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Antimicrobial Effects of Essential Oils against Uropathogens with Varying Sensitivity to Antibiotics

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Abstract: In the present study, the antimicrobial activity of five essential oils namely, basil, chamomile, geranium, lemongrass and thuja were determined against microorganisms isolated from patients having urinary tact infections. The inhibitory effect was evaluated for antibiotic sensitive and resistant bacterial urinary isolates and yeast isolate (*Candida albicans*). Geranium oil exhibited antimicrobial activity against all the isolates, highest diameter of inhibition zone was observed against *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates. The lowest values of minimum inhibitory concentrations were determined for geranium oil against *S. aureus* (8.96 mg mL^{-1}), *Proteus mirabilis* (17.92 mg mL^{-1}), *K. pneumoniae* (35.88 mg mL^{-1}) and *P. aeruginosa* (35.88 mg mL^{-1}). Geranium essential oil also exhibited a strong bactericidal activity against the uropathogens, hence presents a preliminary justification for its therapeutic use for urinary tract infections.

Key words: Antimicrobial activity, essential oils, minimum inhibitory concentration, multi-drug resistance, uropathogens

INTRODUCTION

Urinary Tract Infections (UTIs) are among the most common bacterial infections, accounting for morbidity and mortality in all the human populations, from neonate to the geriatric age group (Hooton, 2000; Acharya, 1992). Despite the realm of antibiotics, these infections have remained a significant problem in medicine. The indiscriminate use of antimicrobial drugs has led to the resistance in uropathogens globally. Concurrent resistance to antibiotics of different structural classes has given rise to multi-drug resistance in uropathogens, which also complicates the therapeutic management of UTIs (Akram *et al*., 2007; Gupta *et al*., 2001). In addition, antibiotics are also associated with adverse effects on host, which include depletion of beneficial gut flora and mucosal microorganisms, immunosuppression, hypersensitivity and allergic reactions (Patel, 2007). In the present scenario of high antimicrobial resistance, alternative nonantibiotic agents are urgently required. Down the ages, plant products have been used for treatment of a number of ailments in humans (Calixto, 2000). Among the natural products, berry juices, fermented milk products containing probiotic bacteria (Kontiokari *et al*., 2003) and some herbal formulations (Pandey and Dwivedi, 2001) are now widely favoured for treatment of UTI.

Essential oils are fragrant volatile substances contained in several plant organs (Cowan, 1999). The aroma, flavour and anti-oxidant properties of essential oils have been

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exploited in manufacture of various cosmetic and food products since millennia. These oils have been in folkloric use for treatment of skin problems, sleep and nervous disorders (Singh and Malik, 2008; Buchbauer and Jirovetz, 1994). Incorporation of essential oils of *Ocimum basilicum* and *O. gratissimium* in tooth pastes and mouth washes showed remarkable antibacterial activities against aerobic dental isolates (Akonkhai *et al*., 2009). Some plant volatile oils have shown antimicrobial activities, comparable to well known bacteriostatic agents, hence were approved to be used as fragrance raw material for soap preparation (Morris *et al*., 1979). The inhibitory activity of essential oils and their components have been reported against bacteria, fungi, viruses and cancer by various researchers such as, Silva *et al*. (2008), Jirovetz *et al*. (2006a, b), Mahboobi *et al*. (2006), Svoboda and Hampson (1999). Some plant essential oils have also been studied for Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) by broth dilution methods. Low inhibitory concentration and lethal activities have been determined in a number of studies (Mahboubi *et al*., 2006, b; Hammer *et al*., 1999). Hence, the biological and antimicrobial properties of essential oils have been proved by various researchers. However, there are a few reports of their activity against uropathogens (Pereira *et al*., 2004). Essential oils being natural and also have aesthetic properties, can be a suitable alternative for treatment of UTIs, provided they also elaborate antimicrobial properties against uropathogens. The purpose of this study was to investigate the antimicrobial potential of essential oils against microorganisms isolated from the patients of UTIs, which also included multi drug resistant bacterial isolates.

