

Antibacterial Activity of the Essential Oil Isolated from *Origanum vulgare* L. (Lamiaceae) Against Multi-Drug Resistant Bacteria

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ABSTRACT

Objective: The current study is designed to evaluate the activity of essential oil of *Origanum vulgare* against multi-drug resistant bacteria and both G+ and G- bacteria.

Material and Methods: *O. vulgare* essential oil was isolated by Clevenger-type apparatus according to the procedure described in the British pharmacopeia volume II. Five species of bacteria were used to evaluate the antibacterial activity of *O. vulgare* constituent in this study, Two Gram negative (*Escherichia coli*, and multidrug resistance *Klebsiella pneumoniae*) and three Gram positive bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis*). All bacteria isolated from different clinical sources. The stock solution (1-mg /16 mL) concentration had been prepared as follow: 1 mg of essential oil dissolved in 16 mL of (DMSO) dimethyl-sulfoxide. The serial dilution of 1-mg/mL was the following concentrations: 1000, 500, 250, 125 and 62.5 µg/mL were prepared for antibacterial activity by agar well diffusion. Ampicillin and Amoxicillin were used as positive control for all strains, while DMSO as negative control.

Conclusion: The essential oil of *O. vulgare* shows a good antibacterial activity against MDR bacteria as well as the other studied types of bacteria.

Keywords: Antibacterial activity, *Origanum vulgare*, Multi-drug resistant bacteria.

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INTRODUCTION

Origanum vulgare is an aromatic perennial herb of the Lamiaceae family known for its flavorful dried leaves and flowering tops. It is native to the Mediterranean region, central European, India, Iran, Iraq, Ireland, Irkutsk, Italy and Turkey.¹ Aromatic and volatile liquids, including leaves, flowers, stems, bark, seeds, peels, fruits, wood, and whole plants, are essential oils. Essential oils are odiferous, highly volatile substances present in plants. These compounds due to their volatility it may be separated by steam distillation from an aromatic plant of a single botanical species and can be identified by both smell and taste.²

Essential oil from *O. Vulgare* has been shown to have antibacterial effects against (*Escherichia coli*, *Bacillus subtilis*, *Enterobacter cloacae*, *Micrococcus flavus*, *Mirabilis proteus*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Salmonella epidermidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Helicobacter pylori*).³

Studies of hexane extract of *O. vulgare* leaves showed that essential oils derived from it known to possess antifungal, insecticidal, and antimicrobial activities.⁴

Oregano possesses considerable antibacterial properties due primarily to their Carvacrol and Thymol content.⁴

Study on aqueous and methanolic extracts of oregano leaves show some anti-radical activity, while ethanolic extracts demonstrated antioxidant activity.⁵

The current study design to examine the anti-bacterial activity of *O. vulgare* against resist bacteria other types of bacteria.

MATERIAL AND METHODS

Extraction and Identification of Essential Oil

Aerial dried parts of the *O. vulgare* L (150 g) were ground and suspended in 1300 mL distilled water and submitted to hydro distillation for about 3 hours by using a Clevenger-type apparatus according to the procedure described in the British pharmacopeia volume II.⁷

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Preparation of Essential Oil

The stock solution (1-mg: 16 mL) concentration had been prepared as follow: 1 mg of essential oil dissolved in 16 mL of (DMSO) dimethyl-sulfoxide.⁶ The serial dilution of 1-mg/mL was the following concentrations: 1000, 500, 250, 125 and 62.5 µg/mL were prepared for antibacterial activity by agar well diffusion.⁷

Ampicillin and amoxicillin were used as positive control for all strains, while DMSO as negative control.

Tested Bacteria

The antibacterial activity of essential oil was done in the educational laboratory of College of Pharmacy, Mustansiriyah University. A preliminary antibacterial activity was conducted according to well diffusion method.⁸

Five species of bacteria were used to evaluate the antibacterial activity of *O. vulgare* constituent in this study, Two of them are Gram negative (*E. coli*, and Multidrug Resistance *K. pneumoniae*) and the other three were Gram positive bacteria (*S. pneumonia*, *S. aureus* and *E. faecalis*). All bacteria isolated from different clinical sources. The bacterial diagnosis was according to morphological examination, biochemical tests and diagnostic kits.

