REPORT

ANTIBACTERIAL ACTIVITY OF OREGANO (*ORIGANUM VULGARE* LINN.) AGAINST GRAM POSITIVE BACTERIA

SABAHAT SAEED AND PERWEEN TARIQ

Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

The present investigation is focused on antibacterial potential of infusion, decoction and essential oil of oregano (*Origanum vulgare*) against 111 Gram-positive bacterial isolates belonging to 23 different species related to 3 genera. Infusion and essential oil exhibited antibacterial activity against *Staphylococcus saprophyticus, S. aureus, Micrococcus roseus, M. kristinae, M. nishinomiyaensis, M. lylae, M. luteus, M. sedentarius, M. varians, Bacillus megaterium, B. thuringiensis, B. alvei, B. circulans, B. brevis, B. coagulans, B. pumilus, B. laterosporus, B. polymyxa, B. macerans, B. subtilis, B. firmus, B. cereus* and *B. lichiniformis*. The infusion exhibited maximum activity against *B. laterosporus* (17.5 mm mean zone of inhibition ± 1.5 Standard deviation) followed by *B. polymyxa* (17.0 mm ± 2.0 SD) and essential oil of oregano exhibited maximum activity against *S. saprophyticus* (16.8 mm ± 1.8 SD) followed by *B. circulans* (14.5 mm ± 0.5 SD). While all these tested isolates were found resistant to decoction of oregano.

Keywords: Oregano (*Origanum vulgare*), antibacterial activity, disc diffusion method, Gram-positive bacteria.

INTRODUCTION

A wide variety of antibiotics are commonly used for the treatment of serious infections caused by bacteria (Tumah, 2005). In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antimicrobial resistance is a threat to mankind because most of the infection causing bacteria has become multidrug resistant (Saeed *et al.,* 2007). Antibiotic resistant bacteria may keep people sick longer, and some times people are unable to recover at all. Children, the elderly and those with weak immune system, including cancer, HIV/AIDS and transplant patients, are particularly vulnerable because their immune system is not very vigrous as those of healthy adults (Plumbi, 2001). Because of the concern about the side effects of conventional medicine, the use of natural products as an alternate to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Saeed and Tariq, 2007).

Oregano plays a primary role among temperate culinary herbs in world trade (Autuono *et al.,* 2000). It is native of Southern Europe. It is cultivated in European countries and is one of the most popular herbs in Mediterranean cooking. It is traded both as 'whole' dried leaves and in ground form (Nybe *et al.,* 2009). The leaves and dried herb of oregano as well as its essential oil are used medicinally (Hammer *et al.,* 1999). The volatile oil of oregano has been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis and urinary tract disorders (Ertas *et al.,* 2005). Carvacrol is a major active component of oregano and has potential uses as a food preservative (Veldhuizen *et al.,* 2007). Other chemical constituents include limonene, gamma-cariofilene, rho-cymenene, canfor, linalol, alpha-pinene and thymol (Arcila-Lozano *et al.,* 2004). Earlier studies have demonstrated antihyperglycaemic (Lemhadri *et al.,* 2004), antioxidant (Ayumi *et al.,* 2003), antimutagenic (Rocha *et al.,* 2008), antifungal (Soylu *et al.,* 2007), antiviral (Meschino *et al.,* 2005) and potent antibacterial properties of oregano (Naim and Tariq, 2006; Lopez *et al.,* 2007). In view of this, it was aimed to conduct the study to evaluate the antibacterial activity of infusion, decoction and essential oil against 100 isolates belonging to 23 different species and three genera of Gram-positive bacteria. These include *Staphylococcus saprophyticus* (24), *S. aureus* (34), *Micrococcus roseus*(2), *M. kristinae* (1), *M. nishinomiyaensis* (3), *M. lylae* (2), *M. luteus* (3), *M. sedentarius* (2), *M. varians* (2), *Bacillus megaterium* (1), *B. thuringiensis* (2), *B. alvei* (2), *B. circulans* (2), *B. brevis* (2), *B. coagulans* (2), *B. pumilus* (3), *B. laterosporus* (2), *B. polymyxa* (2), *B. macerans* (2), *B. subtilis* (2), *B. firmus* (2), *B. cereus* (2) and *B. lichiniformis* (2).

MATERIALS AND METHODS

Maintenance of isolates

A total of 100 isolates belonging to 23 different species of 3 genera of Gram-positive bacteria (table 1) were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of infusion

The infusion was prepared by taking 10 g dried leaves of oregano in 100 ml distilled water and left for 24 hours at

**Corresponding author*: e-mail: sabahatsaeed2003@yahoo.com

room temperature with ocasional shaking and filtered to obtain clear infusion.

Preparation of decoction

The decoction was prepared by boiling 10 g dried leaves of oregano in 100 ml distilled water in a flask for 20 minutes. The flask was removed from heat and allowed to cool. The content of flask was filtered to obtain clear decoction.

Essential oil

Essential oil of oregano was purchased from a local market of Karachi, Pakistan.

Antibacterial activity

Antibacterial activity was performed by standard disc diffusion method (Saeed *et al.*, 2007). Hundred sterilized discs of filter paper (6 mm diameter) were soaked in 1 ml of infusion, decoction and oil, separately, 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, 2007). 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 using software MINITAB 13.

