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Research Article

Antimicrobial Activity of Rose Geranium (*Pelargonium roseum*) Essential Oil on Bacteria of Veterinary Clinical Origin

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ABSTRACT

In the study on antimicrobial activity of rose geranium oil (GEO), 167 bacterial strains belonging to 22 genera and 49 species were tested. In total only seven strains were sensitive to GEO. Out of 15 reference strains included in the study only one (*Streptococcus equi* ssp. *equi* MTCC-3522) and six of the 120 isolates from clinically sick animals were found sensitive. None of the 26 isolates from environment (soil, water and air) and six from healthy animals was sensitive to GEO. All resistant strains (160) had minimum inhibitory concentration (MIC) more than 2µL of GEO / mL of medium. Sensitive strains' GEO MIC ranged between 0.2 µL/ mL to 2µL/ mL. The minimum MIC was for *Pasteurella langaensis* (0.2 µL/ mL) strain followed by strain of *Streptococcus equi* ssp. *equi* MTCC-3522 (0.4µL/ mL), *Strept. intestnalis* and *Strept. pyogenes* (0. 4µL/ mL), *Strept. equi* ssp. *equi* (0.8µL/ mL), *Bacillus polymyxa* (1.5 µL/ mL) and *Pseudomonas aeruginosa* (2.0µL/ mL). The study emphasizes need for evolution of some short of central universally accepted guidelines to perform and report antimicrobial activity of herbal antimicrobials so that the comparable data can be analyzed for future meta-analytical and clinical purposes.

Key-words: *Pasteurella, Brucella, Klebsiella, Escherichia, Streptococcus, Staphylococcus*, Geranium oil

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Introduction:

Geranium oil is extracted from fragrant plants of *Pelargonium* species especially *P. graveolens,* however, much appreciated rose fragrance of rose geranium may also be obtained from *P. roseum*. Main constituents of geranium essential oil[1] include citronellol (~26.7%) and geraniol (~13.1%), nerol (~8.7%), citronellyl formate (~7.1%), isomenthone (~6.3%) and linalool (~5.2%). Composition does not vary significantly among geranium essential oil (GEO) of different origin[2].

Due to its antidepressant, anti-inflammatory, antiseptic, astringent, cicatrisant, cytophylactic, diuretic, deodorant, haemostatic, insect repellent, styptic, tonic, vermifuge and vulnerary properties GEO has widely been used for therapeutic purposes[3]. The GEO is oil of choice in aromatherapy to treat acne, sore throat, anxiety, depression, and insomnia. Due to its anti-inflammatory property GEO is used to reduce pain and inflammation. For its antiseptic properties it has been used on wounds, burns, frostbites, fungal infections, athlete's foot, eczema and hemorrhoides. It not only protects as a natural insect repellent but its topical application helps to heal insect bites and stop itching[4].

Antibacterial, antifungal, and antioxidant properties of GEO have been explored all over the globe. It has been shown to inhibit many food-borne microbes and reference strains of *Aspergillusniger*, *Candida albicans*, *Basillus subtilis, Brevibacterium linens, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Mycobacterium smegmatis, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella* Enteritidis, *Salmonella* Typhi*, Salmonella* Typhimurium, *Staphylococcus aureus, Staph. epidermidis, Streptococcus mutans* and *Yersinia enterocolitica*[5-11]. However, contrasting reports of its non activity on gram positive bacteria[12] are also cited in literature. The wide range of variation in its affectivity as antimicrobial leads to confusion due to use of only limited number of bacterial strains to test and also variation in amount of GEO used (1.5 µL to 15µL) in disc diffusion assay[1, 8, 9, 11]. Therefore, this study was planned to do an elaborate testing using 15 reference, 26 environmental, 120 clinical and six commensal (from healthy animals) bacteria isolates and testing them all at the same level of GEO concentration (2 µL) of discs.

Material and Methods:

Bacterial isolates tested: Bacterial strains either reference (15) or isolated from different sources (from apparently healthy animals 6, sick animals 120, air 10, water 7, soil 9) and available in different laboratories at Indian Veterinary Research Institute, Izatnagar (Table. 1) were revived and checked for purity and identity using standard bacteriological techniques[13, 14]. Pure cultures were stored on nutrient blood agar (BBL, Difco) slants for the period of testing for sensitivity assay for GEO.

Sensitivity assay for GEO: All the bacterial strains were tested in duplicate using disc diffusion method[15, 16] on Mueller Hinton (MH) agar (BBL, Difco). However, for *Streptococcus, Brucella* and *Pasteurella* strains brain heart infusion (BHI) agar (BBL, Difco) was used for sensitivity assays. The GEO discs were prepared to contain 2µL of GEO in each disc as described earlier[16]. The rose geranium oil (GEO) used in the study was purchased from Shubh Flavours and Fragrances Pvt. Ltd. New Delhi.

