

## Antifungal Activity of Essential Oil of Oregano (*Origanum Vulgare*), Marjoram (*Origanum Majorana*) and Synergy of Two Essential Oils against *Candida Albicans*

Bouchra Lakhrissi<sup>1\*</sup>, Asmaa Boukhraz<sup>1</sup>, Mariam Barrahi<sup>1</sup>,  
Hajar EL Hartiti<sup>1</sup> et Mohammed Ouhssine<sup>1</sup>

<sup>1</sup>Laboratoire de Biotechnologie, Environnement et Qualité (LABEQ), Département de Biologie, Faculté des Sciences, Université Ibn Tofaïl, BP 133, 14000 Kenitra, Maroc.

**Abstract:** The work is realized on two known medicinal plants in Morocco oregano and marjoram. The *origanum vulgare* and *Origanum majorana* are respectively collected in the regions of Ouazzane and salé. The objective of this study is to evaluate the antifungal Activity of essential oils extracted from two plants and synergy of these two essential oils against *Candida albicans*. The essential oils are obtained by hydro distillation in a Clevenger type apparatus. The yield of essential oils obtained from the leaves of marjoram and oregano is respectively 2.50% and 2.34%.

The determination of the antifungal power was realized by the method of diffusion on gélose sabouraud, the Essential oils showed a strong antifungal activity against fungal strain *Candida albicans*. However, the largest zone of inhibition of the strain *Candida albicans* is observed by the essential oil of *origanum vulgare* (30 mm).

### 1. INTRODUCTION

The candidiasis is due to a mushroom which has undergone a transformation (processing) that the most common of which is the *Candida albicans* which has increased during that last years. The *Candida albicans* is the pathogenic agent the most associated with a grave fungal infection which represents more than 90 % of the cases [1, 2].

*Candida albicans* is an opportunistic pathogen which can cause local and systematic infections at the amenable people, usually affecting the patients those immunologically damaged and who undergo a treatment prolonged antibiotic [3]. The infections at *albicans Candida* formerly opportunist, begin to constitute a major concern due to the increasing number of fragile patients (multi-operated, immunosuppressed, etc.) [4, 5].

The problems of systemic cytotoxicity [6] and resistance of certain strains to drugs begin to feel [7, 8]. Indeed, the search for new antifungal turned out to be necessary and turned to the use of essential oil extracted from medicinal and aromatic plants [9, 10].

The essential oil extracted from aromatic and medicinal plants is since antiquity known by their biological, antibacterial and antifungal properties [11, 12]. The species of the genre *Origanum* (Lamiaceae) are generally aromatic, medicinal and food plants. They are used in most of the African countries. We have chosen it in this work two species (*origanum vulgare* and *origanum majorana*) to estimate the antifungal activity of their essential oils. A third test is performed; it is about the combination of this both essential oils and determination of its effect on the growth of *Candida albicans*.

### 2. MATERIALS AND METHODS

#### 2.1. Material

##### 2.1.1. Plant material

The *Origanum majorana* and *Origanum vulgare* were collected respectively in the regions of salé and Ouazzane. The samples were drying in dark and well airy area at room temperature for 15 days to facilitate their storage. The leaves used for the extraction of essential oils were separated from the rest of the plant and kept in clean and airy bags.

## Antifungal Activity of Essential Oil of Oregano (*Origanum Vulgare*), Marjoram (*Origanum Majorana*) and Synergy of Two Essential Oils against *Candida Albicans*

### 2.1.2. Micro-organisms studied

Evaluation of antifungal activity against *Candida albicans*.

**Table1.** Tested micro-organisms

Microbial group	Tested strains	Origin of strain
Yeast	<i>Candida albicans</i>	Vaginal infection

The antifungal strain is grown and maintained in gélose of Sabouraud and incubated for 48 h in at 37°C; they are to cause of several local and systematic infections [13].

## 2.2. Methods

### 2.2.1. Extraction of essential oils

The extraction of essential oils of *Origanum majorana* and *Origanum vulgare* was carried out by hydro-distillation in a Clevenger-type apparatus [14]. The essential oil yield is estimated according to the dry vegetal matter. The oils obtained were dehydrated using Sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and stored in a refrigerator at 4°C in dark bottles to protect them from light and heat. [15]. the method applied was that of Clevenger which is described in the European Pharmacopoeia and the 9th edition of the French Pharmacopoeia. The yield of obtained essential oils was calculated by the following formula [16]:

$$(\text{EOY}) \text{ Essential oils yield (\%)} = \text{W1} / \text{W2} \times 100$$

W1 = net weight of oils from dried leaves (grams);

W2 = total weight of fresh leaves (100grams).

