

# Phytochemical Study and Antimicrobial Activity of *Origanum Vulgare* L. (Lamiaceae) in Boumerdes Mountainous Region (Algeria)

Leila Bendifallah, Yamina Tchoulak, Mourad Djouabi, Mustapha Oukili, and Ramdan Ghezraoui  
Laboratory of Biodiversity and Valorization, Department of Biology, Faculty of Sciences, University of Boumerdes, 1 Avenue de l'Indépendance, Boumerdes, Algeria  
Polytechnic School, Algiers, Algeria  
Email: bendif\_l@yahoo.fr

**Abstract**—*Origanum vulgare* L. (Lamiaceae) is widespread in the Mediterranean basin. It is among the most important medicinal plants in Algeria that is known for its antifungal and antimicrobial properties. Despite its plethora of uses for treating various diseases, it has garnered very little scientific interest so far, particularly in Algeria. For this study, the leaves were collected in spring (in March) from the mountainous region of Boumerdes, in northern Algeria. In such a propitious context, the aim of this study was to enhance *Origanum vulgare* as a medicinal herb. The phytochemical screening methods are used. For their antimicrobial activity, extracts of tannin and polyphenols were screened against four pathogenic bacterial strains and two pathogenic yeast strains. The phytochemical analysis results showed a remarkable combination of chemical components including a high content in tannins, in anthocyanins, flavonoids, saponosids and in starch. The tannins and the polyphenols have strong antimicrobial activity against all the species. The maximum zone of inhibition was noted for polyphenol and tannin extracts against *Escherichia coli* (18 mm, 12.33 mm) and an antifungal activity against *Aspergillus* sp (12.33 mm, 12mm). These results indicate to some benefits of *Origanum vulgare* leaves which can use to treatment the microbial infection.

**Index Terms**—*Origanum vulgare*, Leaves, Phytochemistry, Antimicrobial activity, Mountainous region, Algeria

## I. INTRODUCTION

The *Origanum vulgare* is widespread in the Mediterranean basin [1]. It is known for its antifungal and antimicrobial properties. Its leaves are traditionally used as antiseptics and disinfectants. This is the very aromatic plant [2]. Given the limitations of therapeutic chemical drugs [3], the development of research on medicinal plants has been directed towards the achievement of herbal medicines. These are presented in various dosage forms, responding to specific regulations for the evaluation of safety, therapeutic efficacy and stability.

Natural products obtained from plants; alkaloids, flavonoids, saponins, anthraquinones, tannins, glycosides, cardiac glycoside, phenolic acids, steroids which are

secondary metabolites and their derivatives are used for the discovery and production of drugs. Thus, certain plants are classified as medicinal plants because of their established pharmacological activity and they contain substances that can be used for therapeutic purposes or used as precursors for the synthesis of drugs [3].

Many plant families have been reported to have ethno medicinal application. The genus *Origanum* has been particularly studied by Ietswaart in 1980 [4]. It recognizes three groups, 10 sections, 38 species, 6 subspecies, 3 varieties and 16 hybrids. The genus *Origanum* is widely present in the Canary Islands and the Azores in Northern Europe and to East Asia and the Tell of North Africa [2]. It can be found also in culture in Cuba and the island of Reunion, but the Mediterranean is its largest distribution area. The plant *Origanum vulgare* has garnered very little scientific interest so far, particularly in Algeria.

The works done concern only the antimicrobial activity [5]. The aim of this study was evaluate the antimicrobial activity of some tannins and polyphenolic compounds of extracts leaves of *Origanum vulgare* collected from the mountainous region of Boumerdes against a diverse range of microorganisms comprising bacteria and yeasts.



Figure 1. Location of the Lakhdaria area

## II. MATERIALS AND METHODS

### A. Plant Material

The leaves of *Origanum vulgare* were collected in April 2013 from the region of Boumerdes at the

Guerrouma Mountain situated in the eastern part of Algeria (Fig. 1, Fig. 2, Fig. 3). There were identified by Dr. Abelkrim, Professor at National School of Agronomy, Department of Botany. The leaves were shade dried at room temperature for fifteen days.

### B. Phytochemical Screening

The phytochemical screening methods used are those described by [6]. The phytochemical components analysed are: tannins (catechin, gallic), free quinones, coumarins, flavonoids, alkaloids, saponins, glycosids, anthocyanins, leucoanthocyanins, starch and iridoids.

