



## **THE *IN VITRO* EVALUATION OF ANTIBACTERIAL EFFECT OF *MARRUBIUM VULGARE* L. LEAF EXTRACT GROWN IN ALGERIA**

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### Abstract

The emergence of antibioresistance bacteria has become a public health problem at the global level. This problem is of growing concern in hospitals. It is therefore necessary to make use of new active substances from medicinal plants. In order to validate their traditional uses, our work aims to evaluate the antibacterial activity of methanolic extract of *Marrubium vulgare* L. against resistant pathogenic microorganisms. The study was carried out by diffusion method and micro-dilution. Results obtained showed that the antibacterial activity varies with the extract concentration and the nature of the germ. The MIC ranged for most strains between 125 and 250 mg/ml.

**Keywords:** antibioresistance, Antibiogram, *Marrubium vulgare*, methanolic extract.

### 1. Introduction :

The resistant of pathogenic bacteria to antibiotics is one of the most important problems in controlling and treatment of bacterial infectious diseases (Younet *al.*, 2010; Pulcini *et al.*, 2010). With the increase of this threat, the emergence of new infectious agents and therapeutic failure, it is necessary to look for new active substances. One of the strategies is to explore aromatic and medicinal plants (Vanden, 1991).

Plants are a valuable source of many molecules. Besides vitamins, minerals... the plants synthesize other bioactive substances especially polyphenols, which are the subject of several researches (Merghem, 2011). Plants belonging to the *Lamiaceae* family are the best known and the most widely used in medicine. In contrast, only 1% of all species have been studied for their pharmacological activity (Meyre-Silva and Cechinel-Filho, 2010).

*Marrubium vulgare* L. which is an aromatic plant commonly known as "White Horehound" in Europe and "Mariouet" in Algeria is naturalized in North and South America, Western Asia and the Mediterranean region (Benfreha Temmouriet *al.*, 2014; Melendez *et al.*, 2006). In this region, the plant is frequently used in traditional medicine to cure a variety of diseases. It is known for her activities hypoglycemic (Romanet *al.*, 1992), antihypertensive (El-Bardai *et al.*, 2004), analgesic (DeSouza, 1998), anti-inflammatory (Schlemper *et al.*, 1996) and many other biological activities.

In this context, this work aims to promote the spontaneous plant in Algeria *M. vulgare*. We contributed to possible use of the methanolic extract of leaf of this plant as an antibacterial agent against pathogenic bacteria.

### 2. Materials and methods

#### *Methanolic extract*

A semi solid brown crude extract of *M. vulgare* was prepared and provided to us by the Bioconversion Research Laboratory, Engineering Microbiology and Health Safety, Faculty of SNV, University of Mascara, Algeria.

#### *Bacterial strains*

Several samples were taken at surgery services of Maslam Taib hospital in Mascara/Algeria. Bacterial strains were then isolated and identified.

#### *Antibiotics*

Amoxicillin (AX), Amoxicillin + Clavulanic acid (AMC), Cefazolin (CZ), Oxacillin (OX) and Gentamicin (CN) were used in the form of discs of 6mm diameter.

#### *Antibiogram*

The strains isolated were subjected to disk diffusion method using 06 different antibiotics according to Stephen and the susceptibility was determined by the measure of diameter of

inhibition zone in mm (Stephenet *al.*, 2012). Comparing this latter with (OMS, 2008) and the recommendations of the French Society for Microbiology (2008), we determined whether the bacteria are sensitive, resistant or intermediate.

#### **Disc diffusion assay**

The antibacterial activity of *M. vulgare* methanolic extract was firstly determined by disc diffusion method (Boutelilis Djahraet *al.*, 2012). Sterile Whatman paper discs of 6 mm diameter containing substances (500, 250, 125, 62.50 and 31.25 mg/ml) were placed on the surface of agar plates previously seeded by spreading of 0.1 ml from overnight culture. The plates were then incubated at 37 °C for 18 to 24 hours. If the substance is effective, it forms inhibition zones around the disks. Moreover, this area is great, more bacterial species are sensitive (Wadeet *al.*, 2001).