### 2.2.2. Microbiological procedure

The used methods are summarized in TABLE 2

Methods	Description	Incubation	Play
Diffusion disk method	The sterile filter paper discs of 6 mm in diameter, previously impregnated with essential oils is deposited on the agar of Mueller Hinton, by means of sterile tweezers [17].	30°C/ 48h	Measurement of zone inhibition (diameter in mm)
Method contact direct	From a stock solution, a series of dilutions were made. Amounts of these dilutions were added to test tubes containing the gélose of sabouraud. The process is done by streaking using a platinum loop to take the same volume of inoculums [18].	30°C/ 48h	Presence or absence of <i>Candida albicans</i> growth
Nature of the antifungal activity	We determine the minimum fungicidal concentration by seeding a sample of the tubes having no growth of yeast on gélose of sabouraud.	30°C/ 48h	Presence or absence of <i>Candida albicans</i> growth

## 3. RESULTS AND DISCUSSION

**Table3.** The essential oil content of *origanum vulgare* and *origanum majorana*

Plant	marjoram	oregano
Quantity	100 g	100 g
Yield	2, 50%	2 ,34%

The essential oil of marjoram obtained is pale yellow to dark color and a sweet smell, fine, warm and delicate. The yield of essential oils obtained from the leaves is 2.50%. This yield obtained is considered significant compared to that given by Quer (1988) 1.20% [19].

The essential oil of oregano is yellowish to dark brown color and it has a herbaceous smelling. The Yield of essential oils obtained from the leaves is 2.34%. This yield is higher than that indicated by

OJHA and al [20] which obtained an oil yield varies from 0.16 to 2.07%. The large value of the yield achieved can be attributed not only to the origin of the plant, or even to the extraction technique, but probably the collection period of the plant.

The results of antifungal activity of essential oils of this two plants *Origanum majorana* and *Origanum vulgare* are showed in TABLE 4.

The experimental results show that the essential oils have a very good activity against the strain *Candida albicans*. The maximum inhibition of oregano essential oil is 30mm; the combination of two essential oils gave a zone of inhibition of 28mm. While, the essential oil of the marjoram gave only 18 mm as zone of inhibition against *Candida albicans*. It was the lowest zone of the three inhibition tests.

**Table4.** Antifungal activity of the essential oil of *Origanum vulgare*, *Origanum majorana* and the synergy of two essential oils against *Candida albicans*.

Plant	oregano	marjoram	synergy of two essential oils
Diameter of inhibition Zones (mm)	30mm	18mm	28mm

The experimental results show that the oregano, marjoram and their combination has a very good activity against *Candida albicans*. The zone of inhibition is 30 mm for the oregano and of 18mm for the marjoram. It is 28 mm for the mixture.

We also found that the inhibition potential of three essential oils, expressed by the minimum inhibitory concentration (MIC) is high. The results are summarized in Table 5.

**Table5.** The results of minimum inhibitory concentration (MIC) of the essential oil of *Origanum vulgare*, *Origanum majorana* and the synergy of two oils against *Candida albicans*

Concentration (V/V) Plant	1/10	1/25	1/50	1/100	1/200	1/300	1/500	Control
oregano	-	-	-	-	+	+	+	+
marjoram	-	-	-	+	+	+	+	+
synergy of two essential oils	-	-	-	-	+	+	+	+

(-): inhibition (+): growth

The essential oil of oregano inhibited the growth of *Candida albicans*. The minimum inhibitory concentration is 1/100 (V/V). The same result is obtained with the mixture of two oils 1/100 (V/V). It is not the same with marjoram. The MIC of marjoram essential oil against *Candida albicans* is 1/50 (V/V).

He comes out of the above that the essential oil of oregano is the best form to recommend for use biotherapeutic. The mixture of two oils gave no noticeable improvement. It may be that this is only the action of oregano that was manifested. The essential oil of the marjoram can constitute a remedy of help in case of shortage of others oil.

Regarding fungicide action, the minimum fungicidal concentration (MFC) is our tool of decision making. The results of the MFC of the essential oil of *Origanum vulgare*, *Origanum majorana* and their combination are summarized in Table 6.

**Table6.** Results of minimum fungicidal concentration (MFC) of the essential oil of *Origanum vulgare*, *Origanum majorana* and the synergy of two oils against *Candida albicans*

Plant	oregano	marjoram	synergy oregano and marjoram
(MFC)(V/V)	1/50	1/10	1/50

The essential oil of the oregano inhibited the growth of *Candida albicans*. The minimum fungicidal concentration is 1/50 (v/v). The same result is obtained with the mixture of both oil (1/50 v/v). it is not the same with the marjoram there. The MFC of the essential oil of the marjoram against *Candida albicans* is 1/10 (v/v). It is necessary to have a solution more concentrated to have a positive answer.