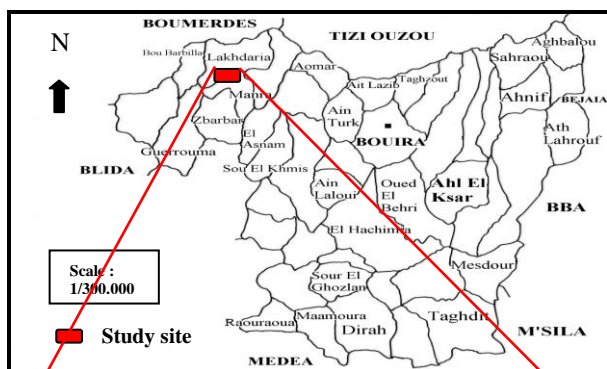


Figure 2. Location of the *Origanum vulgare* collection station



Figure 3. Mountain Guerrouma, *Origanum vulgare* collection region

### C. Preparation of Plant Extracts

#### 1) Chemicals and reagents

The following chemicals and reagents were used : acetic acid, acetone, methanol, Ether, chloroform, hydrochloric acid, Bismuth nitrate, Hydrogen peroxide, Iodine sublimated pure, Lead acetate, Sodium acetate, KOH, NaCl, Sodium carbonates, Potassium iodine, Iso amyl alcohol, Folin ciocalteu reagent, Folin and Denis, Tannic acid, gallic acid, Ammonia nutrient agar slope, Sabouraud agar slope, agar OGA and Muller Hinton.

#### 2) Equipment and apparatus

Precision balance, autoclave, rotoevaporator , Bain marie Steaming, Bunsen burner, spectrophotometer, magnetic stirrer, biological hood, UV lamp, electric grinder, vortex, refrigerator and Stove.

#### 3) Polyphenols extract

The powdered of *Origanum vulgare* (30g) were extracted with methanol (100 ml) for 72 hours. After this step, the decoction was filtered. The filtrate was concentrated by evaporation in vacuo at 40 °C. using a rotavapor and the residue was kept at 4 °C (Harbone, 1976).

#### 4) Tannins extract

Tannins extract obtained by extraction of 15 g of powdered for 24 h in about 100 ml of solvent used (Acetone). This extract was concentrated to dryness and the residue was kept at 4 °C (Harbone, 1976).

#### 5) Microorganisms used

The test organisms used included: five bacteria strains: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aerogenosa*, *Escherichia coli*, and two yeast strains *Candida albicans* and *Aspergillus* ssp.. These strains were collected from the Pasteur Institute (Algiers - Algeria).

### D. Culture Media and Antimicrobial Assay

Mueller-Hinton agar (MH) and Sabouraud Dextrose agar (SD) were respectively used for bacteria and yeasts growth. Microbial cultures, freshly grown at 37 °C/30 °C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 105 CFU: ml.

To evaluate antimicrobial activity, an agar well diffusion method was used as described by [6]. The organisms were spread on MH and SD agar plates by cotton swab. Wells of 6 mm diameter

TABLE I. PHYTOCHEMICAL SCREENING RESULTS OF *ORIGANUM VULGARE*

Substances	<i>Origanum vulgare</i>
Total tannins	+++
Catechic tannins	++
Gallic tannins	+++
Flavonoids	+++
Anthocyanins	+
Leucoanthocyanins	---
Alkalo ïls	---
Starch	+++
Glucosids	---
Saponins	+++
Irido ïls	---
Free quinons	---
Coumarins	---

## III. RESULTS

### A. Phytochemical Tests

The phytochemical results (Table I) show that *Origanum vulgare* is very rich in total tannins, gallic tannins, leucoanthocyanins, flavonoids, glucosids, saponins and mucilage. It is moderately rich in catechic tannins. This plant contains a low content of coumarins and irido ïls. Finally, we must point out that *Origanum vulgare* has no free quinone, starch, alkaloids and anthocyanins.

The phytochemical screening tested on *Origanum vulgare* leaves shows satisfactory results approximately 60% (Fig. 4). It is also richer in total polyphenols than in tannins (Fig. 5).

**B. Results of the Extraction of Total Polyphenols and Tannins from *Origanum Vulgare***

The yield of total polyphenols and tannins from *Origanum vulgare* are showed in the Table II.

TABLE II. YIELD OF TOTAL POLYPHENOLS AND TANNINS FROM *ORIGANUM VULGARE*

Product extracts	Total polyphenols	Tannins
Yield (%)	13,27	8,85

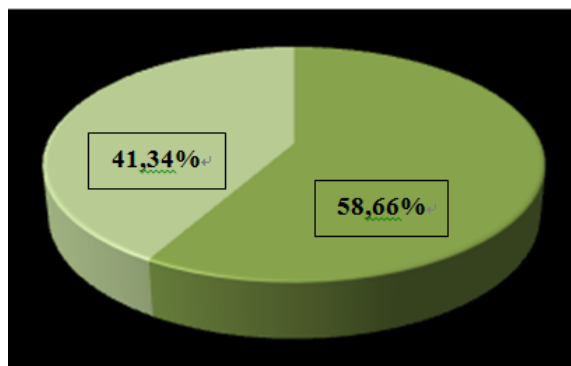


Figure 4. Graphical representation of the results of phytochemical screening of *Origanum vulgare*