Antibacterial Activity Assay by Agar Diffusion Method

The agar well diffusion method was used to determine antibacterial activity of extracts. A suspension of the tested microorganisms was spread on an appropriate solid media plates and incubated overnight at 37°C for 18–24 hours.

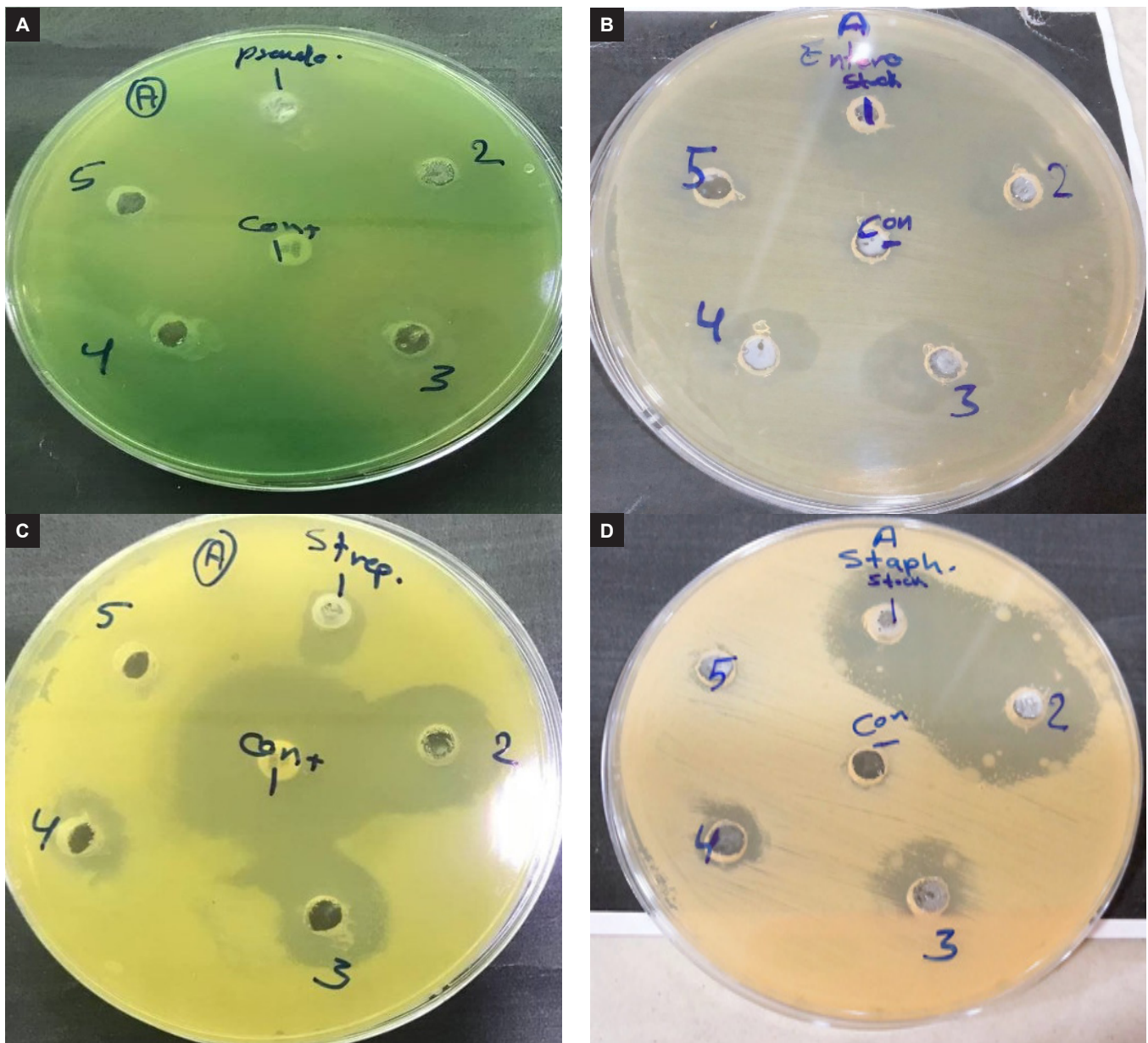
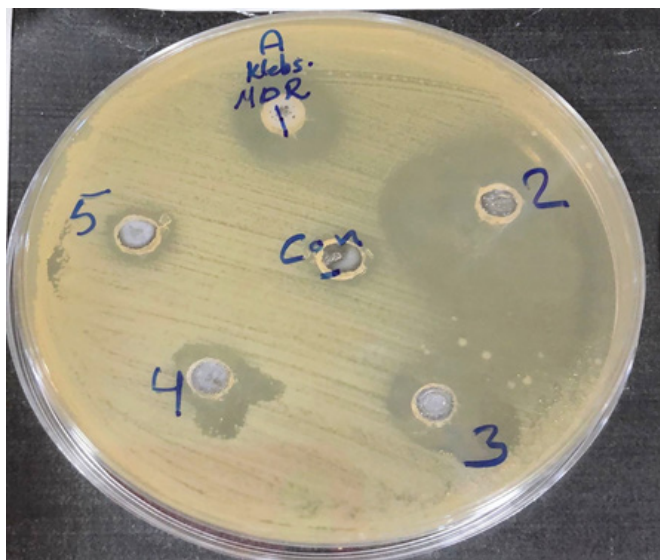


