placed on the Muller Hinton agar plates which was pre-seeded with the lawn culture of test bacterium earlier matched with 0.5 Mc-Farland turbidity standards. The plates were then left undisturbed for 30 min to allow diffusion of the essential oil into the agar. The plates then incubated at 37^0C for 24 hrs. After completion of incubation period zone size was measured. The procedure was repeated for all isolates against each of the oil in triplicate.

RESULTS AND DISCUSSION

In the present study drug resistance profile of *E. coli* isolates and its sensitivity to essential oils was studied. According to the drug resistance profile the *E. coli* isolates from blood and vaginal fluid showed high resistance to more antibiotics, followed by the *E. coli* isolated from oral thrush and ear swab. These data suggest the over-use of the common antibiotics in the treatment of blood stream infections, urinary tract infections, upper respiratory tract infections and common ear infections. On the similar way *E. coli* isolated from pus showed more or less equal resistance nearest to the blood stream *E. coli* isolate. The *E. coli* isolate from oral thrush was found to be more resistant than the stool isolate (Table 1). Marwa et al. (2012) reported much higher prevalence of *E. coli* in clinical specimens than in food samples and also recorded highest antibiotics resistance of *E. coli* clinical isolate against conventional Beta-lactam antibiotics.

Table1. Growth inhibitory zones of *E. coli* isolates against synthetic antibiotics

Isolate No	Specimen	Zone of Inhibition (Expressed in mm)							
		A10	**OF5**	**T10**	**E10**	**NX10**	**S10**	**CP30**	**CF5**
EC32	Ear Swab	0	11	0	0	9	16	0	9
EC15	Pus	23	12	12	0	7	0	19	9
EC19	Vaginal Fluid	0	0	12	11	0	0	0	0
EC27	Urine	0	22	11	13	19	12	0	23
EC36	Catheter Tip	23	13	14	0	9	0	24	7
EC43	Blood	0	0	0	9	0	10	0	0
EC24	Stool	0	14	9	10	22	11	0	20
EC38	Oral Thrush	0	9	7	0	0	14	0	0

Table2. Growth inhibitory zones of *E. coli* isolates against essential oils.

Isolate No	Specimen	Zone of Inhibition (Expressed in mm)				
		Cumin seed	**Clove**	**Allspice**	**Cardamom**	**Coriander**
EC32	Ear Swab	20	17	14	0	13
EC15	Pus	20	16	14	0	14
EC19	Vaginal Fluid	23	19	14	0	15
EC27	Urine	21	18	16	0	12
EC36	Catheter Tip	22	18	15	0	12
EC43	Blood	21	17	14	0	13
EC24	Stool	20	17	14	0	13
EC38	Oral Thrush	20	16	14	0	12

All the isolates of *E. coli* from different clinical specimen were inhibited by cumin seed oil with 20 - 23 mm inhibition zone range (Table 2). The maximum inhibitory zone of 23 mm was recorded against *E. coli* (*EC19*) isolated from vaginal fluid, which was equivalent to the inhibitory zones of ampicillin 10µg against the pus and catheter tip isolates i.e. *EC15 and EC36,* and for rest of the isolates the antibiotic did not show any inhibitory zones. This suggests the efficacy of cumin seed oil as an effective antibacterial agent over the ampicillin10µg synthetic antibiotic. Many components of cumin seed oil were characterized (Derakhshan *et al*., 2010; Shankaracharya and Natarajan, 1971), but the major ones were cumin aldehyde, β-Pinene, p-cymene and carvacrol. Hence, the anti- *E. coli* effects of cumin seed oil may be closely related to their high percentage of these compounds.

Among the isolates, the *E. coli* isolate from vaginal fluid (*EC*19) showed higher sensitivity to the essential oils in the order of activity as cumin seed > clove > coriander >allspice. Zone of inhibition for tested clinical isolates of *E. coli* exposed to the oil of clove ranged from 16 to 35 mm. The clove oil exhibited strong anti- *E. coli* activity against clinical isolate isolated from vaginal fluid and moderate activity (18 mm inhibition zone) against isolates obtained from catheter tip and urine. Smith-palmer *et al*. (1998) reported strong inhibitory activity of clove oil to food-borne pathogens including *E. coli.* The antibacterial activity of clove is attributed to eugenol (2-methoxy-4- allyl phenol), which is considered as an antiseptic and often employed as a preservative (Suresh *et al.*, 1992).

Zone of inhibition for tested clinical isolates of *E. coli* exposed to the oil of allspice ranged from 14 to 16 mm. The oil exhibited strong action against *EC27*isolate obtained from urine with inhibition zone of 16 mm. *EC36* isolate from catheter tip showed inhibition zone of 15 mm, while all other isolates of *E. coli* showed similar sensitivity with inhibition zone of 14 mm towards essential oil of allspice. Previously allspice oil has been reported to be the most active oil against *E. coli* O157:H7 (Mendel *et al.*, 2002**).** . Eugenol has been reported to be the major and inhibitory component of allspice oil. Allspice, a versatile condiment may aid in the control of bacterial growth and rancidity in food. Patil and Kamble (2011) reported moderate antibacterial action of allspice oil against control strains of *Escherichia coli* (MTCC-119) and *Escherichia coli* (NCIM-2066). Coriander oil showed inhibitory effect against all *E. coli* isolates tested in our study, but degree of inhibition was found to be least in comparison to other essential oils. Coriander oil inhibited isolates with inhibition zone ranging from 12 to 15 mm. Multidrug resistant isolate of *E. coli* (*EC19*) from vaginal fluid was inhibited with highest 15 mm inhibition zone. Coriander essential oil has been reported to inhibit a broad spectrum of micro-organisms (Duman *et al*., 2010; Lo Cantore *et al*., 2004). Antimicrobial activity of coriander essential oil against reference strain of *Escherichia coli* ATCC 25922 have been reported recently (Filomena *et al*.2011). In the present study, the essential oil of cardamom was found to be totally ineffective against all tested different clinical isolates of *E. coli*. Essential oil of cardamom also previously had been reported (Patil and Kamble, 2011) to be inactive against *E. coli* (MTCC-118, MTCC-119 and NCIM-2066 strains). However previous studies by Sema *et al.* (2005) reported least inhibitory effect of cardamom seed extract against *E. coli* (ATCC 25922).

CONCLUSION

It is evident from present data that all the essential oils except cardamom showed varying inhibitions of the tested MDR *E.coli* clinical isolates of health significance. The results of present studies provide evidence that some essential oils might indeed be potential sources of new antibacterial agents in the therapeutic management of MDR *E.coli*. But certain clinical trials are needed to determine the usefulness of essential oils *in vivo*.

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