Minimum inhibitory concentration (MIC) determination: It was determined using agar well diffusion assay[16] as it is done for antibiotics. Geranium oil (GEO) dilutions (8) were made in sterile dimethyl sulphoxide (DMSO) to contain 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 µL of GEO in 50 µL of DMSO. The eight dilutions of GEO in DMSO were aseptically transferred to 8 peripheral wells of pre-inoculated (with 1:1000 diluted overnight growth of test strain) while central well was filled with 50 µL of sterile DMSO. Plates were incubated under desired atmosphere (5% CO2 enriched for *Brucella* and aerobic for other bacteria) for 24 h. Plates were read for growth inhibition zone and results were interpreted[16].

To find out the statistical significance in association of sensitivity of bacteria and source of bacteria χ^2 test was performed using MS Office Excel-2007.

Results

Of the 167 bacterial strains tested with disc diffusion assay for GEO sensitivity inhibition zone of 6 mm to 20 mm was evident for only seven strains. Only one of the 15 reference strains of 11 species belonging to nine genera tested, a strain of *Streptococcus equi* ssp. *equi* (MTCC-3522) was sensitive to GEO (Table. 1). Out of 120 isolates of 32 species belonging to 16 genera of potentially pathogenic bacteria from clinically sick animals only six isolates were sensitive to GEO. None of 26 isolates from environment (soil 9, water 7, and air 10) belonging to 8 genera and 9 species was sensitive to GEO in the study. Similarly of the six isolates from apparently healthy animals (belonging to 6 species of four genera) none was sensitive to GEO. Of the 101 Gram

negative bacterial isolates and 66 Gram positive isolates only two and five strains were sensitive to GEO. Maximum zone of inhibition (20 mm) was recorded for *Pasteurella langaensis* and minimum (6-7 mm) for *Bacillus polymyxa* and *Pseudomonas aeruginosa* while for all the four streptococci, sensitive to GEO, inhibition zone was 7mm to 10 mm.

All GEO resistant strains (160) had GEO MIC more than 2µL/ mL while for sensitive strains it ranged between 0.2 µL/ mL to 2µL/ mL (Table. 1). Minimum MIC was for *P. langaensis* followed by that for *Strept. equi* ssp. *equi* MTCC-3522 (0.4µL/ mL), *Strept. intestnalis* and *Strept. pyogenes* (0. 4µL/ mL), *Strept. equi* ssp. *equi* (0.8µL/ mL), *B. polymyxa* (1.5 µL/ mL) and *Pseudomonas aeruginosa* (2.0µL/ mL).

Table 1. Sensitivity of some reference and bacterial isolates for rose geranium (*Pelargonium roseum*) essential oil (GEO)

Source of Strains (Nos.)	No. of strains sensitive to GEO	GEO MIC for test strains	Bacterial strains resistant to GEO(Nos.)	Bacterial strains sensitive to GEO
[4]Apparently healthy animals (6)	0	>2µL/mL	One strains each of *Staphylococcus capitis* ssp. *urealyticus, Staph. caseolyticus, Staph. chromogenes, Agrobacterium tumefaciens, Klebsiella pneumoniae, Pseudomonas aeruginosa*	Nil
[4]Clinically sick animals (120)	6	0.2µL/mL to 2µL/mL for sensitive strains and >2µL/mL for resistant	*Aeromonas caviae* (1), *A. sobria* (1), *Bacillus marcerans* (1), *B. polymyxa* (1), *Bacillus* spp. (5), *Brucella abortus* (3), *Burkholderia* spp. (5), *Dermatophilus congolensis* (2), *Enterobacter agglomerans* (2), *Erwinia ananas* (1), *Escherichia coli* (37), *E. fergusonii*(3), *Hafnia alvei* (1), *Klebsiella oxytoca* (1), *Pasteurella canis* (1), *Proteus mirabilis* (1), *Proteus vulgaris* (3), *Pseudomonas aeruginosa* (2), *Staphylococcus aureus* (3), *Staph. capitis* ssp. *urealyticus* (2), *Staph. haemolyticus* (1), *Staph. hyicus* (1), *Staph. intermedius* (2), *Staph. sciuri* (6), Streptobacillus moniliformis(1), *Streptococcus equi ssp. zooepidemicus* (16), *Strept. Intestinalis* (4), *Strept. Milleri* (1), *Strept. pneumoniae*(1), *Strept. Pyogenes* (5)	One strains each of *Streptococcus intestinalis, Streptococcus pyogenes, Streptococcus equi ssp. equi, Pseudomonas aeruginosa, Bacillus polymyxa* and *Pasteurella langaensis*
[1-4]Reference strains (15)	1	0.4 µL/mL for sensitive and for others >2µL/mL	*Bordetella bronchiseptica* (MTCC3838), *Brucella abortus* strain 19 (S-19, and BS-19)[1], *Burkholderia cepacia* (MTCC438), *B. gladioli* (MTCC1888), *B. pseudomallei* (MTCC7183 and MTCC7259), *Erwinia herbicola* (MTCC7100 and MTCC7100/1), *Pasteurella multocida* (P-52 and SORON)[2], *Salmonella Gallinarum* (E-79)[3], *Staph. aureus* (BM-100)[4], *Yersinia enterocolitica* (MTCC3100)	*Streptococcus equi* ssp. *equi* (MTCC3522)
[4]Air (10)	0	>2µL/mL	*E. coli* (1), *Pseudomonas aeruginosa* (1), *Staph. epidermidis* (8)	Nil
[4]Soil (9)	0	>2µL/mL	*E. coli* (3), *Enterobacter agglomerans* (2), *Proteus mirabilis* (1), *Pseudomonasaeruginosa* (3)	Nil
[4]Water (7)	0	>2µL/mL	*Citrobacter freundii* (1), *E. coli* (1), *Erwinia chrysanthemii* (1), *Klebsiella pneumoniae* (3), *Proteus vulgaris* (1)	Nil