MATERIALS AND METHODS

Microorganisms

The urinary isolates were obtained from the patients of UTI following the detection of significant bacteriuria, by urine culture and sensitivity method. The urine samples were collected from different pathology laboratories of Dehradun (Uttarakhand), India, during February 2007 to June, 2007. Ten bacterial isolates and an isolate of *Candida albicans* were isolated and identified, listed as follows:

- *Escherichia coli* ET1 (sensitive)
- *E. coli* ET4 (resistant to K, A, E)
- *Pseudomonas aeruginosa* PT2 (sensitive)
- *P. aeruginosa* PT3 (resistant to C, K, T, A)
- *Klebsiella pneumoniae* KT2 (sensitive)
- *K. pneumoniae* KT6 (resistant to K, T, Co)
- *Proteus mirabilis* PRT3 (sensitive)
- *P. mirabilis* PRT7 (resistant to K, S, E, Co)
- *S. aureus* ST2 (sensitive)
- *S. aureus* ST4 (resistant to K, A, Cf)
- *C. albicans* CT1 (sensitive to amphotericin)

The following standard cultures were procured from Microbial Type Culture Collection (MTCC, Chandigarh), were sensitive to the tested antibiotics:

- *E. coli* MTCC 443 (ATCC 25922)
- *S. aureus* MTCC 96 (ATCC 9144)
- *P. aeruginosa* MTCC 741 (ATCC 25668)
- *Candida albicans* MTCC 3017 (ATCC 90028)

The standard cultures were maintained on the culture media as recommended by MTCC. These cultures were used as control strains for determination of antimicrobial sensitivity testing and were sensitive to all the antibiotics tested. (A- Ampicillin, C-Chloramphenicol, Cf-Ciprofloxacin, Co-Clotrimoxazole, E-Erythromycin, K-Kanamycin, S- Streptomycin, T-Tetracycline).

Essential Oils

Fresh mature leaves of *Ocimum basilicum*, *Matricaria chamomile, Pelargonium graveolans*, *Cymbopogon flexosus* and *Thuja orientalis* were used for the extraction of basil, chamomile, geranium, lemongrass and thuja essential oil, respectively. The leaves were collected from Demonstration Farm, Centre for Aromatic Plants (CAP), Selaqui, Dehradun. The leaves were threshed into small pieces and were thoroughly washed with distilled water at least two times. The excess water was drained out and leaves were dried in shade for 2 days on paper towel. The leaves were hydrodistilled for 4 h using a clevenger apparatus. The oil was collected after removal from water oil surface and then dried over anhydrous sodium sulphate. The extracted oil was stored in glass bottle covered with aluminium foil to prevent the effect of direct sunlight and was kept at 4°C till further use.

Screening for Antimicrobial Activity of Essential Oil

The antimicrobial activity against selected bacterial and yeast isolates was determined by disc diffusion method as described in previous studies (Burt, 2004; Prabuseenivasan *et al*., 2006). Briefly, Muller Hinton Agar plates (Himedia, Mumbai) were inoculated with 100 μL of inoculum (NCCLS, 2002). The sterile filter paper discs (HiMedia, 6 mm) were placed on Muller Hinton Agar plate, 10 μL of respective oil was impregnated to each disc. After 18 h (bacteria) or 48 h (yeast) of incubation at 37°C in dark, the diameter of zone of inhibition (three different readings) was noted for each disc. If the diameter was found to be ≥7 mm then it was considered to be positive (Prabuseenivasan *et al*., 2006; Nascimento *et al*., 2000).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC)

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of reference antimicrobial ciprofloxacin (HiMedia, Mumbai)/amphotericin (HiMedia, Mumbai) and essential oils was carried by microbroth dilution method (Hammer *et al*., 1999; Chander, 2002). Briefly, the test was carried out in 96-well microtitre plates, wells were dispensed with 95 μL of muller hinton broth (bacteria) or RPMI-1640 (yeast) and subsequently 5 μL of inoculum. Oil was serially diluted in the wells of single row. The lowest concentration of antimicrobial/oil showing no visible growth was considered as MIC. The bactericidal or bacteriostatic activity was determined by subculturing 5 μL of contents of the wells on solid agar medium. The lowest concentration showing absence of growth was considered as MLC.

Statistical Analysis

The means of inhibition zone were analyzed by one way Analysis of Variance (ANOVA) followed by post hoc Least Significant Difference (LSD) test at 5% level of significance, using SPSS software package 12 version for windows. The results were expressed as the Mean±SE of mean and Critical Difference (CD) values.