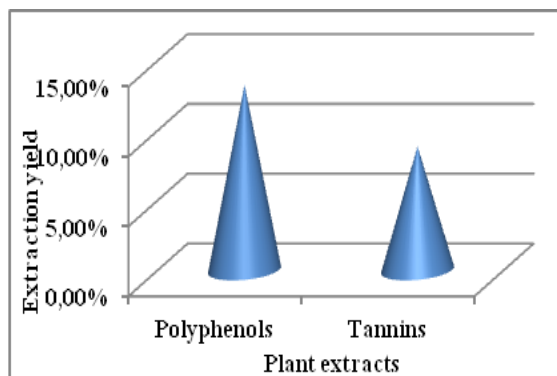


Figure 5. Graphic representation of extracts total polyphenols and tannins of *Origanum vulgare*

TABLE III. SCREENING ACTIVITY OF *ORIGANUM VULGARE* LEAVES EXTRACTS COLLECTED FROM BOUMERDES MOUNTAINOUS REGION (ALGERIA)

Plant extracts	Polyphenols	Tannins
<b>Microbial strains tested</b>		
<b>Bacterial strains</b>		
<i>Escherichia coli</i>	15,66 ± 1.41	12,33 ± 0.58
<i>Staphylococcus aureus</i>	13,66 ± 3.54	9,33 ± 1.15
<i>Klebsiella pneumonia</i>	10 ± 0.58	10 ± 0.62
<i>Pseudomonas sp</i>	12,33 ± 1.33	10,33 ± 0.58
<b>Yeast strains</b>		
<i>Candida albicans</i>	10 ± 1.17	11,66 ± 1.34
<i>Aspergillus sp</i>	12,33 ± 2.03	12 ± 1.42

**C. Results of Antimicrobial Activity**

The extracts leaves of *Origanum vulgare* have inhibitory action on all microbial strains tested, but the diameter of the zone of inhibition varies from one strain to another. The screening antibacterial activity shows that *Escherichia coli* is extremely sensitive to the methanol

extract and acetone extract with a zone of inhibition (18 ± 1.24) mm for polyphenols (12.33 ± 1.35) mm for tannins (Table III).

**IV. DISCUSSION**

**A. Phytochemical Screening of *Origanum Vulgare***

[7] and [8] reported reported 3,53 % for *Origanum vulgare*. These results are similar to those [9]. These authors reported that the leaves of myrtle are very rich in tannins. Also, Novaes et al (2001) point out that the confirm the results of the present study. They also found that this plant is rich in tannins, flavonoids. The latter is in low proportion in our study may be due to the physiological state of the plant and the harvest period.

The difference in yield of total polyphenols and tannins of plant species is a function of intrinsic and extrinsic factors: genotype, environment, geographical origin, sunshine duration, humidity, rainfall, soil type, harvest time, temperature and drying time [10]-[14].

**B. Antimicrobial Activity of *Origanum Vulgare***

The two extracts *Origanum* (polyphenols and tannin) have strong antimicrobial activity against all tested strains. Our results are better than those obtained by [4]. Toshitsigu et al (2004) conclude that the sensitivity of microorganisms to polyphenols, depending on the species itself and the structure of polyphenols.

**V. CONCLUSION**

The extracts leaves of *Origanum vulgare* could be good antimicrobial agents. In perspective, it would be desirable to complete this study by the extraction of active ingredients from different parts of the plant (roots, stems, leaves, flowers and seeds) and evaluation of their antimicrobial activity of several strains of pathogen.

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**Leila Bendifallah**, Ph.D, Empowered to search, Associate professor, Laboratory of Biodiversity and Valorization. Teacher and researcher at the Department of Biology, Faculty of Sciences, University of Boumerdes, Algeria, Bendif\_l@yahoo.fr  
Areas of interest: Entomology, botany, phytochemistry, environment, ecology, plant – bee interactions, pollinators, entomophilous pollination. Biological control, biodiversity.

Research Associate in both two laboratories: The Applied Entomology Laboratory, National School of Agricultural Sciences, Algiers; The Laboratory of Biosystematic and Arthropods Ecology, Constantine University, Algeria.

I have held several positions such as Head of Department responsible for Studies and Research at the National Institute of Plant Protection at El Harrach – Algiers. I have over 10 publications, most in refereed journals, participation in more than 40 international conferences, membership of Scientific Committee of International Conferences such as: ICMAP 2014: International Conference on Medicinal and Aromatic Plants. Malaysia, D cembre 4-5 2014, *reviewer and Editor in several International Scientific Journals*.

**Yamina Tchoulak**, Polytechnic School, Algiers, Algeria, Tchoulak.1999@yahoo.fr

**Mourad Djouabi**, Department of Biology, Faculty of Sciences, University of Boumerdes, Algeria

**Mustapha Oukili**, Department of Biology, Faculty of Sciences, University of Boumerdes, Algeria

**Ramdan Ghezraoui**, Department of Biology, Faculty of Sciences, University of Boumerdes, Algeria.