Strains numbers prefixed with MTCC were procured from The Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, [1]National *Brucella* Centre, [2]*Pasteurella* Laboratory, [3]National *Salmonella* Centre (Vet.), [4]Division of Epidemiology, Indian Veterinary Research Institute, Izatnagar, Bareilly.

Discussion

It was apparent from the results that all six isolates sensitive to GEO were isolated from sick animals only and none from the healthy animals or their environment. It might be due to the fact that none of the type of bacteria found sensitive to GEO, except *P. aeruginosa*, was included in the study was from healthy animals and environment. The *P. aeruginosa* strain sensitive to GEO had MIC just near the cut off limit to decide sensitivity. Therefore, the results cannot be compared on the basis of source except for strains of those bacteria which were detected in healthy as well as sick animals and their environment. Statistical analysis indicated that source of bacteria could not be associated (p, >0.1) with their sensitivity to GEO.

Both of the *Streptococcus equi* ssp. *equi* (one reference and other from strangled horse), only one of the five *Streptococcus intestinalis,* one of 6 *Streptococcus pyogenes* and a *Streptococcus pneumoniae* isolates, were sensitive to GEO. However, none of 16 *Streptococcus equi* ssp. *zooepidemicus,* one *Streptococcus milleri,* all from clinically sick animals, irrespective of the animal source was sensitive to GEO. The observation indicated that it cannot be predicted even for the streptococci that which one of the strain may be sensitive to GEO. Probability of being sensitive to GEO can also not be defined for strains of other bacteria. Even for the most sensitive *P. langaensis,* it cannot be generalized as it was the lonely isolate in the study and testing of more number of strains is required. However, from the earlier studies (mostly on reference strains) one may get a false impression that GEO is an effective antimicrobial acting against wide range of microbes[5-11]. Observations in this study corroborate with only few studies[12] indicating the fact that GEO possess very limited antimicrobial utility similar to so many other herbal antimicrobials[17-19].

Our study seems to contrast earlier observations[5-11] but the difference in observations might be due to the fact that most of the earlier studies were done on reference sensitive strains using discs impregnated in to GEO or disc containing more amount of oil ranging between 3.5 µL to 15 µL[1, 8, 9, 11]. Besides, in earlier studies different concentrations of GEO were used in discs for different bacteria in contrast to uniform concentration used in the present study (2 µL/ disc). Therefore, it is now the time to decide over fixing the amount of herbal product(s) to be used for determining herbal drug sensitivity similar to the standards available for antimicrobial drugs[15]. In lack of standards, confusing literature will keep on emerging giving false impression of affectivity of herbal drugs on microbes. Besides, the lot of data generated in different labs using different standards poses difficulty in meta-analysis of the information to draw a useful conclusion for future clinical use of herbal antimicrobials.

In the present study using cut off limit of 2 µL/ disc or 2 µL/ mL for deciding sensitivity by disc diffusion assay or MIC through agar well method, respectively was used throughout the study irrespective of microbes tested. The uniformity in testing provides more lucidity in understanding the comparative sensitivity of different bacteria to GEO. In earlier studies GEO MIC has been reported to be the lowest for *Streptococcus mutans* in range of 0.06µL/ mL to 1.25µL/ mL[9, 11], the figures are quite close to our observations on GEO sensitive *Streptococcus* strains with MIC ranging from 0.4-0.8 µL/ mL. However, none of the 27 staphylococci and majority of the streptococci tested in our study was sensitive to GEO and had MIC ≥2 µL/ mL which is contrast to earlier observation on MIC (0.25–2.50 µL/ mL) for *Staph. aureus*[1]. It might be due to several reasons including the variation in the genetic background of the strains and origin of GEO used in the study.

Conclusion

The study conclude that GEO is effective as antimicrobial only on few strains of some bacteria. For evolution of consistent and clinically useful literature on antimicrobial activity of herbal antimicrobials some central agency should be constituted to guide the researchers to use the standard concentrations of different preparations based on the scientific data on, method of using (topical/ systemic/ perentral), toxicity in target tissues, organs and animals/ host, biological availability, serum levels and diffusion in agar or any other testing media etc. Similarly for determining MIC and minimum bactericidal concentration (MBC) of herbal antimicrobials, often insoluble in broth media and aqueous environment even after use of tween and other solvents, suitable methodology needs to be standardized.

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