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Original article

Extraction and Antistaphylococcal Study of the Essential Oil of *Origanum vulgare* L. (Guelma-Algeria) [1]

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Abstract

Antimicrobial properties of plant essential oils (EO) have been investigated through several observations and clinical studies which purpose them as potential tools to overcome the microbial drug resistance (MDR) problem. The aim of this research was to study the antibacterial effect of a traditional plant EO, *Oraginum vulgare* L., against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) through disk diffusion and agar dilution methods.

The EO showed very effective bactericidal activity towards the majority of the tested bacterial strains with inhibition zone diameters in the range of 9.9-31.9 mm and a minimum inhibitory concentration (MIC) ranging from 0.314 to 0.628 mg/ml.

These results suggest that the essential oil of *Origanum vulgare* L. may be a useful alternative to antibiotics for the control of the infections caused by *Staphylococcus aureus*.

Keywords: *Origanum vulgare* L., Essential oil, Antibacterial activity, *Staphylococcus aureus* MRSA.

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INTRODUCTION

The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy (Boucher et al., 2009). MDR bacterial infections often lead to increased mortality, longer stays in hospitals, and higher cost of treatment and care (Boucher et al., 2009; Giamarellou, 2010).

The resistance to antibiotics by microorganisms has increased because, generally, bacteria have the genetic ability to transmit and acquire resistance to them.

Bacteria that belong to the *Staphylococcus* genus constitute one of the most serious epidemiological problems. *S. aureus* has the strongest virulence potential among all the staphylococcal species. It may become a part of the human bacterial flora (*S. aureus* nasal carriage) but increases the risk of infection development, both nosocomial and community-acquired (DeLeo et al., 2010; Rafee et al., 2012; Wang et al., 2010).

Methicillin-resistant Staphylococcus aureus (MRSA) is a major pathogen causing nosocomial infections, but it has emerged as a problematic pathogen in the community setting as well. These strains called Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CAMRSA) cause infections in healthy individuals without predisponent risk factor and outside the hospital setting. MRSA and CA-MRSA present a significant threat to public health and are difficult to manage (Li et al., 2009). The therapeutic options for these pathogens are extremely limited and physicians are forced to use expensive or previously discarded drugs that are associated with significant side effect to the patients health (Boucher et al., 2009). Therefore, it is necessary to search the other alternatives that can potentially be effective in the treatment of these problematic bacterial infections.

For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant compounds for pharmaceutical purposes has gradually increased in the world according to World Health Organization (Santos et al., 1995). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Ellof, 1998).

The use of essential oils has been shown to possess potential in the treatment of infections, and is safe in terms of human and animal health. Essential oils generally regarded as safe show antimicrobial proprieties and antibacterial resistance that has not been reported after prolonged exposure (Pozzo et al., 2012).

Among many plants scientifically studied regarding their antimicrobial properties, *Origanum vulgare* L. (Oregano), Lamiaceae, has showed prominent results (Skandamis et al., 2002; Souza et al., 2007). *O. vulgare* L. essential oil presented interesting results in inhibiting the growth of bacteria and

synthesis of microbial metabolites, including the pathogen *Staphylococcus aureus* (Barros et al., 2009; Baydar et al., 2004; Souza et al., 2006).

Oregano oil and its major phenolic components, carvacrol and thymol, are known for their wide spectrum of antimicrobial activity, which has been the subject of several investigations *in vitro* (Dorman et al., 2000; Lambert et al., 2001) and *in vivo* (Adam et al., 1998, Manohar et al., 2001).

In this context, the aim of this study was to evaluate the antimicrobial activity *in vitro* of the essential oil of oregano from the region of Guelma (Algeria) against eight (8) methicillin-resistant *Staphylococcus aureus*.

Material and Methods

Plant material

Leaves of *O. vulgare* were harvested at flowering stage in mid-June, 2014 from wild grown plants at Guelma (East of Algeria) situated at latitude: 36° 36′ 41″, longitude: 7° 30 ′48″ where the climate is mild and rainy in winter and hot in summer with an annual average temperature of 17.3°C. Dried leaves of *O. vulgare* were subjected to hydro distillation using a Clevenger-type apparatus and has been analyzed by GC/MS, as described in our latest publication (Mahfouf et al., 2017).

Bacterial Strains

Bacterial strains of MRSA used were clinically isolated from specimens of different infectious diseases obtained from Hakim Okbi hospital in Guelma city. They were provided by laboratory of microbiology, faculty of medicine, university of Badji Mokhtar, Annaba.

