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ANTIBACTERIAL EFFECTS OF OREGANO (*ORIGANUM VULGARE*) AGAINST GRAM NEGATIVE BACILLI

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Abstract

The oil, aqueous infusion and decoction of oregano (*Origanum vulgare),* of the family Limiaceae, were asessed for antibacterial activity against 11 different genera of Gram–ve bacilli viz., *Aeromonas hydrophila, Citrobacter* sp., *Enterobacter aerogenese, Escherichia coli, Flavobacterium* sp., *Klebsiella ozaenae, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, S. paratyphi B, Serratia marcescens* and *Shigella dysenteriae,* by disc diffusion method. Oregano oil exhibited the highest activity against *Citrobacter* species with mean zone of inhibition of 24.0 mm ± 0.5. The aqueous infusion also showed significant inhibitory activity against *Klebsiella pneumoniae* (20.1 mm ± 6.1 SD), *Klebsiella ozaenae* (19.5 mm ± 0.5 SD) and *Enterobacter aerogenes* (18.0 mm). Besides, all isolates were found resistant to the aqueous decoction of oregano seeds.

Introduction

Microorganisms occur nearly everywhere in nature and affect the well being of people in a great many ways. Many different microbial species normally inhabit various parts of our bodies, such as the oral cavity, skin and intestinal tract. A variety of Gram negative bacilli (GNB) are members of the intestinal microbiota of most animals and humans as normal commensals or pathogens. They are mostly members of the family *Enterobacteriaceae* but members of other taxonomical groups of *Vibrionaceae* are also considered in this category. The GNB emerged rapidly, caused a large outbreak of serious infections, and contributed to mortality (Herbert *et al*., 2007). The GNB vary in the frequencies that they cause either intestinal and extra-intestinal infections. The example of intestinal disorders are enteric fever, inflammatory and non-Inflammatory enteritis. Among the extra-intestinal diseases, such as nosocomial urinary tract infections (Yu *et al*., 2006), chronic obstructive pulmonary disease (Lin *et al*., 2007), neonatal septicaemia (Iregbu *et al*., 2006), wound infections, meningitis, pneumonia, bloodstream infection and bacteremia with septic shock are the most prevalent diseases (Donskey, 2006). During the past 20 years, changes in health care, infection-control practices, and antimicrobial use and resistance may have influenced the frequency that these GNB are associated with hospital-acquired infection (Gaynes & Edwards, 2005).

Previous research studies have documented increasing rates of antimicrobial resistance of GNB (Gupta *et al*., 2006). Misuse of antibiotics and ineffective infection control have been implicated in the development and spread of resistant GNB pathogens which are associated with increased mortality and morbidity, prolonged hospitalization and increased costs. It is evident from literature that new multi-drug-resistant GNB strains have emerged and proliferated in the world. For example, the occurrence of fluoroquinolone-resistant GNB colonizing community-dwelling people with spinal cord dysfunction is common (Roghmann *et al*., 2006). It has also been reported that ciprofloxacin-resistant GNB are becoming increasingly important and that they may cause serious infections in children (Qin *et al*., 2006).

Problematic organisms including non-fermentative *Pseudomonas aeruginosa* (Wroblewska, 2006), multi-drug resistant Salmonella typhi (Akinyemi *et al*., 2000) and other GNB producing extended-spectrum beta-lactamases (GNB-ESBL), pose a particular difficulty for the healthcare community because they represent the problem of multi-drug resistance to the maximum (Wroblewska *et al*., 2006). These organisms are niche pathogens that primarily cause opportunistic healthcare-associated infections in patients who are critically ill or immunocompromised. Multi-drug resistance is common and increasing among GNB, and a number of strains have now been identified that exhibit resistance to essentially all commonly used antibiotics, including antipseudomonal penicillins, cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole and carbapenems (McGawan, 2006). The impact of antibiotic resistance on the outcome of infections due to GNB remains highly controversial. Therapeutic options for multi-drug resistant GNB strains are limited; for this reason, there are a continuous need for alternative new chemical entities with such activities may be identified through a variety of approaches (Combes *et al*., 2007).

Screening of natural medicinal plants is common because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Naim & Tariq, 2006). In search for alternative ways of infectious disease control; essential oil, aqueous infusion and aqueous decoction from oregano were used in the present study to check their antibacterial properties against GNB using standard disc diffusion method *In vitro*.

Materials and Method

Maintainance of isolates: A total of 100 bacterial isolates belonging to 11 different genera of GNB viz., *Aeromonas hydrophila* (2), *Citrobacter* sp. (3), *Enterobacter aerogenes* (2), *Escherichia coli* (22), *Flavobacterium* sp. (4), *Klebsiella ozaenae* (12), *Klebsiella pneumoniae* (20), *Proteus mirabilis* (7), *Pseudomonas aeruginosa* (15), *Salmonella typhi* (5), *S. paratyphi* B (2), *Serratia marcescens* (1) and *Shigella dysenteriae* (5); were maintained on Nutrient Agar medium (Oxoid).

Preparation of aqueous infusion: Aqueous infusion of oregano seeds was prepared by soaking 20g in 100ml sterile distilled water in sterile flask. The flask was kept for two days with occasional shaking. The contents of flasks were filtered.

Preparation of aqueous decoction: Aqueous decoction of oregano seeds was prepared by boiling 20g in 100ml sterile distilled water for 15minutes. The flask was then plugged and removed from heat and allowed to cool. After cooling the contents of flask were filtered.