#### **MIC determination**

Minimum Inhibitory Concentrations (MIC) was determined using a broth dilution method as described by Cosentino *et al.* (1999). All wells were filled with 50 µL of Muller Hinton broth (MHB). Extracts were dissolved in DMSO and added to the first well (50 µL). Serial two-fold dilutions were made then. An over-night culture of bacteria suspended in MHB was adjusted to turbidity equal to 0.5 McFarland standards. The plates were inoculated with bacterial suspension (50 µL/well) and incubated at 37°C for 24 h. Each test included two growth controls consisting of the medium with the solvent (DMSO) and medium with bacterial suspension. Then the turbidity was measured every two hours using micro-plate reader (TECAN brand) at 620 nm wavelength. The lowest concentration showing no culture was considered as the MIC and it's expressed as (mg/ml) (Side Larbiet *al.*, 2016).

#### **Statistical analysis**

All results were the average of three experiments.

### **3. Results and discussion**

#### **Antibiogram**

Comparing to (WHO, 2008), results of Antibiogram are shown in table 2. For all strains, mainly antibiotics are inactive. This resistance for most bacteria was acquired.

#### **Disk diffusion assay and MIC determination**

According to figure 1 and table 3 below, the antibacterial effect is more or less important depending on the nature of the strain and on the concentration of active substance. Generally, the extract appears to be effective against strains tested. This result was confirmed by an earlier study (Fleurette *et al.*, 1989). However, *S. aureus* displays a high resistance, compared to other strains, which differed to the previous study. Regarding the *Enterobacteriaceae*, the extract showed moderate effectiveness, *E. coli* was more resistant than *Enterobacter*. Indeed, the essential oils of *M. vulgare* showed a similar effect (Mubashir *et al.*, 2008). As for *Pseudomonas aeruginosa* strain, it showed larger inhibition zones than those caused by antibiotic. Our results on this multidrug resistant bacterium are closer to other results achieved on the same plant (Boutelilis Djahraet *al.*, 2012).

Generally, the MIC ranged between 125 and 250 mg/ml, whereas it reached 61.50 mg/ml for *S. epidermidis*. However, for inhibiting the growth of *S. aureus*, the extract concentration should be higher (500 mg/ml).

Comparing the results of two techniques applied in the present work, we deduce that there is an inverse relationship between the diameters of inhibition and MIC. More zones of inhibition are greater, more the MIC is lower and the antibacterial substance is active.

The biological activity of *plant extracts* against tested bacteria could be attributed to the presence of biologically active components such as flavonoids and phenolic acids. These components are able to cross the cell wall and bind to certain proteins and enzymes, which induces cell lysis (Zarai *et al.*, 2011).

#### 4. Conclusion :

The antibacterial activity of phytochemicals varies according to their content. The latter is affected by the genotype (variety of the plant), growing conditions, maturity and the extraction methods. The study of the antibacterial activity of the methanolic extract from leaves of *M. vulgare* showed efficacy against resistant pathogenic bacteria.

Further, studies can be made like isolation and purification of bioactive compounds. The study of the synergistic effect between these compounds and antibiotic drugs can be also made.

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**Table 1.** Pathogenic bacteria used for the antibacterial assay

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*Enterobactersp.*

*E. coli*

*Acinetobacterbaumani*

*Pseudomonas aeruginosa*

*Staphylococcus epidermidis*

*Staphylococcus aureus.*

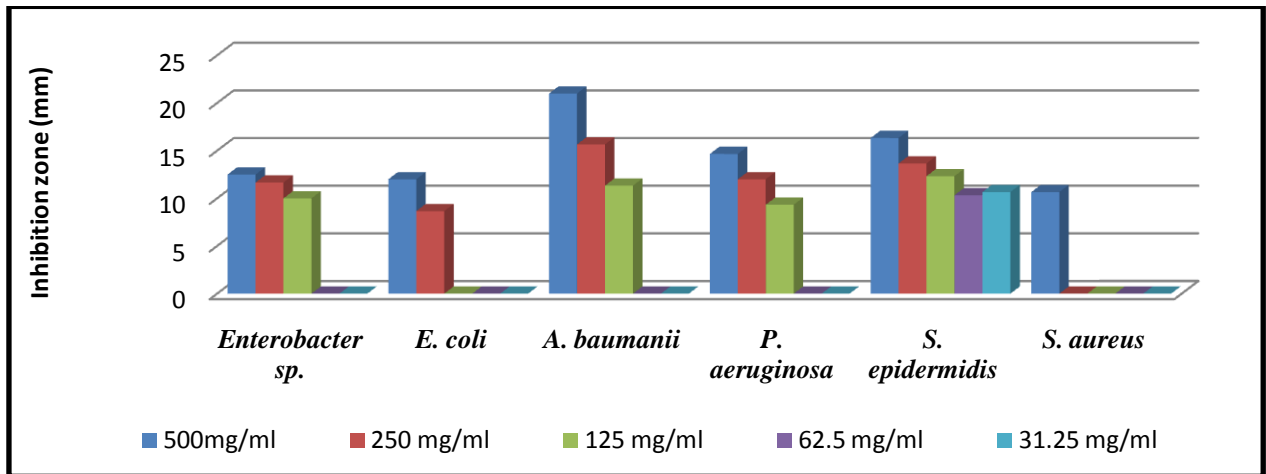
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**Table 2.** Effect of antibiotics on selected strains