The isolates were identified on the basis of Gram's staining, mobility, cultural characterization and biochemical screening routine methods were used. The antibiotic susceptibility was tested by the antibiogram method.

Diffusion method in agar

Disc diffusion assay

A 24 h culture was diluted with sterile physiological saline solution with reference to the McFarland standard, to achieve an inoculum of approximately 106 CFU ml. A 5ml portion of this inoculum was placed on to the surface of pre-dried Mueller–Hinton agar Petri dishes, and allowed to remain in contact for 1 min. Excess inoculums was removed using a sterile syringe and the Petri dishes, were allowed to dry for 20 min at room temperature. Sterile 6 mm filter paper discs were placed on the plates and immediately 20 µl of the essential oils were added. Sterile paper disc was used as control. After allowing 1 h at room temperature for the essential oil to diffuse across the surface, Petri dishes were incubated at 37°C for 24 h. The inhibition zone was measured in millimeter (Ahmad et al., 1999).

Dilution method

The essential oil to be tested was incorporated into a semi-solid agar medium with different concentrations of essential oil diluted in dimethyl sulfoxide (DMSO). After incubation, the absence of microbial growth in Petri dishes was determined by the naked eye.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of essential oil inhibiting any visible growth to the naked eye after 16 to 20 hours incubation at 37 ° C. Microorganisms, however, remain viable.

The MIC values were evaluated according to published procedures (National Committee for Clinical Laboratory Standard, 2000). The MICs were determined only with micro-organisms that displayed inhibitory zones. Dilutions of the oil within a concentration range of 0.314-10.05 mg/ml.

Results and Discussion

Chemical composition of the essential oil of O. vulgare L.

Fifty five volatile constituents were identified in this EO, representing 98.7% of the total composition (Table 1).The most abundant component was thymol (32.58%). Other components were identified as γ-terpinene (18.76%), phenol (17.92%),1,2,3,4-tetramethylfulvene (11.40%), isodiprene(2.79%), β thujene (1.94%), caryophyllene (1.80%) , β sesquiphellandrene (1.43%) and linalool (1.22%) (Mahfouf et al., 2017).

Antistaphylococcal activity

Table 1. Antibacterial activity of *O.vulgare* L. essential oil against the bacterial strains tested based on MIC and disc diffusion method

Micro-organisms	****Disc diffusion assay (inhibtion zone mm)**	***MIC (mg/ml)**
MRSA 1	25.2	0.628
MRSA 2	31.9	
MRSA 3	25.1	
MRSA 4	9.9	
MRSA 5	19.3	0.314
MRSA 6	26.2	
MRSA 7	28.9	
MRSA 8	26.1	

* MIC: Minimal Inhibitory Concentration, concentration range: 0.314-0.628 g/ml

** Disc diameter 6 mm average of three consecutive trials

Among the 8 strains of MRSA tested, 6 strains were very sensitive to the essential oil tested with diameters of inhibition zones ranging from 25.1 mm to 31.9 mm, one strain had average sensitivity with a diameter of inhibition zone of 19.3 mm and another strain with a limited sensitivity with diameter of inhibition zone of 9.9 mm. The MIC was relatively low, ranging from 0.314 mg/ml to 0.628 mg/ml (Table 1, Fig.1).

The essential oil from oregano was inhibitory to the growth of all the bacteria under test (Table 1). The obtained results, in accordance with the literature, showed that Oregano essential oil has antibacterial properties. The activity is due to the high content of phenolics compounds such as thymol, which account for over 32% of the ingredients of the oregano oil (Bouhdid et al., 2012).

Generally, the major compounds determine antimicrobial activity. Carvacrol, terpinen-4-ol and thymol are the major compounds in oregano EO (Barros et al., 2009). The antimicrobial activity of oregano oil is mostly attributed to the action of its principal phenolic components, carvacrol and thymol, which exhibit significant bactericidal activity when tested separately (Juven et al., 1994; Ultee et al., 1998; Lambert et al., 2001; Friedman et al., 2002). Due to their hydrophobic nature, carvacrol and thymol interact with the lipid bilayer of cytoplasmic membranes causing loss of integrity and leakage of cellular material such as ions, ATP and nucleic acid (Helander et al., 1998; Ultee et al., 1999; Lambert et al., 2001; Trombetta et al., 2005).

Studies on the antibacterial mechanism of phenolic compounds found in essential oils focused on their effects on the cellular membrane, changing its structure and permeability (Shetty et al., 2004). Lin et al. (2004) stated that the damage to cell membrane might explain the observed effects, since phenolics could cause sublethal injury to cell membranes, causing disruption of proton motive force due loss of H^+-ATPase. This could make bacteria more susceptible to acid environment. Moreover, at low pH, the hidrophobicity of an essential oil increases, enabling it to more easily dissolve in the lipids of the cell membrane of target bacteria (Juven et al., 1994).

