



Antibacterial and Antifungal Activity of Essential Oil of *Origanum Majorana* on a Few Bacteria and Fungi

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Abstract

The objective of this study is to assess the fungal and antimicrobial activity of the essential oil (ET) of marjoram (*Origanum majorana* L.). This strategy can contribute to the sustainable development of our country. Preliminary tests performed on the essential oil of *Origanum majorana* showed that this oil has antibacterial activity vis-à-vis the bacterial strains (*Enterococcus faecalis*, *Enterobacter cloacae*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and antifungal (*Fusarium sporotrichioides* and *Fusarium graminearum*). The culture medium used was nutrient broth Muller Hinton. The interaction between the bacteria and the essential oil is expressed by a zone of inhibition with diameters of MIC indirectly expression of. And we used the PDA medium to determine the fungal activity. The extraction of the aromatic fraction (essentially oil hydrolat) of the fresh aërian part of the *Origanum majorana* was performed by hydrodistillation. The average essential oil yield is 0.99%. The antimicrobial and fungal study of the essential oil and hydrosol showed a high inhibitory effect on the growth of pathogens.

Keywords: Essential oil; *Origanum majorana*; Bacteria and Fungi

Introduction

Essential oils have many therapeutic properties. In herbal medicine, they are used for their antiseptic properties against infectious diseases of fungal origin, against dermatophytes, those of bacterial origin. Humans use plants for thousands of years to treat various ailments, in many developing countries; most of the population relies on traditional doctors and their collections of medicinal plants to cure them [1].

The MAP are plants that have grown or have picks in his natural environment for its medicinal and had an infinite variety of jobs, to report the therapeutic area, food, cosmetics, industrial, etc.. Herbs can play an important role in conserving biodiversity. These plants are actually very familiar to rural people who are very sensitive to

their scarcity and their disappearance. Indeed, medicinal plants play an important role of health care population and represent a significant source of income for many families in the countryside and cities [2]. Throughout history, the plant kingdom has provided the essential human resources to its feeding, hygiene and health. Since ancient times, the fragrances of these same plants are associated with mystic rites, artistic and aesthetic.

It is known that some plants emit odors to attract insects to defend themselves. These smells come from small glands on the surface of leaves, stems or flowers that contain the essential oil. The essential oil is a volatile aromatic substance extracted from the plant. Little or no fat, it is called oil because it does not mix with water. Like gasoline, it ignites. Once extracted from plants, essential oils are used in perfumery,

cosmetics, in food and other industries.

Essential oils have many therapeutic properties. In herbal medicine, they are used for their antiseptic properties against infectious diseases of fungal origin, against dermatophytes, those of bacterial origin. In this work we try to study the antibacterial and antifungal activity of essential oils on the plant: *Origanum majorana*.

Work Methodology

Hardware Plant

The aromatic plant harvested in the month of May 2018 is among the most abundant species in the region northwest of Algeria. The *Origanum majorana*: has been selected for testing antibacterial and fungal activity.

Marjoram or Garden Oregano (*Origanum majorana*.L.) is an annual plant of the Lamiaceae family, cultivated as a condiment plant for its aromatic leaves. It is a species very close to the Common Oregano (*Origanum vulgare*). It is sometimes called Marjoram of the gardens. Other common names: officinal marjoram, shell marjoram [3].

Marjoram is relatively close to thyme, both in terms of its smell and its chemical composition. It belongs to the Labiaceae family. About two feet tall, marjoram is characterized by small white or purple, calyx-shaped flowers and downy, oval-shaped, gray-green leaves, growing in pairs. Wild, it is commonly known as oregano, due to its Greek etymology organon meaning "mountain and joy" or "love the mountain". It can also be called shell marjoram, from Crete, dictame, shepherd's thyme, red tea [4].

Marjoram (*Origanum majorana*) is an annual plant of the Lamiaceae family, cultivated as a condiment plant for its aromatic leaves. It is a species, very close to oregano, which has leaves 1 to 2cm long, opposite, of a grayish green, of entire oval shape. Its flowers are small, white or mauve, arranged in tight groups in the leaf axils with two spoon-shaped bracts [5].

This herb is used in the form of fresh or dried leaves, alone or in mixture with other herbs, to flavor many culinary preparations. Marjoram is also known for its anaphrodisiac properties. It is an aromatic plant very much used in cooking, especially in Mediterranean culinary dishes, its essential oil is known for its antiseptic property [6].

Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: *Origanum*

Species: *Origanum majorana*

Extraction of Essential Oils by Hydrodistillation

The hydrodistillation of *Origanum majorana* (leaves dry) is performed using a Clevenger-type device (1928) [2]. The setup used is shown in Figure 1.

