Original article

Inhibition of swarming behaviour in *Proteus mirabilis* by *Pelargonium graveolens* essential oil.

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Abstract:

Objective: The purpose of the present study was to evaluate the anti-swarming potential of *Pelargonium graveolens* essential oil against the Proteus isolate. ***Materials and methods:*** The effect of sub-minimal inhibitory concentrations of *Pelargonium graveolens* essential oil and aroma-chemicals was studied on swarming differentiation of urinary *Proteus mirabilis* isolate. The parameters under study were number of concentric rings and diameter of swarm fronts. ***Results and Discussion***: The concentrations of *P. graveolens* essential oil ranging from 1.12-8.96 mg/ml showed a pronounced reduction in the diameter of the colony as a function of time. Evident reduction in the number of concentric rings was also observed due to the incorporation of *P. graveolens* essential oil at 8.96 mg/ml and 4.48 mg/ml concentrations. ***Conclusion***: Hence, the inhibition of swarming by *P. graveolens* essential oil suggests its potential to be developed as a product for preventing *P. mirabilis* infections.

Keywords: anti-swarming; geranium essential oil; Proteus mirabilis; urinary tract infections (UTIs)

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Introduction:

Proteus constitutes of cosmopolitan microorganisms; inhabits polluted water, soil, manure and intestinal tract of mammals[1]. *Proteus mirabilis* is an important opportunistic pathogen, second most common causative agent of UTIs, causes cystitis and pyelonephritis in individuals with indwelling catheters or structural abnormalities of the urinary tract [2]. It possesses a unique arsenal of virulence factors which includes adhesins, swarming motility mediated by flagella, outer membrane protein, urease and haemolysin production[2, 3]. *Proteus mirabilis* exhibits a form of multicellular behaviour known as swarming migration, a cyclical differentiation process in which typical vegetative rods (2-4μm) at the colony margin differentiate into long (up to 80μm) aseptate filaments that possess up to 50 fold more flagella per unit cell surface area. These swarming cells migrate rapidly and coordinately away from the original colony [4].

The ability of *Proteus mirabilis* to differentiate into swarming cells facilitates rapid infections which involve colonization of the lower urinary tract followed by the ascending migration of bacteria. In addition, co-ordinately regulated expression of virulence factors during swarm cell differentiation and population migration of *Proteus mirabilis* has been also observed. Although, the association between swarming and virulence of *Proteus* has

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been confirmed[5,6]. However, there are only few reports in the literature on the effect of natural anti-swarm agents, which can be applied for treatment of urinary tract infections caused by *Proteus* sp[7-9].

Essential oils are odoriferous hydrophobic mixtures of aroma compounds derived from plants[10,11] that have wide applications in cosmetics, food and pharmaceuticals owing to their versatile chemical and biological properties[12,13]. Numerous studies have proved their antimicrobial potential against different types of pathogenic microorganisms[14-16].

Pelargonium sp. (geranium oil) has wide application in aromatherapy, perfumery, cosmetics and flavour industry[17-20]. It also possess anti-candidial[21], antibacterial activity [16, 22] and insect repellant properties[23]. Anti-*Proteus* activity of *Pelargonium sp.* essential oil has also been previously reported[16, 24].

Further, it may represent an insurmountable anti-*Proteus* agent if it interferes with the distinctive swarming characteristic of *Proteus* too. Hence, the objective of this study was to evaluate the anti-swarming potential of *Pelargonium graveolens* essential oil against the *Proteus* isolate.

Materials and methods

Proteus mirabilis isolate- *Proteus mirabilis* isolate PRT3, previously isolated from a female outpatient suffering from the urinary tract infection at Department of Microbiology, Kanya Gurukul Mahavidyalaya, Haridwar, Uttarakhand, was used in the present study. The bacterium was routinely cultured on Luria Bertani medium[16].

Essential oil – The essential oil was obtained from the fresh mature leaves of *Pelargonium graveolens* L'Her (common name-Geranium) by the method of hydrodistillation using the Clevenger apparatus. The leaves were collected from the 'Demonstartion Farm', Centre for Aromatic Plants (CAP), Selaqui, Dehradun. The leaves were threshed into small crumbs, washed thoroughly with distilled water and dried in shade. The leaves were hydrodistilled for 5h, the oil was collected from oil-water interface and dried over anhydrous sodium sulphate. The extracted oil was stored in a glass bottle at 4 °C, in the dark conditions[25].