Although the antibacterial activity of oregano EO is more pronounced against Gram-positive than Gram-negative bacteria (Marino et al., 2001).

Overall, we can conclude that oregano EO is one of the most promising natural compounds that can be used to develop safer antibacterial agents and that its effective combination with antibacterials may be used in the future to treat diseases caused by *Staphylococcus aureus*.

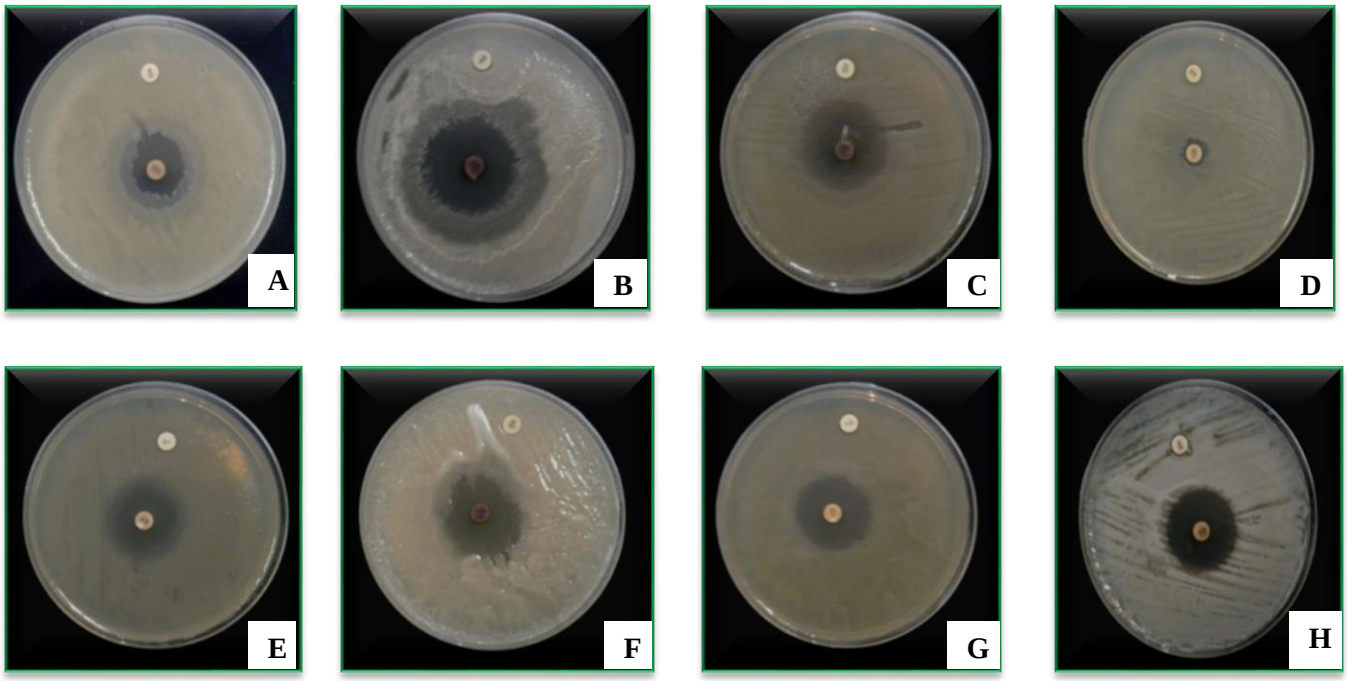

Figure 1. Aromatograms: qualitative effects of the essential oil of oregano observed on the strains of *Staphylococcus aureus* by diffusion from impregnated discs on agar medium. (A) MRSA 1; (B) MRSA 2; (C) MRSA 3; (D) MRSA 4; (E) MRSA **5**; (F) MRSA **7**; (G) MRSA **6**; (H) MRSA 8.

Conclusion

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

The results ofthis study suggest that *O. vulgare* essential oil possesses antibacterial properties due to its richness in thymol. It gave inhibition zone diameters in the range of 9.9-31.9 mm and MIC ranging from 0.314 to 0.628mg/ ml.

This EO could be used as bacterial growth inhibitory agent in new drugs in therapy of Staphylococcus diseases. Although this study has investigated *in vitro* antibacterial activity, extensive *in vivo* studies could confirm the potential usefulness of this plant's essential oil in combating antimicrobial resistance.

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