RESULTS AND DISCUSSION

The results of disc diffusion experiments revealed that the essential oils showed antimicrobial activity in varying magnitudes, which can be classified as strong, moderate or weak (Table 1). Basil, geranium, lemongrass and thuja oil showed activity against all the bacterial isolates and *Candida albicans* isolate, which were isolated from urine samples. The strongest activity (26.5 and 26.3 mm) was observed for geranium oil against antibiotic sensitive and antibiotic resistant *S. aureus* respectively. *K. pneumoniae* and *P. mirabilis* were also strongly inhibited by geranium oil. The direct comparison of the results obtained in the present study with previously published reports cannot be appropriate as the climatic, environmental and experimental conditions causes variation in composition of plant oils. The results of inhibition of *P. aeruginosa* by geranium oil can be compared with the findings of Mahboobi *et al.* (2006b), who have reported the diameter of zone of inhibition in the range of 9.3-13.6 mm for different multiresistant isolates of *P. aeruginosa.* Basil oil inhibited all the pathogens except *P. aeruginosa* in the present study. However, inhibition of *E. coli*, *S. aureus, P. aeruginosa* and *P. vulgaris* has been previously observed for basil oil, but no inhibition was found against *K. pneumoniae* (Prabuseenivasan *et al.*, 2006). Another study showed that volatile oils of *Ocimum basilicum* and *Ocimum gratissimum* independently inhibited the growth of *K. pneumoniae, Staphylococcus albus*, *P. aeruginosa* at the concentration of 0.51, 1.10 and 10%, respectively. *Proteus vulgaris* was inhibited at 0.53% by essential oil of *O. gratissium* and 0.67% by *O. basilicum* (Akonkhai *et al.*, 2009). In the present study lemongrass oil showed a moderate activity against *P. aeruginosa, K. pneumoniae, S. aureus,* a weaker activity against *P. mirabilis* and no activity against *E. coli.* The complete lack of activity of lemongrass oil against *E. coli* isolates can be directly corroborated to the findings of Prabuseenivasan *et al.* (2006). Chamomile oil did not inhibited most of the pathogens undertaken in the present study, although it showed a moderate activity against *S. aureus* isolates in the present study, similar inhibition (10.5 mm) of *S. aureus* isolates has been previously reported by Yonzon *et al.* (2005). In the present study, thuja oil showed weak activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*

Table 1: Inhibition zone diameters of essential oils against microorganisms

	Inhibition zone diameter of essential oil (mm)					
Microorganism	Basil	Chamomile	Geranium	Lemon grass	Thuja	CD (p = 0.05)
E. coli ET1 (S)	20.3±0.01	nz	23.7±0.44	nz	7.73±0.29	0.34
E. coli ET4 (R)	15.6±0.93	nz	21.4±0.69	nz	7.6±0.14	0.36
P. aeruginosa PT2 (S)	nz	nz	19±0.33	14.9±0.24	8.03±0.49	0.26
P. aeruginosa PT3 (R)	nz	nz	18.6±0.36	14.9±0.66	8.1±0.41	0.38
K. pneumoniae KT2 (S)	17.9±0.43	nz	25.8±0.53	14.2±0.41	7.4±0.16	0.33
K. pneumoniae KT6 (R)	17.3±0.21	nz	25.3±0.58	13.5±0.47	7.2±0.12	0.36
P. mirabilis PRT3 (S)	13.5±1.3	8.8±0.27	21.2±0.85	8.8±0.45	10.6±0.37	0.47
P. mirabilis PRT7 (R)	12.8±0.23	8.4±0.47	20.8±0.52	8.9±0.21	11.5±0.4	0.52
S. aureus ST2 (S)	21.0±0.47	12.0±0.43	26.5±0.99	17.5±0.33	10.5±0.25	0.35
S. aureus ST4 (R)	20.6±0.4	11.8±0.16	26.3±0.91	15.5±0.33	10.0±0.41	0.48
C. albicans CT1 (S)	18.±0.24	nz	27.5±1.43	16.5±0.49	7.4±0.52	0.60

Data are Mean±SD (n = 3), nz: No inhibition zone

Table 2: Minimal Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of essential oils against microorganisms