Minimal inhibitory concentration of *P. graveolens* essential oil for *P. mirabilis* was determined previously by the microbroth dilution method[16]. Briefly, the wells of 96-well microtitre plates were dispensed with 95µl of sterile muller hinton broth and subsequently 5 µl inoculum was added in each of the well. The oil was serially diluted in the concentrations of 35.84-0.0287mg/ml, the plate was incubated at 37 °C, for 18 h. After incubation, 10µl resazurin solution was added in all the wells, incubated at 37 °C for 2 h, the wells were visually examined for blue/purple colour. The highest dilution remaining blue was considered as MIC. For determination of MBC, 5 µl from each well was subcultured on the nutrient agar. The lowest concentration of the essential oil showing absence of growth was considered as MBC. Data from five replicates were evaluated and modal results were calculated.

Effect of essential oil on swarming differentiation: The effect of sub-minimal concentrations (8.96-0.14 mg/ml) of *P. graveolens* essential oil was studied on the swarming behaviour of *P. mirabilis* PRT3 isolate. The inhibition of swarming was measured in terms of concentric rings, reduction in the diameter of 1st ring and last ring. 5µl of the overnight culture of *P. mirabilis* was inoculated centrally on the surface of the dried Luria Bertani (LB) agar (1.5%) plates, with or without different sub-minimal concentrations of oil. The plates were incubated at 37 ºC and the swarming migration distance was determined by measuring the swarm fronts of the bacterial cells at regular time intervals. The swarming inhibition was determined by number of concentric rings, diameter of 1st swarm and last swarm [26].

Statistical analysis

The means of diameters of both first and last swarm ring were analyzed by one way analysis of variance (ANOVA) followed by post hoc 'Least Significant Difference' (LSD) test at 5% level of significance, using the SPSS software package version (12.0) for Windows. A set of critical difference (CD) values was determined for each swarming parameter and concentration.

Ethical approved was taken from Department of Microbiology, Kanya Gurukul Mahavidyalaya, Haridwar.

Results:

The swarming parameters which are expressed in terms of number of concentric rings, diameters of swarm rings were determined to be reduced by different sub-minimal concentrations of *P. graveolens* essential oil. Pronounced reduction was observed in the colony diameter by *P. graveolens* essential oil, in concentrations ranging from 1.12-8.96 mg/ml (Fig. 1).

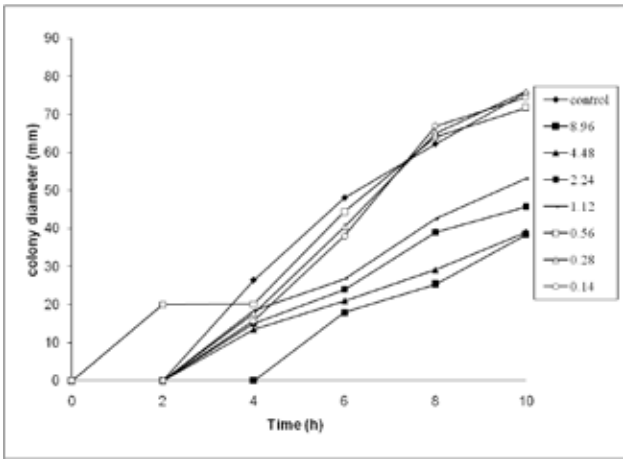

Fig1- Effect of different concentration of *P. graveolens* oil on swarming behaviour of *Proteus mirabilis*

The control plate (without *P. graveolens* essential oil) showed 5 concentric rings. Number of rings was reduced to 2 in the medium having 8.96mg/ml and 4.48mg/ml of *P. graveolens* essential oil (Table 1).

