

Antibacterial activity of aqueous infusion and decoction of dried leaves of oregano (*Origanum vulgare*) on clinical bacterial isolates

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Abstract

Introduction: Oregano (*Origanum vulgare*) is a common culinary herb that is known to inhibit the growth of several bacterial strains.

Aim: To assess the antibacterial effect of crude aqueous infusion and decoction of dried leaves of *Origanum vulgare* against common clinical isolates.

Materials and Method: Crude extracts of Oregano with a standard concentration of 0.2 g/ml, were tested for their antibacterial activity against 10 isolates each of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*, isolated from various clinical samples, by agar well diffusion assay. The standardised antibiotics (Hi Media, India) used for comparing sensitivity pattern were ciprofloxacin (5 µg/disc) for all strains except *P. aeruginosa* for which gentamicin (15 µg/disc) was used.

Results: In the present study, both the aqueous infusion and decoction showed inhibitory potential against 3 out of the 5 organisms tested. Highest activity was noted against *Staphylococcus aureus* with 8 out of 10 isolates tested being sensitive with wide range of zone of inhibition between 10-19 mm, followed by *Escherichia coli*, 6 out of 10 sensitive with a zone of 12-16 mm. 3 out of 10 isolates of *K. pneumoniae* were sensitive with a zone of 7-10 mm. Both infusion and decoction did not show any activity against *Salmonella typhi* and *Pseudomonas aeruginosa*.

Conclusion: The data presented confirm the antibacterial potential and highlight the promising role of oregano as new lead structure in the search for novel antibacterial agents. Therefore, further experiment involving a larger sample size and extract concentrations is worthy of evaluation.

Keywords: *Origanum vulgare*, Antibacterial activity, Crude extracts, Clinical bacterial isolates

Introduction

Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Since the 1940s, antibiotics have greatly reduced illness and death. However, antibiotic-resistant bacteria, known as “superbugs” are becoming more numerous and more virulent due to the widespread and inappropriate use of antimicrobials such as their use as growth enhancers in animal feed. Herbal medicine offers an alternative to these increasingly ineffective drugs and today plant materials play a major role in primary health care as therapeutic remedies in many developing countries.⁽¹⁾ Recently, consumer’s demand for natural food ingredients has increased because of their safety and availability.⁽²⁾ Plant materials containing Essential oils have shown wide antimicrobial activity spectra and may act as natural food preservatives when added to fresh food products.⁽³⁾

Oregano (*Origanum vulgare*) is a common species of Origanum, a genus of the mint family (*Lamiaceae*). Among the chemical compounds contributing to its flavour is *carvacrol*. It inhibits the growth of several bacterial strains, e.g. *Escherichia coli*⁽⁴⁾ and *Bacillus cereus*. The cause of its antimicrobial properties is believed to be disruption of the bacterial membrane.⁽⁵⁾ The antimicrobial and antioxidant effects of the seeds of oregano species were also studied and found effective.⁽⁶⁾

Oregano is an important culinary herb, used for the flavour of its leaves. It is cost efficient and readily available. Its low toxicity, pleasant taste and smell suggests its use as a food additive to prevent bacterial contamination.⁽⁷⁾ This information makes it imperative to study the antibacterial activity of aqueous infusion and decoction of dried leaves of oregano (*Origanum vulgare*) on clinical bacterial isolates. This study was done under ICMR – STS 2016.