Essential oil of oregano: Essential oil of oregano (Planter) was purchased from the local market of Saddar, Karachi, Pakistan.

Screening of antibacterial activity: Screening of antibacterial activity was performed by standard disc diffusion method (Saeed *et al*., 2006). Hundred sterilized discs of filter paper (6 mm diameter) were soaked in 1 ml of infusion, decoction and oil, seperately, for 1-2 minutes and then used for screening. Thus potency of each disc was 10 µl. Mueller-

Hinton agar (MHA) (Merck) was used as base medium and Mueller-Hinton broth (MHB) was used for the preparation of inoculum. Four to five isolated colonies of tested organisms were picked by sterile inoculating loop and inoculated in tubes of MHB (5 ml in each). The inoculated tubes were incubated at 35-37^{o}C for 24 hours and matched with 0.5 McFarland nephelometer turbidity standard (Saeed & Tariq, 2005). A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate entire surface of a MHA plate. Discs of infusion, decoction and oil were placed on the surface of inoculated plates with the help of sterile forcep. The inoculated plates were incubated at 35-37^{o}C for 24 hours. After incubation inhibition zone diameters were measured to the nearest millimeter (mm).

Statistical analysis: Mean zone of inhibition and standard deviations were calculated.

Results and Discussion

Oregano has been a valuable source of natural products for maintaining human health for a long period of time, especially in last decade, with more intensive studies for natural therapies (Force *et al*., 2000). The volatile oil of oregano has been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis, and urinary tract disorders. As a medicinal plant, oregano has been used as antifungal, anticoccidial, antispazmolytic, antibacterial (Ertas *et al*., 2005), antioxidant (Lamaison *et al*., 1991), antiaggregant (Okazaki *et al*., 2002) and antiinflammatory (Kelm *et al.*, 2000) agent. In addition, it has stimulating effect of digestion and antiseptic (Cabuk *et al*., 2003). The most important components of oregano are the limonene, gamma-cariofilene, rho-cymenene, canfor, linalol. Alpha-pinene, carvacol and thymol (Arcila-Lozano *et al*., 2004). Among them thymol and carvacrol are the main components of the essential oil of oregano (Tian & Lai, 2006), which are responsible for its antioxidative, antimicrobial and antifungal effects (Proestos *et al*., 2005).

Antibacterial activity of aqueous infusion, aqueous decoction and oil of oregano seeds was evaluated against 11 different GNB that are known to cause infections in human. In the present study, the oregano oil revealed the highest antibacterial activity. It was followed by aqueous infusion while aqueous decoction of oregano did not show inhibitory potential against tested bacterial isolates (Table 1).

The oregano oil exhibited significant inhibitory activity against *Citrobacter* spp., (24.0 mm ± 0.5 SD), *Salmonella typhi* (22.4 mm ± 1.5 SD) and *Escherichia coli* (19.0 mm ± 2.2 SD) (Table 1). It has long been acknowledged that oregano oil is among the most active against strains of *E. coli* and also presents antimicrobial activity against pathogenic microorganisms like *Salmonella. choleraesuis*, *S. typhi*, *S. typhimurium* and many others related GNB strains of *Enterobacteriaceae* family (Penalver *et al*., 2005) and *H. pylori* (Stamatis *et al.*, 2003). Similarly, the results of present study are in accordance with the reports on oregano oil against GNB viz., *Proteus vulgaris, Aeromonas hydrophila, Klebsiella pneumoniae* and *Escherichia coli* (Baydar *et al*., 2004).

In the present study, the antibacterial effect of aqueous infusion of oregano was next to oil and exhibited significant inhibitory activity against *Klebsiella pneumoniae* (20.1 ± 6.1SD), *Klebsiella ozaenae* (19.5 ± 0.5SD) and *Enterobacter aerogenes* (18.0 ± 00SD). It is interesting to note that aqueous infusion of oregano inhibited all type of tested bacterial strains. The present study also described the antibacterial activity of aqueous decoction of oregano seeds. It was found that the aqueous decoction of oregano seeds did not posses any antibacterial effect against tested GNB.

Table 1. Antibacterial activities of oil, aqueous infusion and aqueous decoction of oregano seeds against Gram negative bacteria

Organisms	No. of isolates	Mean zone of inhibition in mm ± standard deviation		
		Oil	Infusion	Decoction
Aeromonas hydrophila	2	12.8 ± 3.3	10.5 ± 1.5	---
Citrobacter sp.	3	24.0 ± 0.5	10.0	---
Enterobacter aerogenes	2	---	18.0 ± 0.0	---
Escherichia coli	22	19.0 ± 2.2	15.8 ± 1.5	---
Flavobacterium sp.	4	11.6 ± 0.9	8.8 ± 1.5	---
Klebsiella ozaenae	12	---	19.5 ± 0.5	---
Klebsiella pneumoniae	20	11.7 ± 1.6	20.1 ± 6.1	---
Proteus mirabilis	7	---	10.2 ± 1.3	---
Pseudomonas aeruginosa	15	---	10.8 ± 1.0	---
Salmonella typhi	5	22.4 ± 1.5	16.1 ± 3.8	---
Salmonella para typhi B	2	12.0 ± 1.0	13.0 ± 1.0	---
Serratia marcescens	1	12.0	13.0	---
Shigella dysenteriae	5	9.5 ± 0.5	11.0 ± 0.7	---

The study demonstrated that oregano represents an economic source of natural mixtures of antibacterial compounds that can be as effective as modern medicine to combat pathogenic microorganisms and safe alternative to treat infectious diseases.

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