RESULTS AND DISCUSSION

One hundred isolates belonging to 3 different genera of Gram-positive bacteria, *Staphylococcus*, *Micrococcus* and *Bacillus*, and 23 species i.e. *Staphylococcus saprophyticus* (24), *S. aureus* (34), *Micrococcus roseus*(2), *M. kristinae* (1), *M. nishinomiyaensis* (3), *M. lylae* (2), *M. luteus* (3), *M. sedentarius* (2), *M. varians* (2), *Bacillus megaterium* (1), *B. thuringiensis* (2), *B. alvei* (2),

Table 1: Antibacterial activity of infusion, decoction and oil of oregano

S. No.	Organisms	No. of isolates	Mean zone of inhibition in mm ± Standard deviation		
			Infusion	Decoction	Oil
01	*S. saprophyticus*	24	10.9 ± 0.8	-	16.8 ± 1.8
02	*S. aureus*	34	12.3 ± 1.2	-	11.7 ± 1.5
03	*M. roseus*	02	11.8 ± 1.5	-	8.0 ± 0.0
04	*M. kristinae*	01	13.0	-	8.0
05	*M. nishinomiyaensis*	03	12.7 ± 1.9	-	11.3 ± 0.9
06	*M. lylae*	02	15.5 ± 0.5	-	10.5 ± 0.5
07	*M. luteus*	03	15.3 ± 2.5	-	11.7 ± 0.5
08	*M. sedentarius*	02	11.5 ± 0.5	-	9.5 ± 0.5
09	*M. varians*	02	13.0 ± 1.0	-	12.0 ± 0.0
10	*B. megaterium*	01	16.0	-	11.0
11	*B. thuringiensis*	02	14.0 ± 2.0	-	12.5 ± 2.5
12	*B. alvei*	02	10.5 ± 0.5	-	11.0 ± 1.0
13	*B. circulans*	02	14.5 ± 0.5	-	14.5 ± 0.5
14	*B. brevis*	02	13.5 ± 1.5	-	8.0 ± 0.0
15	*B. coagulans*	02	8.0 ± 0.0	-	8.0 ± 0.0
16	*B. pumilus*	03	14.7 ± 2.5	-	9.3 ± 0.5
17	*B. laterosporus*	02	17.5 ± 1.5	-	11.0 ± 0.0
18	*B. polymyxa*	02	17.5 ± 2.0	-	9.0 ± 1.0
19	*B. macerans*	01	9.0	-	9.0
20	*B. subtilis*	02	1.0. ± 0.0	-	9.0 ± 0.0
21	*B. firmus*	02	10.5 ± 0.5	-	8.5 ± 0.5
22	*B. cereus*	02	8.0 ± 0.0	-	9.5 ± 0.5
23	*B. lichiniformis*	02	8.5 ± 0.5	-	8.5 ± 0.5

- No activity

B. circulans (2), *B. brevis* (2), *B. coagulans* (2), *B. pumilus* (3), *B. laterosporus* (2), *B. polymyxa* (2), *B. macerans* (2), *B. subtilis* (2), *B. firmus* (2), *B. cereus* (2) and *B. lichiniformis* (2), were used in the present study. The results of antibacterial activity of infusion, decoction and oil of oregano are presented in table 1.

The results showed great variation in antibacterial activity of selected forms of oregano. The infusion exhibited maximum activity against *B. laterosporus* (17.5 mm mean zone of inhibition ± 1.5 Standard deviation) followed by *B. polymyxa* (17.0 mm ± 2.0 SD). The minimum activity of infusion was found against *B. cereus* and *B. coagulans* with 8 mm mean zone of inhibition. While essential oil of oregano exhibited maximum activity against *S. saprophyticus* (16.8 mm ± 1.8 SD) followed by *B. circulans* (14.5 mm ± 0.5 SD). The minimum activity was found against *M. roseus, M. kristinae, B. brevis* and *B. coagulans* with 8 mm mean zone of inhibition. These forms (infusion and oil) also exhibited potent activity against all tested bacteria. Baydar *et al.* (2004) also reported inhibitory activity of oregano oil against *Bacillus amyloliquefaciens, B. brevis, B. cereus, B. subtilis, Staphylococcus aureus, Micrococcus luteus, Aeromonas hydrophila, Coryenebacterium xerosis , Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Mycobacterium smegmatis, Yersinia enterocolitica* and *Listeria monocytogenes.* Similarly, Firouzi *et al.* (2007) reported that oregano oil was found effective against pathogenic strains of *Yersinia enterocolitica* and *Listeria monocytogenes.* In another study oregano oil exhibited antibacterial activity against methicillin-sensitive and methicillin-resistant bacteria (Naim and Tariq, 2006). Lin *et al.* (2005) have reported the antibacterial activity of oregano oil against *Vibrio parahaemolyticus*. Another study also reported the antibacterial potential of oregano oil against *E. coli* O157:H7 (Moreira *et al.*, 2005).

In the present study, the antibacterial activity of decoction of oregano was also evaluated. All tested isolates were found resistant to decoction of oregano. It might be due to the heat labile nature of active components of oregano. Our findings are in fair correlation with the study carried out by Naim and Tariq (2006) who found that decoction of oregano did not show antibacterial activity against Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus.*

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