Note:

i) CD stands for critical difference at 5% level

ii) Conc.-concentration

The inhibition in swarming was evidenced by a reduction in the diameter of first and last ring. About 50% reduction in the diameter of both first and last ring was observed for 8.96mg/ml (1%) concentration of oil. The inhibitory effect of *P. graveolens* essential oil on the diameter of the last ring was significant till the concentration 0.28 mg/ml. While for the first ring, the reduction was significant till the concentration of 0.56mg/ml. The expansion of the colony over time was also found to be reduced in the LB medium added with *P. graveolens* essential oil. The highest reduction was observed at 2.24mg/ml.

Discussion:

Reduction in swarming parameters by *P. graveolens* essential oil in the present study can be correlated with the former research works in which the anti-swarming properties of some chemicals and natural products has been elucidated.

The dose-dependent inhibition of swarming in *P. mirabilis* by *P. graveolens* essential oil is in accordance with the effect of monoterpenes on swarming differentiation in *Proteus mirabilis*[26]. The oxygenated monoterpenes effectively inhibited swarming which was evidenced by the reduction in the colony diameter, number of concentric rings and ring width, and it was found to be dependent on concentration of terpenes. The hydroxyl group was found to be important in the antibacterial activity and swarming inhibition of *P.mirabilis* by monoterpenes. Complete inhibition was observed in media containing 0.25 mg/ml and 0.75 mg/ml of geraniol, 0.75 mg/ml citral and citronellol. In the present study, 50% inhibition in swarming has been observed for 8.96 mg/ml of *P. graveolens* essential oil. The essential oil of both *Rosadamascene* (2%v/v), *Anethum graveolens* (concentration not mentioned) and *Artemisia sieberi* (2%v/v) inhibited the swarming of two strains of *P. vulgaris*, one strain of *P. penneri* and two strains of *P.mirabilis*. The most active plant materials tested were the essential oil of *Zataria mulitflora* and a mixture containing equal amount of essential oil of *Lavandula officinalis* plus *Ferula gumosa* Boiss, whereas the essential oil of *Lavandula officinalis* (2%) plus *Ferula gumosa* (2%) was not antibacterial but inhibited the swarming

Table 1- Effect of sub-minimal concentrations of *P.graveolens* essential oil on swarming parameters of *Proteus mirabilis* PRT3

Concentration (mg/ml)	No. of concentric rings	Mean value of diameter (± S.D) of last ring in mm	Mean value of diameter (± S.D) of 1st ring in mm
Control	5	82.6±0.5	6.46±0.4
8.96	2	41.66±0.3	3.63±0.2
4.48	2	42.96±0.8	4.43±0.6
2.24	4	46.96±0.5	4.66±0.8
1.12	4	57.53±0.6	4.93±0.3
0.56	4	78.8±0.7	6.23±0.6
0.28	4-5	83.86±0.4	6.13±0.8
0.14	4-5	82.86±0.6	6.36±0.9
CD(p=0.05)		Conc. 3.3200649	Conc 0.68084732

of all *Proteus* strains[7]. Remarkable antibacterial activity of ethanol extracts of *Physalis minima, Azardirachta indica, Asparagus racemosus, Phyllanthus emblica, Urena labata* and *Tamarindus indica* against different gram positive and gram negative bacteria including multidrug resistant *Proteus* isolates have been reported. Complete inhibition of swarming of *Proteus* isolates by *Urena lobata* root extract (500µg/ml) was also verified [10]. Egyptian clover honey has also been suggested as an alternative therapy for diabetic foot infections; was found to be bactericidal to planktonic cells, inhibited both swarming and biofilm formation in *Proteus mirabilis*[9].

As the connotation between swarming, virulence factors and UTIs has been proved earlier by researchers [5, 6]. Hence, the inhibition of swarming by *P. graveolens* essential oil may also reduce the expression of virulence factors in *P. mirabilis*, which will further decrease the acquisition of UTIs. Moreover, geranium oil (Geranium robertianum L.) has also been determined to be a potent quorum sensing inhibitor against sensor bacterial strains which further reinforces its candidature for the treatment of human infections 31. In conclusion, *P. graveolens* essential oil has significant anti-swarming potential against *P.mirabilis.* Therefore, *P. graveolens* essential oil can be applied as a therapeutic swarming inhibitor for the prevention of urinary tract infections. However, these preliminary findings warrants further research on the mechanism and pharmacological aspects of *P. graveolens* essential oil.

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