It stays even for this test to confirm that the essential oil of the oregano is the best shape to recommend for a biothérapeutic use. The use of marjoram essential oil can only be a relief product.

#### **4. CONCLUSION**

The yield of essential oils of oregano and marjoram is respectively 2.34% and 2.50%. The oil yield of marjoram is however significant and can be profitable on an industrial scale. The antifungal activities of essential oil of oregano, marjoram and of their synergy are important.

The essential oils of the two studied plants appear as an exception compared with the other essential oils to the point of view activity anti-candida. This study allows once again the development of the exploitation of these essential oils in the pharmaceutical and cosmetic domains. At the same time, these two plants can be used as conservative in the field of the food-processing industry.

#### **REFERENCES**

- [1] Douglas L.J.(2003):Candida biofilms and their role in infection. Trends Microbial. 11:30-36.
- [2] Edwards J.E. (1995): Candida species. In: Mandell, Douglas, Bennett, editors. Principles and practice of infectious diseases. NewYork, USA: Churchill Livingstone. 2289—2301.
- [3] Zhang Z, Elshohly H.N, Jacob M.R, Pasco D.S, Walker L.A, Clark A.M. (2002): Natural products inhibiting *Candida albicans* secreted aspartic proteases from *Tovomitakrukovii*. Planta Médica. 68: 49–54
- [4] Ascioğlu S, Rex J.H, de Pauw B, et al. (2002) :Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants an international consensus. Clin Infect Dis. 34: 7–14,
- [5] Gudlaugsson O, Gillespie S, Lee K. (2003): Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis .37: 1172-1177
- [6] Lin S.J, Schranz J, Teutsch S.M. (2001): Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis .32: 358–366.
- [7] Granier F. (2003) : Antifongiques, classes thérapeutiques, mécanisme d'action, problèmes de résistance antibiotiques .5: 39–48
- [8] Ramage G, Bachmann S, Patterson T.F, et al. (2002): Investigation of multidrug efflux pumps in relation to fluconazol resistance in *Candida albicans* biofilms. J Antimicrob Chemother. 49: 973–980,
- [9] Butler M. (2004): the role of natural product chemistry in drug discovery. J Nat Prod 67: 2141–2153.
- [10] Newman D, Cragg G, Snader K. (2003): Natural products as sources of new drugs over the period 1981–2002. J Nat Prod. 66: 1022–1037.
- [11] Chanegriha N, Foudil-cherif Y, Baaliouamer A, Meklati B.Y. ( 1998): RivistaItaliana E.P.P.O. 25:11-16.
- [12] Massouti V, Viano J, Gaydou E.M. ( 1998) : Fitoterapia volume LXIX, 5.
- [13] Zhang Z, Elshohly H.N, Jacob M.R, Pasco D.S, Walker L.A, Clark A.M, (2002): Natural products inhibiting *Candida albicans* secreted aspartic proteases from *Tovomita krukovii*. Planta Medica .68: 49–54.
- [14] Clevenger J.F. (1928): Apparatus for the determination of volatile oil, *J. Am. Pharm. Assoc.*17 (4): 346-351.
- [15] Denny E.F.K. (1991): "Field distillation for herbaceous oils", 2<sup>nd</sup> Ed, Denny, Mc Kenzie Associates, Lilydale, Tasmania, Australia.
- [16] AFNOR. (1986) : "Recueil des Normes Françaises "huiles essentielles" AFNOR. Paris. 57.
- [17] Joffin J.N, Leyral G, (2006) : "Microbiologie technique". Tome 1 : Dictionnaire des techniques, 4<sup>ème</sup> édition. Centre régional de documentation pédagogique d'Aquitaine, France. ISBN 2 86617 515 8.
- [18] Courvallin P, Goldstein F, Philippon A. &Sirot J. (1985): "L'antibiogramme". MPC-VIDEOM, Paris, France.
- [19] Quer D.R.P.F.( 1988). Plantas Medicinales-Labiadas-Mayorana, 11th ed. Editorial Labor S.A., Barcelona: p. 696.
- [20] Ojha et al. (2013): "Variation in essential oil composition and anti-microbial activity of Indian Oregano (*Origanumvulgare*L.) Population from Indian Himalayan Region", J. of Medicinal Plants Research. 7(46): 3375-3384.