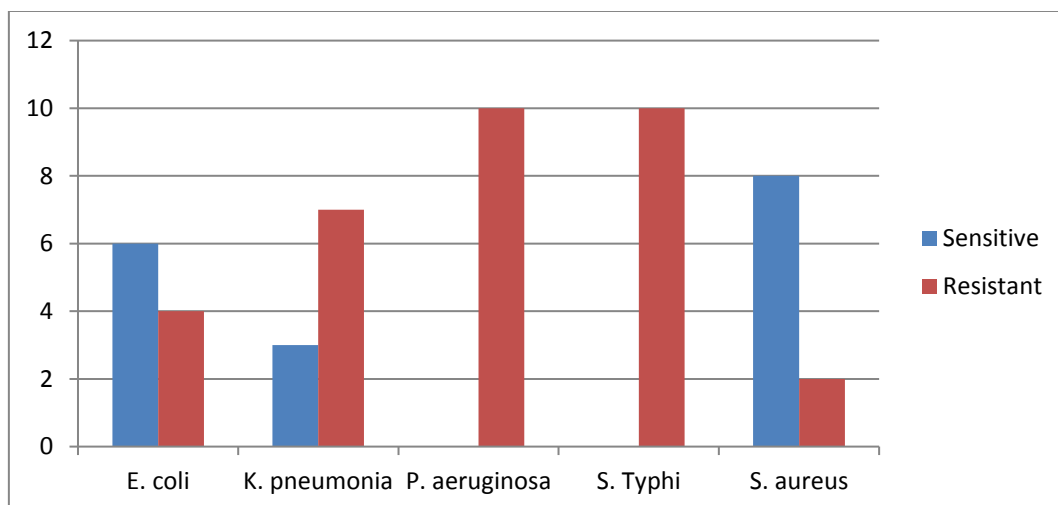


Fig. 1: Sensitivity pattern of the clinical bacterial isolates to the oregano extracts (Based on presence of zone of inhibition)

Materials and Method

Study design: Experimental study

Study Site: Department of Microbiology

Study period: Two months

Study population: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* isolated from various clinical samples

Sample size: 50 isolates

Inclusion criteria: Samples positive for the said organisms from any age group will be taken for the study.

Exclusion criteria: Samples positive for organisms other than the said organisms are excluded.

Statistical Analysis: Statistical mean and standard deviation

Collection and Processing of bacterial isolates:

Ethical committee clearance was obtained with waiver of consent as only samples in laboratory were collected. *Escherichia coli* (10 samples), *Klebsiella pneumoniae* (10 samples), *Pseudomonas aeruginosa* (10 samples), *Staphylococcus aureus* (10 samples) and *Salmonella typhi* (10 samples) were isolated from various clinical samples such as urine, sputum, blood, stool and pus, collected in a sterile container, at a hospital, over a period of 2 months. The samples were processed in Blood agar, Chocolate agar and MacConkey agar at 37°C for 24 - 48 hours and the bacterial isolates were identified by colony morphology and standard biochemical tests.

Plant material: The oregano (*Origanum vulgare*) plant was purchased from Nurserylive, a registered company in Sasane Nagar, Hadapsar, Pune. The identity of the plant was confirmed morphologically and by consulting with an expert botanist in Chennai. The leaves were washed and air dried in well-ventilated rooms, away from sunlight, for 10 days.

Preparation of aqueous infusion: Aqueous infusion of oregano leaves was prepared by soaking 20g of the dried leaves in 100ml sterile distilled water in sterile flask. The flask was kept for two days with occasional shaking. The contents of flasks were filtered.⁽⁸⁾

Preparation of aqueous decoction: Aqueous decoction of oregano leaves was prepared by boiling 20g of the dried leaves in 100ml sterile distilled water for 15 minutes. The flask was then plugged and removed from heat and allowed to cool. After cooling the contents of flask were filtered.⁽⁸⁾ The concentration of the extracts used in this study is the total weight of oregano leaves per millilitre, i.e. 0.2 g/ml (both infusion and decoction). The extract was prepared freshly every week and stored at room temperature (24- 28°C)

Screening of antibacterial activity: Screening of antibacterial activity was performed by agar well diffusion method. Mueller Hinton Agar (Hi Media, India) (pH 7.2-7.4) was poured in plates of 10 cm diameter and depth of agar was about 3 mm. The plates were allowed to dry. Four to five isolated colonies of tested organisms were picked by sterile inoculating loop and inoculated in tubes of peptone water (5 ml in each), preparing a standard turbidity of 0.5 McFarland. The inoculated tubes were incubated at 37°C for 2 to 3 hours. A sterile cotton swab was dipped into the bacterial test suspension to inoculate the entire surface of a MHA plate. Standard wells of size about 6 mm in diameter were made on the Mueller-Hinton agar (MHA) plates with a sterile borer. 100 µl volumes of aqueous infusion and decoction were added in separate wells and in one well distilled water was added as a negative control. The standardised antibiotics (Hi Media, India) used in this study were ciprofloxacin and gentamicin. Ciprofloxacin (5 µg/disc) was used as positive control for all strains except *P. aeruginosa* for which gentamicin (15 µg/disc) was used. The sensitivity pattern of the antibiotics was recorded as per standard

Clinical Laboratory Standards Institute (CLSI) guidelines⁽⁹⁾ (Fig. 2). The inoculated plates were incubated at 35-37°C, ambient air for 24 hours. After

incubation, inhibition zone diameters were measured by a scale to the nearest millimetre (mm) including the well diameter. (Fig. 3).