Figure 1: Disc diffusion method (A: *P. aeruginosa*, B: *S. pneumonia*, C: *E. faecalis*, D: *S. aureus*).

Tables 1: The inhibition zone of *O. vulgare* essential oil.

Compounds	Bacterial species	Inhibitory zone of essential oil(mm)					DMSO	Positive control
		500	250	125	62.5	31.2		Amoxicillin / Ampicillin
<i>O. vulgare</i> essential oil	<i>E. coli</i>	25	20	15	10	8	Zero	18 13
	<i>P. aeruginosa</i>	Zero	Zero	15	Zero	13	Zero	Zero Zero
	<i>S. aureus</i>	15	15	12	Zero	Zero	Zero	30 30
	<i>S. pneumonia</i>	22	20	17	15	Zero	Zero	30 20
	<i>E. faecalis</i>	35	35	20	16	15	Zero	20 25
	<i>K. pneumonia</i> (MDR)	23	20	15	5	Zero	Zero	Zero Zero

**Figure 2:** Disc diffusion method for *K. pneumonia* (Multi-drug resistance)

RESULTS

Oregano is considered a food plant and is used as a flavoring for food, in addition to its therapeutic efficacy that has been proven in previous studies.^{1,2} Since it is classified as a food plant, this feature gives it significant importance in medical applications. Five types of bacteria and multi-drug resistance bacteria were selected to study the effectiveness of *O. vulgare* essential oil isolated from the plant, as shown in Tables 1.

The essential oil showed anti-bacterial activity against all types of bacteria and showed good activity against resistant bacteria.

DISCUSSION

The essential oil composition of *O. vulgare* was analyzed by employing GC-MS, the results show 17 identified components found in the essential oil of *O. vulgare*. Monoterpenes both (hydrocarbons and oxygenated monoterpene). Essential oils consist of compounds (monoterpene and sesquiterpene) characterized by their capacity to generate flavor or aroma and are generally obtained from spices, aromatic herbs,

fruits, and flowers.⁹ GC/MS analysis of essential oils shows that of the different constituent compounds, terpenoids are the most abundant and are present as either monoterpenes, or sesquiterpene and as their derivatives.