	MIC of essential oil (mg mL^{-1})					
Microorganism	Basil	Chamomile	Geranium	Lemongrass	Thuja	MIC of Ciprofloxacin/ amphotericin (μg mL^{-1})
E. coli ET1 (S)	>152	>150	142.4	>147.2	>145.6	4
E. coli ET4 (R)	>152	>150	142.4	>147.2	>145.6	>32
P. aeruginosa PT2 (S)	>152	>150	35.88	73.6	>145.6	16
P. aeruginosa PT3 (R)	>152	>150	35.88	73.6	>145.6	32
K. pneumoniae KT2 (S)	76	>150	35.88	>147.2	72.8	16
K. pneumoniae KT6 (R)	76	>150	35.88	>147.2	72.8	32
P. mirabilis PRT3 (S)	76	>150	17.92	73.6	>145.6	16
P. mirabilis PRT7 (R)	76	>150	17.92	73.6	>145.6	32
S. aureus ST2 (S)	76	>150	8.96	73.6	>145.6	16
S. aureus ST4 (R)	76	>150	8.96	73.6	>145.6	>32
C. albicans CT1 (S)	152	150	142.4	147.2	145.6	4
	MLC of essential oil (mg mL^{-1})					
Microorganism	Basil	Chamomile	Geranium	Lemongrass	Thuja	MLC of Ciprofloxacin/ amphotericin (μg mL^{-1})
E. coli ET1 (S)	>152	>150	>142.4	>147.2	>145.6	4
E. coli ET4 (R)	>152	>150	>142.4	>147.2	>145.6	>32
P. aeruginosa PT2 (S)	>152	>150	71.7	147.2	>145.6	16
P. aeruginosa PT3 (R)	>152	>150	71.7	147.2	>145.6	>16
K. pneumoniae KT2 (S)	152	>150	71.7	>147.2	145.6	16
K. pneumoniae KT6 (R)	152	>150	71.7	>147.2	145.6	>16
P. mirabilis PRT3 (S)	152	>150	35.88	147.2	>145.6	16
P. mirabilis PRT7 (R)	152	>150	35.88	147.2	>145.6	32
S. aureus ST2 (S)	152	>150	8.96	73.6	>145.6	16
S. aureus ST4 (R)	152	>150	8.96	73.6	>145.6	>32
C. albicans CT1 (S)	>152	>150	>142.4	>147.2	>145.6	4

and *C. albicans*, while a moderate activity was observed against *P. mirabilis* and *S. aureus*. Weak or nil activity of essential oil extracted from leaves of different species of *Thuja* has been reported previously by Jirovetz *et al.* (2006b) and Hassanzadeh *et al.* (2001).

The antibiotic sensitive and antibiotic resistant isolates of *S.aureus*, *P.mirabilis* and *K. pneumoniae* were inhibited at the 8.96, 17.92 and 35.88 mg mL^{-1} concentration of geranium essential oil (Table 2), comparable to the findings of Prabuseenivasan *et al.* (2006), who have determined MIC of geranium oil to be >12.8 mg mL^{-1} for both *S. aureus* and *P. vulgaris,* whilst for *K. pneumoniae,* it was reported to be 12.8 mg mL^{-1}. Hammer *et al.* (1999) have observed MIC of geranium oil to be 0.25% v/v and >2.0% v/v for *S. aureus* and *K. pneumoniae*, respectively. Jirovetz *et al.* (2006a) have found MIC of geranium oil Africa, geranium oil Bourbon and geranium oil China to be 60, 600 and 60 ppm, respectively for *S. aureus*. It was found to be 600, 60 and 60 ppm for *P. vulgaris* while for *K. pneumoniae,* the values of MIC were 600, 600 and 60 ppm, respectively.

In the present study, the disc diffusion studies have shown the best inhibition (27.56 mm) of *Candida albicans* isolate by geranium oil, followed by basil, lemongrass and thuja oil and no inhibition by chamomile oil. These findings are in accordance with previous studies of Yonzon *et al.* (2005), Jirovetz *et al.* (2006a) and Silva *et al.* (2008). In the study of Mahboubi *et al.* (2006a,b), the diameter of inhibition zone, MIC and MLC varied between 12 to 30 mm, 62.5 to 250 μg mL^{-1} and 125 to 500 μg mL^{-1}, respectively for 11 different *C. albicans* strains for geranium oil. Hence, the findings of present study conclude that essential oils showed antimicrobial activity in varying magnitude against uropathogens which also included multidrug resistant bacterial isolates. The strongest activity was exhibited by oil of *Pelargonium graveolans* i.e., geranium oil; it may be an alternative choice

for treatment of urinary tract infections. However, further studies would be needed to study its phytochemical, toxicological and pharmacological aspects.

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