Research article

Screening of antimicrobial, anti-quorum sensing activity and cytotoxicity of origanum oil against Gram-positive and Gram-negative bacteria

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ABSTRACT

Introduction and Aim: Essential oils have been used from ancient times to treat different gram-positive and gram-negative bacterial-related infections. The study aims to screen the antibacterial, anti-quorum sensing activity of origanum oil against the common infection causing gram-negative and gram-positive bacteria.

Material and Methods: The MIC (minimum inhibitory concentration) and antibacterial activity of origanum oil against the eight bacterial species, namely, *Pseudomonas aeruginosa, Shigella flexneri, Salmonella enterica, Klebsiella pneumoniae, Streptococcus pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Enterococcus faecalis,* was assessed by broth-dilution method and well diffusion method. The anti-quorum sensing activity was analyzed using bioreporter strain CV026 at sub-MIC concentrations, while the cytotoxicity of the origanum oil was analyzed using the SW480 cell line.

Results: The oil's antibacterial activity was analyzed by measuring the clear inhibitory zone diameter, and maximum inhibitory zone of 36.66 mm zone against *Staphylococcus aureus* (Gram-positive) and 33.33 mm against *Salmonella enterica* (Gram-negative) of origanum oil was measured. And the minimum inhibitory zone of 16.66 mm zone of *Enterococcus faecalis* (Gram-positive) and 19.66 mm against *Klebsiella pneumoniae* (Gram-negative) of oil was noted. And the lowest MIC (0.1 mg/ml) of oil was found against *Streptococcus pneumonia*. The oil significantly inhibited the violacein pigment production (30.29 %) at 0.02 mg/ml concentration (this oil concentration did not significantly affect the growth curve). The *in vitro* cytotoxicity assay shows that the oil inhibited the SW480 cells growth with increasing concentration.

Conclusion: The origanum oil possesses antibacterial and anti-quorum sensing activity and can be used as an alternative for treating tested bacterial infection.

Keywords: Origanum oil; anti-quorum sensing; antibacterial; MIC; cytotoxicity.

INTRODUCTION

Research involving oils (essential) has started getting more attention from both industries as well as scientists. This is because of the increasing concern and trust of consumers in herbal and its related products, quality assurance, and safety (1,2). Essential oils (EOs) isolated from medicinal or aromatic plants are proven to have a great potential as antibacterial agents (3). Apart from the antibacterial activity, EOs essential oils also have antitoxigenic, antiviral, antiparasitic, and insecticidal properties, and these activities are due to the compounds present in plants (1,2). EOs are aromatic liquids extracted from different parts of the plant, including flowers, fruits, leaves, seeds, roots, barks, and herbs by several techniques, such as solvent extraction, water distillation, supercritical fluid extraction, and steam distillation (4). These EOs also contain various secondary metabolites, which play a crucial role in hindering bacterial growth (5). Among several available EOs, the origanum oil of the *Lamiaceae* family may also have a huge potential for research as well as industrial applications, especially in the food industry (6). In origanum oil, also different compounds such as γ-terpinene, p-cymene, and carvacrol were present. In this regard, the antibacterial activities of the origanum oil against four common Gram-negative bacteria such as *Pseudomonas aeruginosa, Shigella flexneri, Salmonella enterica, Klebsiella pneumoniae,* and four-Gram positive bacteria including *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis* and cytotoxicity, and anti-quorum activity were investigated in this research study.

MATERIALS AND METHODS

Origanum oil

The essential origanum oil was obtained from Sigma-Aldrich Inc. (Bengaluru, India). Tween 20 was purchased from SRL, India (surfactant) for the uniform distribution of origanum oil. The 10mg/ml concentration of origanum oil was prepared by using sterile Milli-Q and 0.1% Tween 20.