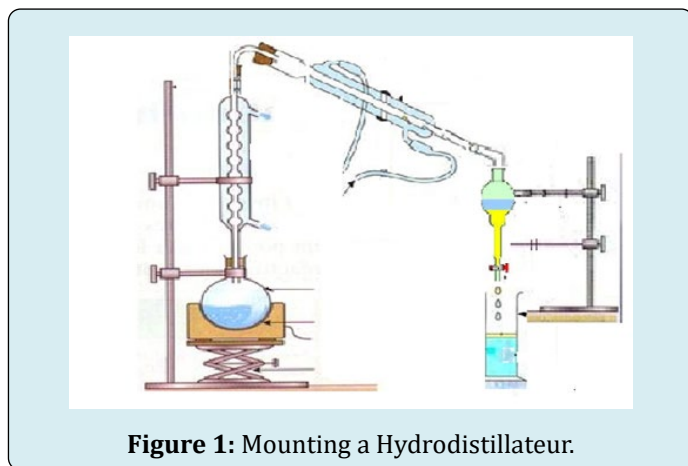


Figure 1: Mounting a Hydrodistillateur.

Study of the Biological Activity of Essential Oil

Five bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus microsilis* and *Pseudomonas aeruginosa*) and two yeasts (*Fusarium sporotrichioides* and *Fusarium graminearum*) were chosen for their high frequency in human infections.

Technique in Solid Medium: Method of Aromatogrammes

The aromatogram is based on a technique used in medical bacteriology, called antibiogram [7,8]. It has the advantage of being very flexible in the choice of products to test and apply to many bacterial species [9,10].

In this method, we use filter paper discs of 6 mm in diameter, impregnated in different concentrations of essential oil diluted in DMSO at 25%, 50% and 75%. These discs we deposit on the surface of an agar medium inoculated with the surface of a bacterial suspension. The incubation was carried out in an oven at 35°C for 24 h for bacteria and at 25°C for 5 days for yeasts.

Preparing Disks: The antibiogram is starting from the preparation of records, including Whatman paper was cut into 6 mm white disc. After sterilizing oven pastor for 20 min at 160°C, the discs were soaked in solutions with paravant prepares. Then, they are placed in Petri dishes where they

suffered a drying before being placed on the culture medium.

Preparation of Microbial Suspension: Preparation of microbial suspension is done by introducing two well isolated pure colonies of each species studied, in 10ml of saline contained in a test tube.

Inoculation: The microbial suspension prepared was cast on the Muller Hinton agar. After soak up of all surface of the medium by the microbial suspension, the supernatant was discarded. Therefore, these plates were left to dry for 15min at 37°C.

Application of Discs: The discs are prepared disposer to the surface of culture medium, pressing lightly with a sterile forceps, and then these dishes are incubated in an oven at T 37°C for 24h.

Reading Results: We have methods to measure the diameter of the zones of inhibition in the case of microbial sensitivity around the discs of 6mm in diameter.

Antifungal Activity: For the realization of the antifungal activity was adopted method of direct contact. To prepare the different concentrations were taken different

concentrations of essential oil of *Origanum majorana* namely (50,10,5,2.5,1.25 μ l) and adjust to 20ml PDA then stirred for 5minutes to homogenize the medium PDA with essential oil.

Results

Antimicrobial Activity

The study of antibacterial extracts of *Origanum majorana* by the agar diffusion method or the method of absorbing disc to determine the antimicrobial activity of this plant in vitro including the diameter disk (6mm) to measurement of the inhibition zones.

The following Figure 2 shows the results of the antimicrobial activity of the EO (classical diffusion method) of the *Origanum majorana* plant on bacterial strains *microsillis* *Proteus*, *Enterococcus feacalis*, *cloaceai* *Enterobacter*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

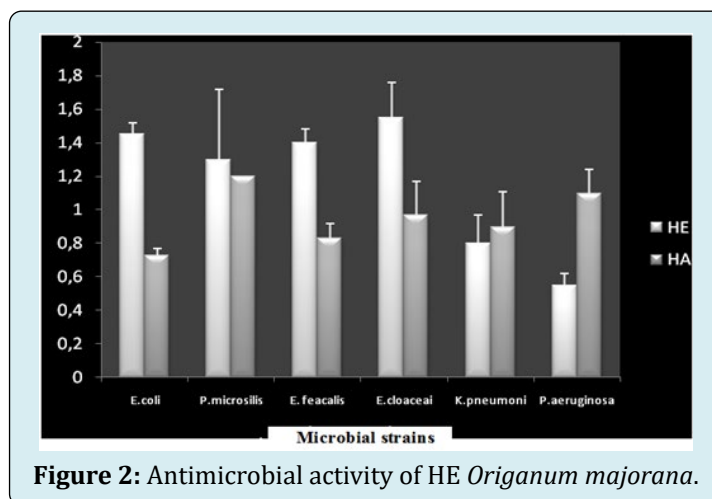


Figure 2: Antimicrobial activity of HE *Origanum majorana*.