Many previous studies focused on the antimicrobial properties of essential oils and of the main monoterpenes found in them. In fact, many terpenes are known to be active against a wide range of microorganisms, including G⁺ and G⁻ bacteria.¹⁰

The antibacterial action of essential oils and their monoterpene components has been attributed to toxic effects on membrane structure and function. In fact, as a result of their lipophilic character, monoterpenes will preferentially partition from an aqueous phase into membrane structures.¹² This will result in membrane expansion, increased membrane permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and also alteration of ion transport processes. Other study¹³ has shown the effects of selected essential oil components on outer membrane permeability in gram-negative bacteria, evidencing that monoterpene uptake is also determined by the permeability of the outer envelope of the target microorganism. However, the particular processes involved in monoterpenes' antibacterial effect are still unknown.

In conclusion, essential oil of *O. vulgare* show an antibacterial activity against different type of bacteria as well as multi-drug resistance bacteria.

REFERENCES

- Oniga I, Pușcaș C, Silaghi-Dumitrescu R, Olah NK, Sevastre B, Marica R, Marcus I, Sevastre-Berghian AC, Benedec D, Pop CE, Hanganu D. *Origanum vulgare* ssp. *vulgare*: Chemical Composition and Biological Studies. *Molecules*. 2018 Aug 19;23(8):2077.
- B, Sudji J, Tešanović K, Janjušević L. *Origanum vulgare* essential oil affects pathogens causing vaginal infections. *J Appl Microbiol*. 2017 May;122(5):1177-1185.
- RN, Abdelgayed SS, Mohamed SS, Farag MA, Ramadan MA. *Origanum vulgare* L. Essential Oil as a Potential Anti-Acne Topical Nanoemulsion-In Vitro and In Vivo Study. *Molecules*. 2018 Aug 28;23(9):2164.

4. Xu JJ, Chung HY, Ye WC, Li YL, Wang GC. Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food Chem.* 2014;152:300-6.
5. Martorell M, Setzer WN, Del Mar Contreras M, Salehi B, Soltani-Nejad A, Rajabi S, Tajbakhsh M, Sharifi-Rad J. Carvacrol and human health: A comprehensive review. *Phytother Res.* 2018 Sep;32(9):1675-1687.
6. Xie Y, Huang Q, Rao Y, Hong L, Zhang D. Efficacy of *Origanum vulgare* essential oil and carvacrol against the housefly, *Musca domestica* L. (Diptera: Muscidae). *Environ Sci Pollut Res Int.* 2019 Aug;26(23):23824-23831.
7. Neto T, Antonio Freitag R, Guerra Lund R. Evaluation of anti-enzyme properties of *Origanum vulgare* essential oil against oral *Candida albicans*. *J Mycol Med.* 2018 Mar;28(1):94-100.
8. Vasconcelos NG, Croda J, Silva KE, Motta MLL, Maciel WG, Limiere LC, Simionatto S. *Origanum vulgare* L. essential oil inhibits the growth of carbapenem-resistant gram-negative bacteria. *Rev Soc Bras Med Trop.* 2019 Jun 27;52:e20180502
9. Sakkas H, Papadopoulou C. Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. *J Microbiol Biotechnol.* 2017 Mar 28;27(3):429-438.
10. Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. Essential Oils of Oregano: Biological Activity beyond Their Antimicrobial Properties. *Molecules.* 2017 Jun 14;22(6):989.
11. Ferraz EO, Vieira MAR, Ferreira MI, Fernandes Junior A, Marques MOM, Minatel IO, Albano M, Sambo P, Lima GPP. Seasonality effects on chemical composition, antibacterial activity and essential oil yield of three species of *Nectandra*. *PLoS One.* 2018 Sep 18;13(9):e0204132.
12. Valdivieso-Ugarte M, Gomez-Llorente C, Plaza-Díaz J, Gil Á. Antimicrobial, Antioxidant, and Immunomodulatory Properties of Essential Oils: A Systematic Review. *Nutrients.* 2019;11(11):2786. Published 2019 Nov 15. doi:10.3390/nu11112786