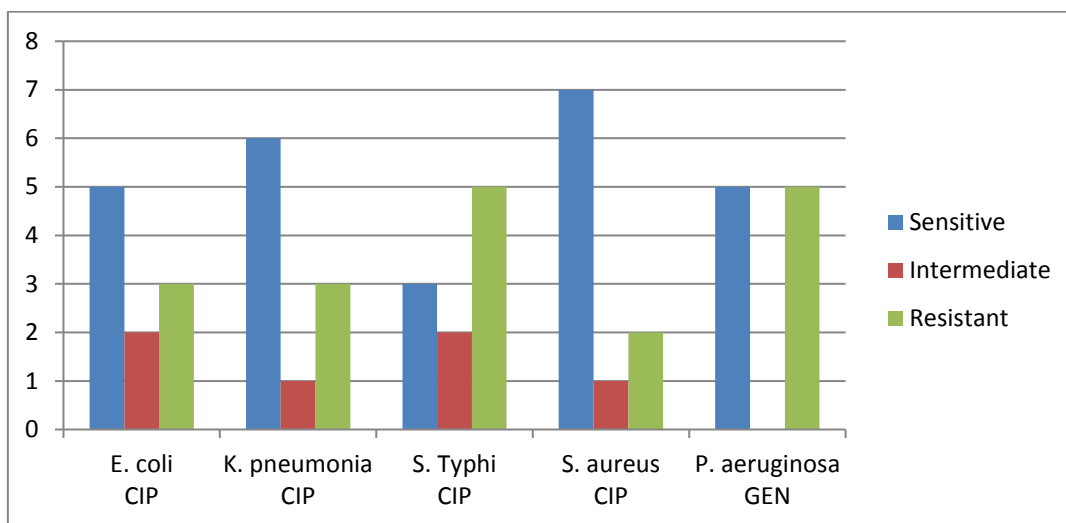


Fig. 2: Antibiotic sensitivity pattern of the clinical bacterial isolates (As per standard Clinical Laboratory Standards Institute (CLSI) guidelines)

CIP - Ciprofloxacin (5 µg/disc); GEN - Gentamicin (15 µg/disc)

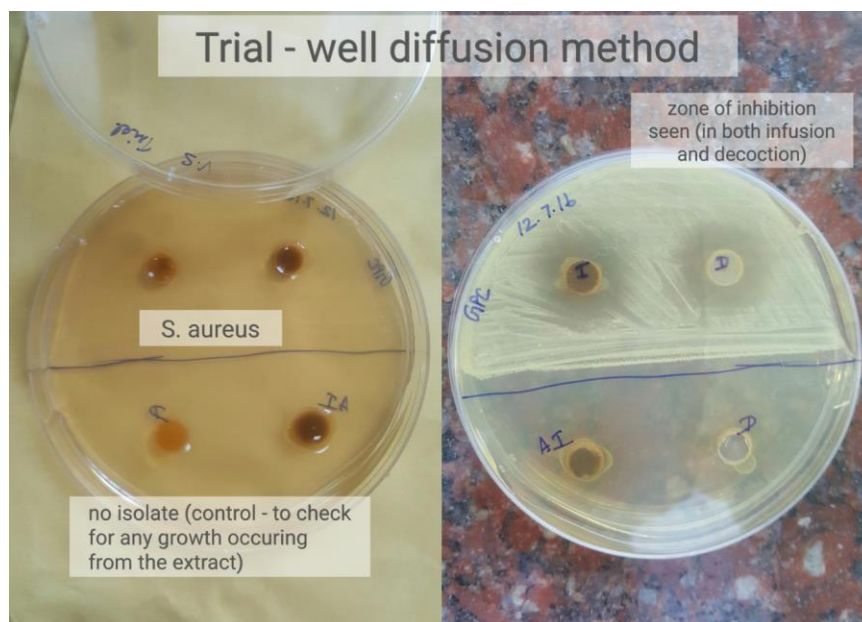


Fig. 3: Trial of well diffusion method using *Staphylococcus aureus*

A trial was done initially, every week with the new extract prepared, to rule out extract contamination. Half the surface of Mueller Hinton Agar plate was inoculated with *S. aureus*. Two wells of size about 6 mm in diameter were made on either sides of the plate for infusion and decoction. The plate was incubated at 35-37°C, ambient air for 24 hours. Zones of inhibition are seen with both infusion (19 mm) and decoction (20 mm) of *O. vulgare* in the tested strain of *S. aureus*. Extract was free from contamination.

Statistical analysis: All the data were analyzed statistically with standard SPSS software (IBM SPSS). Mean zone of inhibition and standard deviations were calculated (the results obtained are expressed as mean \pm standard deviation).

Results and discussion

In the present study, both the aqueous infusion and aqueous decoction of oregano showed antibacterial activity against many bacterial isolates tested (Table 1), which was assessed by evaluating the presence of

inhibition zone. Both extracts have demonstrated nearly equal inhibitory activity against the sensitive organisms.