Bacteria and growth condition

The bacterial strain of *Pseudomonas aeruginosa, Shigella flexneri, Salmonella enterica, Klebsiella*

pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, and *Enterococcus faecalis* was procured from MTCC and was grown in tryptic soy broth (TSB), nutrient broth (NB), and brain heart infusion broth (BHIB) media at 37°C, pH 7.0. *Chromobacterium violaceum* CV026 (ATCC) was purchased from CECT, Spain, and was maintained at 28°C. Kanamycin and hexanoyl homoserine lactone (HHL) were purchased from Sigma-Aldrich, India, and media, such as BHIB, TSB and NB was purchased from HI-media.

MIC (Minimum Inhibitory Concentration)

The MIC (Minimum Inhibitory Concentration) of origanum oil against *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Shigella flexneri, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella pneumoniae,* and *Enterococcus faecalis* was investigated by using the micro broth dilution method. Brief, bacterial culture (OD 1 at 600 nm) was grown for 24 h at 37°C in the MHB (Mueller-Hinton broth) with or without oil in 96 well plates. The lowest oil concentration, which completely inhibits bacterial growth, was noted as the MIC for the oil (7, 8).

Antibacterial activity of origanum oil

In brief, the bacterial inoculums were spread on the agar plate surface by using an autoclaved spreader. Then, aseptically a well of 8 mm is punched with the help of a sterile tip, and 100 µl from the prepared sample is put into the cut wells and incubated for 24 h at 37°C in an upright position. The diameter of the inhibition zone (mm) around the wells was measured, showing the origanum oil antibacterial activity against different bacterial strains (7, 8).

Violacein inhibition assay

Briefly, a 100 µl of *C. violaceum* CV026 culture was seeded into the wells of 96 microtiter plate with 100 µl of LB broth and incubated in the absence or presence of increasing concentrations of origanum oil (0.01, 0.02, 0.03, 0.04 mg/ml). Then the 96 microtiter plate was incubated for 24 h at 28°C. After that, the plate was dried completely at 60°C. Next, 100 µl DMSO was added followed by shaking incubation at 30°C. The absorbance (OD) was noted at 585 nm with an ELISA plate reader (9).

Cytotoxicity of origanum oil by using cell line

Cell line SW480 procured from NCCS, Pune, India, was maintained in a Dulbecco's modified Eagle's medium (DMEM) with 10% Fetal bovine serum (FBS, Gibco) and1% penstrep (penicillin-streptomycin) antibiotic and incubated in a humidified atmosphere of 5% CO_2 at 37°C. For cytotoxic analysis of origanum oil, a stock solution was prepared by dissolving the oil in a culture medium. The cells, after washing with PBS, were incubated for few minutes with 0.25% trypsin-EDTA. Now the cells (10^4) were seeded per well on 96 well plates. After 24 h, SW480 cells were treated with increasing concentrations of origanum oil (1µg/ml, 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml) and incubated for 24 h. For the MTT assay, an MTT solution (5mg/ml) was added into each well and was incubated for 3 h at 37°C. After 3 h, the media was removed, and the 100 µL DMSO was added to dissolve the formazan product. The absorbance (OD) was taken at 540 nm on the ELISA plate reader, and the percentage of cell viability was calculated (10).

Statistical analysis

The analysis was performed in triplicate (n=3) and expressed as average ± standard deviation. All the statistical analysis was performed by following ANOVA using Prism5. At $p \leq 0.05$, the significance value was determined.

RESULTS

Description of Origanum oil

The description related to the oil, used in the study, were mentioned in Table 1. Different physical paramters of origanum oil used in the study was also analysed and mention in Table 2.

Table 1: The detailed description of the selected oil used in the study

Name of oil	Family	Medicinal Uses	Other application	Main chemical components	Reference
Origanum oil	*Lamiaceae*	Bronchitis, cough, whooping cough, fever.	Flavorings, soaps, perfumes, detergents, cosmetics	γ-terpinene, p-cymene, carvacrol.	(11)

Table 2: Physical characters of origanum oil used in the study

Physical parameters	Origanum oil
Colour	Light yellow
Specific gravity	0.937
Odour	Strong aromatic odour (pungent)
Appearance	Turbid
Refractive index	1.508