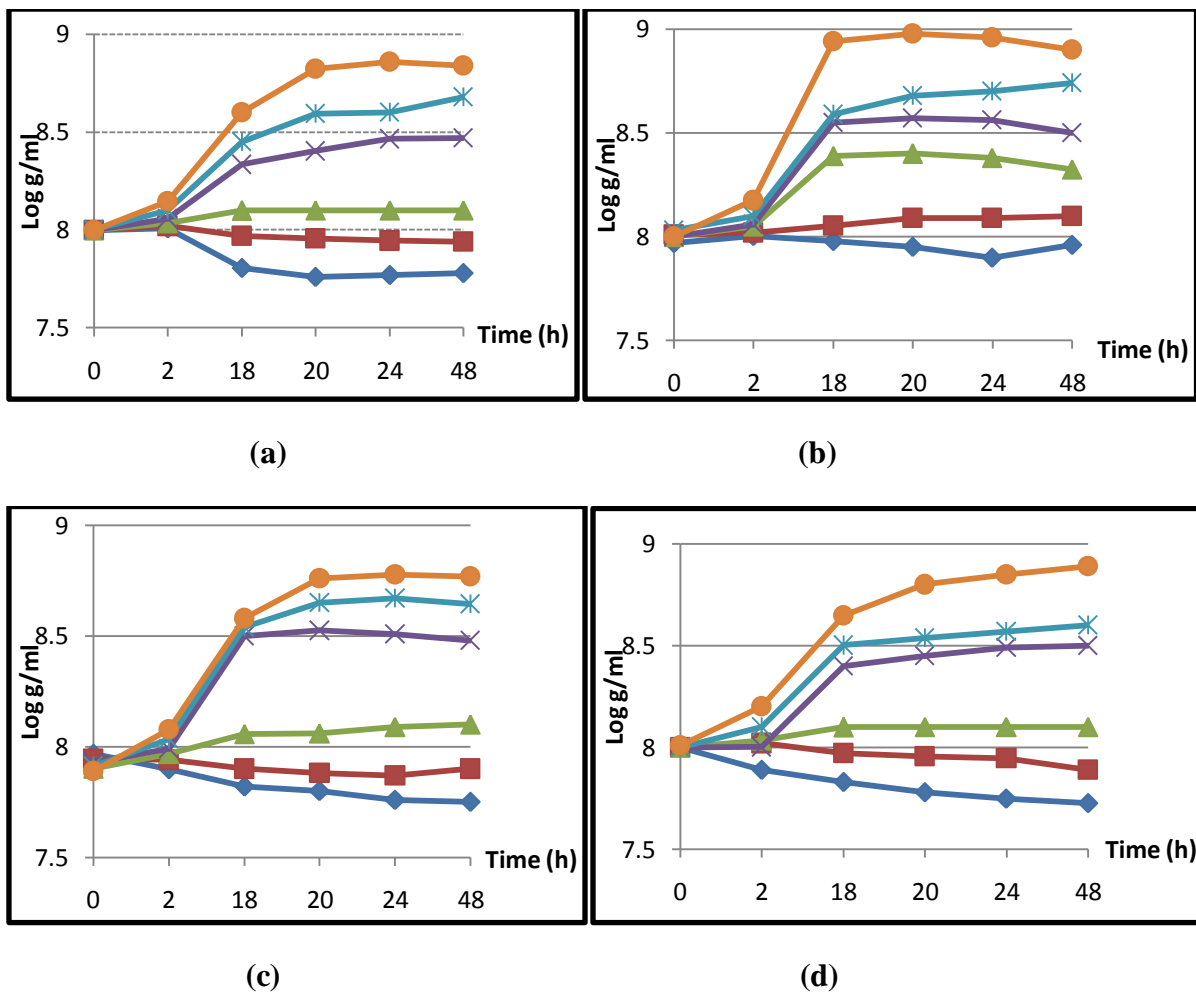
	AX	AMC	OX	CZ	CN
<i>Enterobacter sp.</i>	R	I	R	I	S
<i>E.coli</i>	R	I	R	S	S
<i>Acinetobacterbaumanii</i>	R	R	R	R	R
<i>P. aeruginosa</i>	R	R	R	R	R
<i>S. epidermidis</i>	R	R	R	S	I
<i>S. aureus</i>	R	R	R	I	S