Table 1: Antibacterial activity of aqueous infusion and decoction of dried leaves of *Origanum vulgare* on clinical bacterial isolates

Organism	No. of isolates	Mean zone of inhibition in millimetre \pm standard deviation	
		Aqueous infusion	Decoction
<i>Escherichia coli</i>	10	11.1 \pm 4.6	10.9 \pm 4.4
<i>Klebsiella pneumoniae</i>	10	6.7 \pm 1.1	6.9 \pm 1.5
<i>Pseudomonas aeruginosa</i>	10	-	-
<i>Salmonella typhi</i>	10	-	-
<i>Staphylococcus aureus</i>	10	13.1 \pm 5.6	13.3 \pm 5.7

The aqueous infusion exhibited significant inhibitory activity against *Staphylococcus aureus* (13.1 mm \pm 5.6 SD), *Escherichia coli* (11.1 mm \pm 4.6 SD) and *Klebsiella pneumoniae* (6.7 mm \pm 1.1 SD). The decoction also exhibited inhibitory activity against *Staphylococcus aureus* (13.3 mm \pm 5.7 SD), *Escherichia coli* (10.9 mm \pm 4.4 SD) and *Klebsiella pneumoniae* (6.9 mm \pm 1.5 SD). Both infusion and decoction did not show any activity against *Salmonella typhi* and *Pseudomonas aeruginosa*. The mean zone of inhibition of the control (distilled water) was 6 mm \pm 0.

Out of the tested isolates, even among the sensitive organisms, some strains showed complete resistance to the extract (Fig. 1). It was interesting to note that the resistant strains exhibited resistance to both aqueous infusion and decoction. Taking into account of only the sensitive isolates, the mean zone of inhibition was calculated (Table 2).

Table 2: Zone of inhibition of aqueous infusion and decoction of dried leaves of *Origanum vulgare* in the sensitive clinical bacterial isolates

Organism	No. of isolates	No. of sensitive isolates	No. of resistant isolates	Mean zone of inhibition of sensitive isolates in millimetre \pm standard deviation	
				Aqueous infusion	Decoction
<i>Escherichia coli</i>	10	6 ^a	4	14.5 \pm 1.9	14.0 \pm 1.9
<i>Klebsiella pneumoniae</i>	10	3 ^b	7	8.3 \pm 0.6	9.0 \pm 1.0
<i>Staphylococcus aureus</i>	10	8 ^c	2	14.9 \pm 4.7	15.1 \pm 4.8

a – Out of the 6 strains, 5 were sensitive and 1 intermediately sensitive to ciprofloxacin.

b – All 3 were sensitive to ciprofloxacin.

c – Out of the 8 strains, 7 were sensitive and 1 resistant to ciprofloxacin.

Oregano contains polyphenols, including numerous flavones,⁽¹⁰⁾ fibre, iron, manganese, vitamin E, iron, calcium, omega fatty acids, manganese, and tryptophan. It's most important component is its volatile oil, which contains over 60 different compounds, with the primary ones being *carvacrol* and *thymol*, while lesser abundant compounds include *p-cymene*, γ -terpinene, caryophyllene, spathulenol, germacrene-D, β -fenchyl alcohol and δ -terpineol.⁽¹¹⁾ In addition, the chemical compounds contributing to flavour are *limonene*, *pinene* and *ocimene*. As we all know, *carvacrol* is majorly responsible for the biological activities of oregano⁽¹²⁾ and is considered as a biocidal, resulting in bacterial membrane perturbations that lead to leakage of intracellular ATP and potassium ions and ultimately cell death.⁽¹³⁾ Oregano has shown antimicrobial activity in a number of studies. A team of British and Indian researchers reported that the essential oil of Himalayan oregano has strong antibacterial properties that can

even kill the hospital superbug MRSA.⁽¹⁴⁾ Though the antimicrobial activity of essential oils, ethanolic and methanolic extracts were extensively studied, reports about aqueous extracts, mainly decoction or infusion preparations that are used for therapeutic applications, are scarce.