Antibacterial activity of origanum oil

The antibacterial activity of the origanum oil against the different gram-positive and gram-negative bacteria was measured by using an agar well diffusion assay. The antibacterial property was calculated by measuring the diameter (mm) of the clear zone around the sample well. The results show that the maximum inhibitory zone of 33.33 mm against *Salmonella enterica* (Gram-negative) and 36.66 mm zone against *Staphylococcus aureus* (Gram-positive) of origanum oil was measured. And the minimum inhibitory zone of 19.66 mm against *Klebsiella pneumonia* (Gram-negative) and 16.66 mm zone of *Enterococcus faecalis* (Gram-positive) of origanum oil was noted. The result shows that the origanum oil was found a little bit more effective against gram-positive bacteria (Fig. 1).

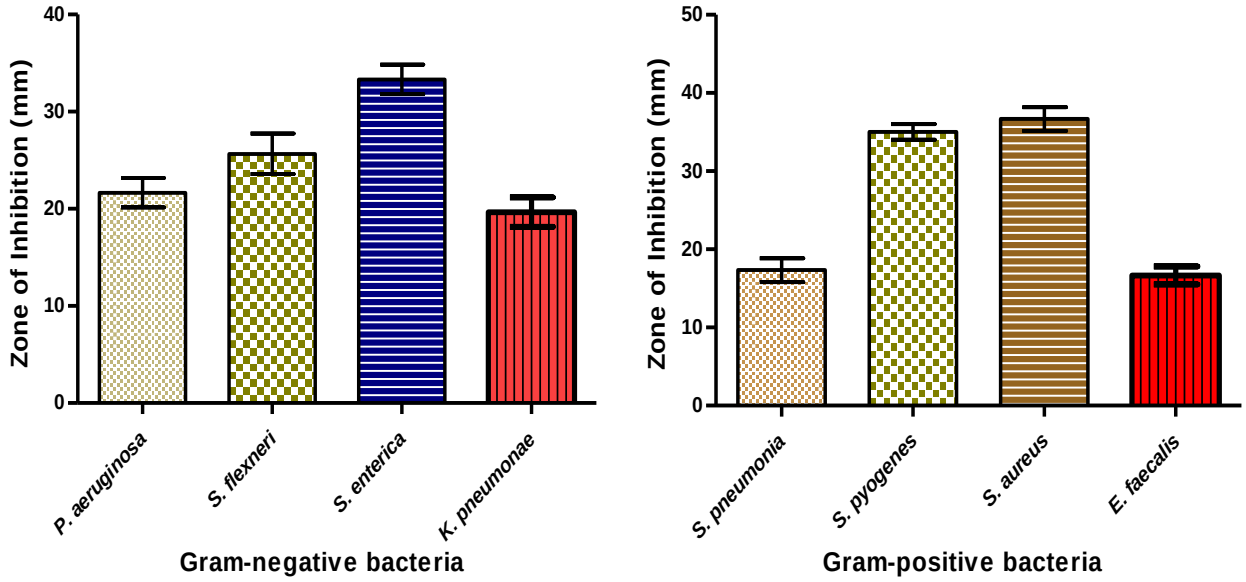

Fig. 1: Antibacterial activity of oil against the different bacterial strain. Values are presented as mean ± SD.

MICs (minimum inhibitory concentrations)

The MICs (minimum inhibitory concentrations) of oil against *Streptococcus pneumonia, Streptococcus pyogenes, Staphylococcus aureus, Shigella flexneri, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella pneumoniae,* and *Enterococcus faecalis* were calculated by using the broth-dilution method in 96 well microplates, and the result was shown in fig. 2.