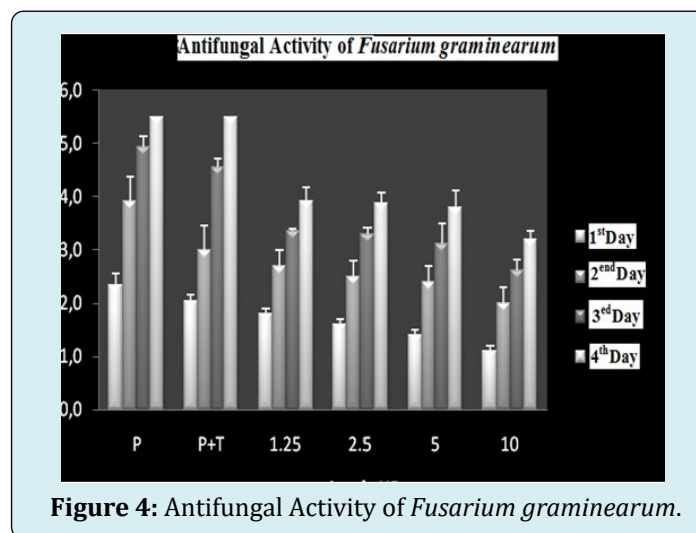
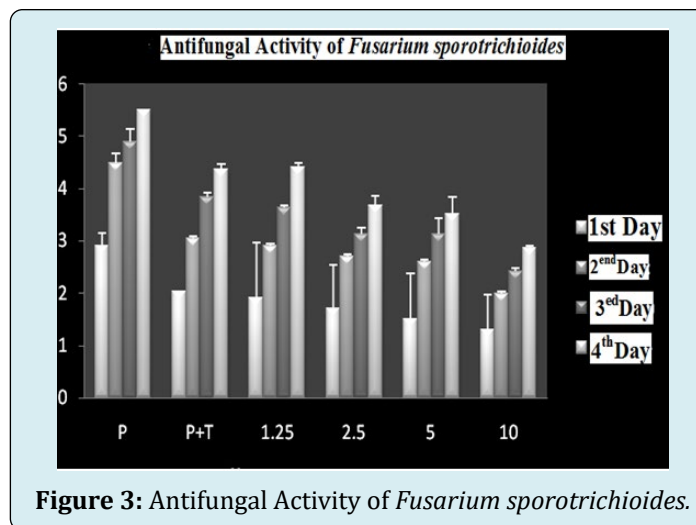
The EO has in vitro inhibitory activity against the bacteria tested. By taking into consideration the inhibition diameters, EO is active on *E. coli*, *P. microsillis*, *E. cloaceai* and *E. feacalis* respectively with a muting area 1.45,1.3,1.4 and 1.55cm. On the contrary, it is less active vis-à-vis *P. aeruginosa* and *K. pneumoni* (0.55 and 0.8cm). In contrast to *P. aeruginosa* and *P. microsillis* do not indicate great inhibitory action (insensitive) on essential oil of *Origanum majorana*.

Similar results were reported by [11]. They show that the essential oil of *Origanum majorana* Sm.a, a broad inhibitor against microorganisms studied power [12]; Also showed that the essential oils of *Clausena anisata*, *Origanum majorana* and *Ocimum basilicum* have biological

activity on microorganisms. Our results are consistent with those reported by [13], which showed that *Origanum majorana* is endowed with an efficient biological activity of microorganisms.

Antifungal Activity

The antifungal activity is revealed by the absence or the presence of mycelial growth. The results of the diameter of antifungal activity of essential oil of *Origanum majorana* are presented in the graph 3. They vary between 13 and 55mm (diameter including the disc) in the *Fusarium sporotrichioides* and *Fusarium graminearum*, the mycelial growth is varied between 11 and 55mm.



With different concentrations of essential oil extracted from *Origanum majorana*, it is observed that mycelial growth is remarkable after 72h for the control and different concentrations of essential oil of *Origanum majorana* namely 1.25, 2.5, 5 and 10 μl , by against at 25 μl no mycelial growth of *Fusarium sporotrichioides* is observed.

According to the graph N°4, which represents the activity antifonguique of *Fusarium graminearum* depending on the incubation time and the concentration of essential oil of *Origanum majorana* we note that there is an increase in mycelial growth over time with the exception of the incubation 50 μl concentration/20ml of PDA that shows no mycelial growth.

Conclusion

Many herbs contain chemical compounds having antimicrobial properties. Several research studies have been focused on the essential oils of these herbs. The search

for new therapeutic herbs character used mainly to show the validity of their use by traditional practitioners. It also showed that our country has to offer a rich and varied plant biomass. This is an immeasurable source for the development and the development of new active molecules for therapeutic purpose.

The use of volatile formulations based on medicinal and aromatic plants may have many advantages over existing products syntheses. Better understanding of our study was to *Origanum majorana* by studying of its aromatic fraction (HE-HA). We were able to evaluate and verify some of its biological properties and highlight its pharmacological potential.

In addition to its potential antimicrobial and antifungal verified on standardized gelose medium germs and vapor phase, the aromatic fraction has an undeniable anti-inflammatory action. Therefore, it may be proposed eventually as an asset of choice in the local treatment of inflammation. It

might be interesting to further phytochemical and biological investigations on these plants including purification of the extracts obtained to isolate the molecules responsible for the antimicrobial activities, which will larger armamentarium of herbal plants.

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