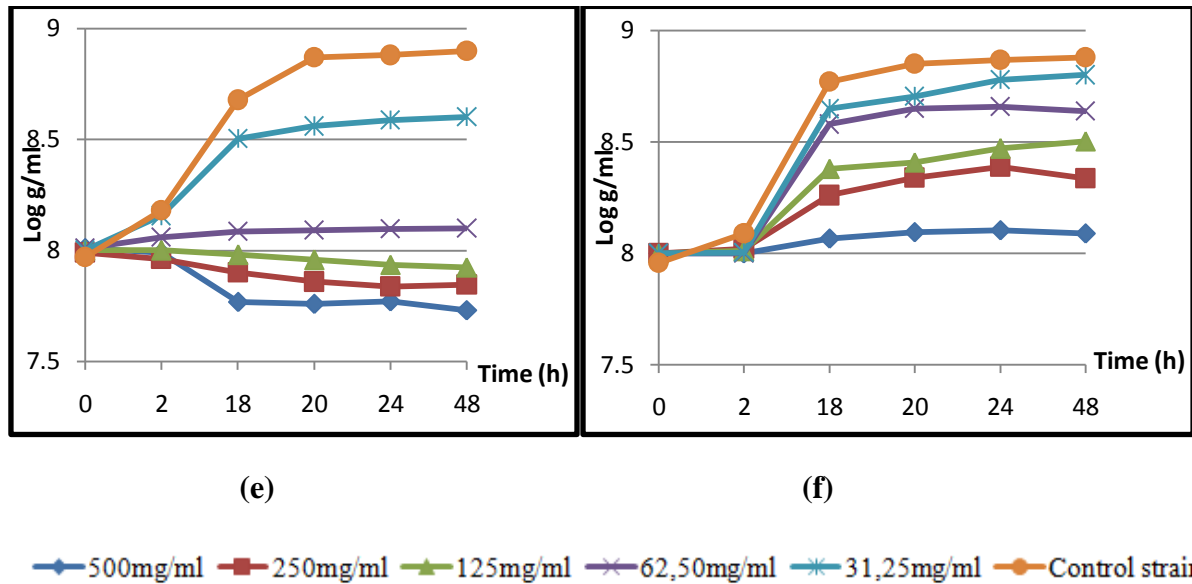
**Table 3.** Effect of *M. vulgare* methanolic extract on selected strains

Strains	Diameter of inhibition zones (mm)					MIC (mg/ml)
	500	250	125	62.50	31.25	
<i>Enterobacter sp</i>	12±0.04	11±0.07	10±0.09	0	0	125
<i>E. coli</i>	12±0.08	07±0.08	0	0	0	250
<i>Acinetobacterbaumanii</i>	20±0.02	16±0.05	11±0.04	0	0	125
<i>P. aeruginosa</i>	15±0.13	12±0.08	09±0.05	0	0	125
<i>S. epidermidis.</i>	16±0.1	14±0.1	12±0.08	10±0.08	15±0.13	62.50
<i>S. aureus</i>	10±0.08	0	0	0	0	500



**Figure 1.** Inhibition diameters (mm) of methanolic extract of *M. vulgare* against bacterial strains tested





**Figure 2.** Antibacterial effect of *M. vulgare* methanolic extract on strains tested  
(a) *Enterobacter sp.*; (b) *Escherichia coli*; (c) *Acinetobacterbaumanii*; (d) *Pseudomonas aeruginosa*; (e) *Staphylococcusepidermidis*; (f) *S. aureus*