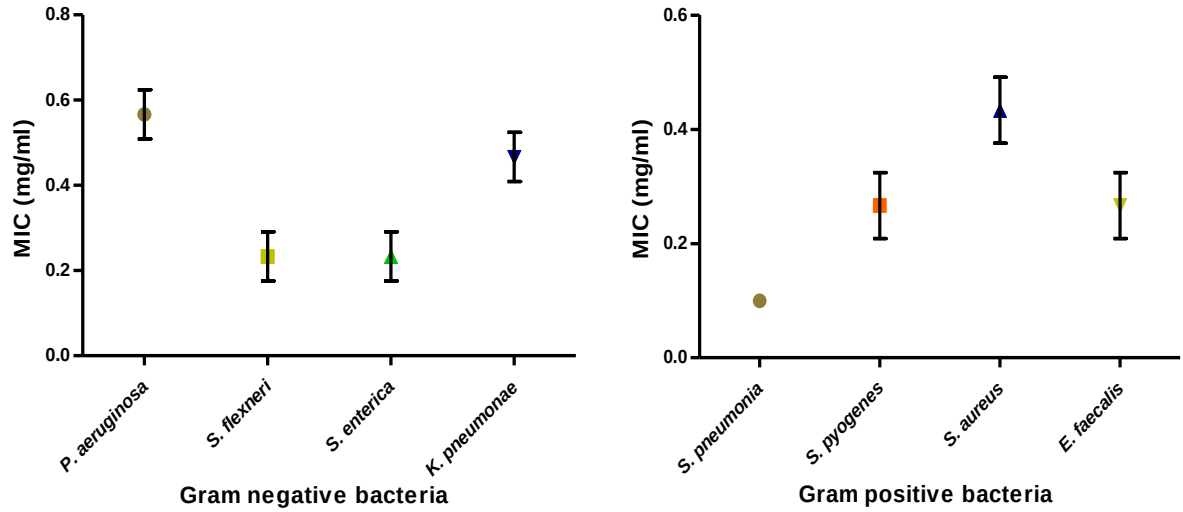

Fig. 2: MIC (Minimum inhibitory concentration) results of origanum oil against different Gram-negative and Gram-positive bacteria. Values are presented as mean ± SD.

Anti-quorum activity of origanum oil

The quorum inhibitory activity of the origanum oil was evaluated by using violacein production (*C. violaceum* CV026 produces violacein when AHL is added exogenously). In quantitative inhibition assay, the violacein production decreases with increasing oil concentration. Violacein production was found to be decreased by 30.29 % at 0.02 mg/ml oil concentration.

The reason behind taking the 0.01, 0.02, and 0.03 mg/ml concentrations of origanum oil because this concentration did not significantly affect the bacterial growth. With increasing oil concentration, the violacein production was observed to be reduced by 59.31 % at 0.04 mg/ml oil concentration (Fig. 3).

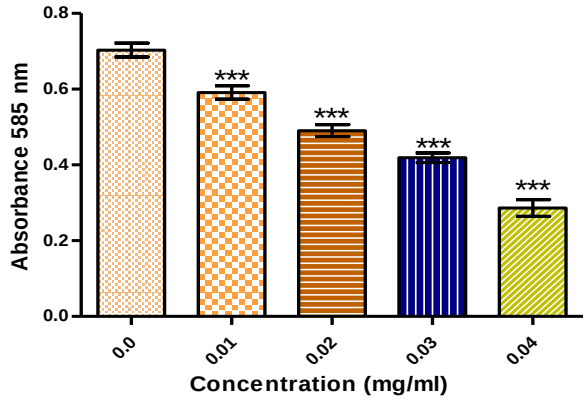

Fig. 3: Inhibition of violacein production by origanum oil. Violacein pigment production was noted spectrophotometrically at 585 nm. Values are presented as mean ± SD. *** indicates the significant samples (one-way ANOVA P<0.001).

Cell viability

In examining the *in vitro* cytotoxicity activity of the origanum oil, human colon cell line SW480 cells were treated with increasing concentrations of origanum oil ranging from 0–100 µg/mL 24 h of treatment. The % cell viability was calculated, and a concentration-dependent decrease in cell viability was observed (Fig. 4).