In 2007, the oil, aqueous infusion and decoction of *Origanum vulgare* were assessed⁽⁸⁾ for antibacterial activity against 11 different genera of Gram negative bacilli by disc diffusion method. Oregano oil exhibited the highest activity against *Citrobacter* species (mean zone of inhibition of 24.0 mm \pm 0.5 standard deviation). The aqueous infusion also showed significant inhibitory activity against *Klebsiella pneumoniae* (20.1 mm \pm 6.1 SD), *Escherichia coli* (15.8 mm \pm 1.5 SD), *Pseudomonas aeruginosa* (10.8 \pm 1.0), *Salmonella typhi* (16.1 \pm 3.8) and few other organisms. Besides, all isolates were found resistant to the aqueous decoction of oregano seeds. In 2009, the antibacterial potential of infusion, decoction and essential oil of oregano (*O.*

vulgare) against 111 Gram-positive bacterial isolates belonging to 23 different species related to 3 genera was studied.⁽¹⁵⁾ The infusion and essential oil of oregano exhibited activity against several organisms including *S. aureus* and *S. saprophyticus* but all the tested isolates were found resistant to decoction of oregano.

From the current study, it is clear that, both aqueous infusion and decoction of *Origanum vulgare* showed the highest antibacterial activity against *S. aureus* followed by *E. coli* and *K. Pneumoniae*, which is in agreement with the above mentioned studies and another experiment⁽¹⁶⁾ evaluating the action of carvacrol against *S. aureus*.

A study⁽¹¹⁾ published in August 2013, aimed to characterise the in vitro antioxidant and antibacterial properties of oregano (*Origanum vulgare*) essential oil and extracts (in hot and cold water, and ethanol). All extracts and essential oil were effective in inhibiting the growth of most of the tested bacteria. The hot water extract had the strongest antioxidant properties and highest phenolic content. The essential oil caused greater reductions on *Listeria* strains (*L. monocytogenes* and *L. innocua*). The study suggested that *O. vulgare* extracts and essential oil from Portuguese origin are strong candidates to replace synthetic chemicals used by the industry.⁽¹¹⁾

In 2014, the antioxidant and antibacterial activities of infusion, decoction and hydro alcoholic extract of oregano were evaluated and compared.⁽¹⁷⁾ The samples were effective against both gram-negative and gram-positive bacteria. The hydro alcoholic extract showed the highest efficacy against *Escherichia coli*. Moreover, the study⁽¹⁷⁾ suggested that the use of infusion/ decoction can avoid the toxic effects shown by oregano essential oil, widely reported for its antioxidant and antimicrobial properties. A similar study in 2017, tested the antimicrobial effect of hot and cold water extracts and infusions of oregano and thyme against 20 pathogenic strains, and all extracts were found effective.⁽¹⁸⁾

In the present study, no activity was seen against the tested samples of *S. typhi* and *P. aeruginosa* and both infusion and decoction have demonstrated nearly equal inhibitory activity against the sensitive organisms. These results are not completely in accordance to the results obtained in similar studies^(8,11,15) where the decoction did not possess any inhibitory activity. This might probably be due to the fact that the study⁽⁸⁾ involved oregano seed extracts or because the method of preparation of extract^(11,15) is different (such as duration of boiling or extract concentration). Further, a research concluded that the antimicrobial activity of the essential oil of certain oregano is not diminished by heating in boiling water.⁽¹⁴⁾

Also, the content of carvacrol in *O. vulgare* (EO) varied between 18% and 63% according to the

geographic origin and time of distillation.⁽¹⁹⁾ This could also contribute to some differences in the observation. Moreover, the current study involved a limited sample size and hence sensitivity of *S. typhi* and *P. aeruginosa* cannot be completely ruled out. The separation and purification of the crude extracts might show an increase in bioactivity than the crude extracts, hence is the enhanced antimicrobial activity shown by certain active components of *O. vulgare*. This might be due to the fact that, there are numerous compounds within the crude extracts, which may have interfered with the actions of one another. Once they were separated by various purification methods, the inhibiting effect of one on the other had reduced significantly.⁽²⁰⁾ Furthermore; the inability of higher concentrations of plant extracts to diffuse through the agar medium may cause impairment in drug diffusion. It is a major limitation in the evaluation of the antimicrobial effects of plant extracts using the agar diffusion method.⁽²¹⁾

Conclusion

The study confirms the antibacterial potential of both the crude extracts of *Origanum vulgare* against some of the common clinical isolates. Highest activity was noted against *Staphylococcus aureus* (13.3 mm \pm 5.7 SD), followed by *Escherichia coli* (11.1 mm \pm 4.6 SD) and *K. Pneumonia* (6.9 mm \pm 1.5 SD). No inhibition was seen against *S. typhi* and *P. aeruginosa*. The results are in conformity with many of the similar studies in the past and emphasise the propitious role of oregano as an antimicrobial agent. The observations noted in this study need further development in terms of increased number of isolates and standardisation of extract concentrations. It may be extended to other microorganisms as well.

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