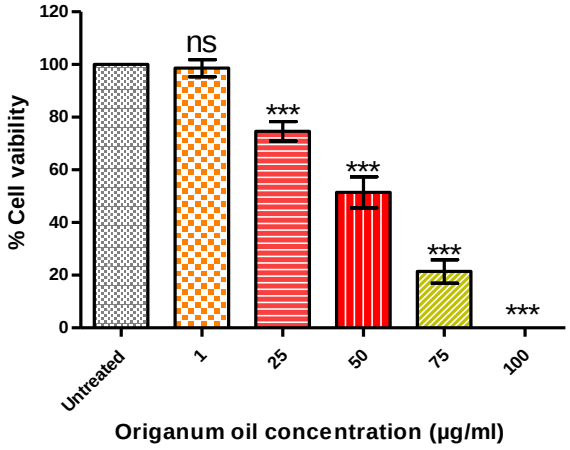

Fig. 4: Cytotoxicity activity of origanum oil determined by MTT assay. Values are presented as mean ± SD. *** indicates the significant samples (one-way ANOVA *P<0.001*).

DISCUSSION

Bacteria including both Gram-negative (*Pseudomonas aeruginosa, Shigella flexneri, Salmonella enterica, Klebsiella pneumoniae* and Gram-positive bacteria *(Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, and Enterococcus faecalis*) are among the common bacteria which are responsible for causing various human infection including respiratory tract infections, bacillary dysentery, shigellosis and many other severe diseases (12-14).

Generally, antibiotics were recommended for the treatment of different bacterial infections caused either by Gram-positive or Gram-negative bacteria. However, in recent times it was commonly reported that several bacteria start showing resistance against some drugs (15). Essential oils contain the phytochemical compounds that pose antibacterial activity and employ therapeutic effects on human health (16). The use of herbal extracts and essential oils (EOs) has started gaining attention in recent years because of their safety, easy availability, efficacy, least side effects, and low cost (8, 17).

The bacteria selected for the study are some common bacteria that are associated and responsible for causing various diseases, such as *S. aureus*, which causes severe bacterial pneumonia, which causes widespread morbidity and mortality (15). *P. aeruginosa* causes meningitis, endophthalmitis, endocarditis, malignant external otitis, and pneumonia (12). *S. flexneri* and *S. enterica* cause shigellosis and dysentery (14). *S. pyogenes* causes rashes, impetigo, scarlet, and pharyngitis (18). *K. pneumoniae* and *S. pneumoniae* causes respiratory tract infection, while E. faecalis causes urinary tract infections, endocarditis, intra-abdominal infection, and wound infection (13). This study investigates the antibacterial activity of origanum oil against some common Gram-positive and Gram-negative bacteria.

Well, diffusion assay technique is a well recognized and widely accepted semi-quantitative method used to determine the antimicrobial activity of compounds, 8 bacteria were selected, and the antibacterial activity of origanum oil was assessed by measuring the size of the halo zone formed (7).

The highest clear halo inhibitory zone of 36.66 mm against *S. aureus* while the minimum inhibitory zone of 16.66 mm of *E. faecalis* of origanum oil was noted (13, 16, 19). The result indicates that the oil contains a bioactive compound that is responsible for bacterial destruction.

From the MIC results, it was observed that all bacteria were susceptible to the action of origanum oil, ranging MIC values from 0.1 to 0.56 mg/mL (19). Results of MIC of origanum oil obtained is similar to the result of thyme oil against *E. coli* and *S. aureus* (20). Further, to analyze the anti-quorum oil activity at sub-lethal concentrations was used because this concentration cannot neutralize the bacteria. The results reveal that the low oil concentration (0.01, 0.02, and 0.03 mg/ml) significantly inhibits the QS (7). The cytotoxicity of the oil was screened by using SW480 cell line by using MTT assay. The *in vitro* cytotoxicity result reveals that the origanum oil show cytotoxicity against the SW480 cell (21).

CONCLUSION

In conclusion, the *in vitro* result shows that the origanum oil possesses antibacterial activity and anti-quorum activity. The evidence shows that the origanum oil effectively inhibited the Gram-positive and Gram-negative bacterial growth. The origanum oil screened in the study has the potential and can be used as an alternative remedy for treating the antibiotic resistance problem and bacterial infection. In the future, despite of encouraging results, the *in vivo* and clinical analysis should be performed.

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CONFLICT OF INTEREST

No conflict of interest exists between the authors.

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