ISSN : 0974 - 7532 Volume 10 Issue 8

Research & Reviews in

BioSciences

Regular Paper

RRBS, 10(8), 2015 [285-289]

# Extraction and evaluation of antibacterial activity of essential oil of marjoram (*Origanum majorana*) in the region of salé, Morocco

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## ABSTRACT

The main objective of this work is to evaluate the antimicrobial activity of the essential oil of marjoram (*Origanum majorana*). *Origanum majorana* is an aromatic plant, widely distributed in Morocco and widely used by the Moroccan people in order to its medicinal characteristics. The constituent of essential oils has a long history as antimicrobial agent, for this purpose and in the framework of the valorization of the Moroccan flora, we are interested in the species of the family Lamiaceae which is one of the most families utilized as a global source of spices and as strong antimicrobial power extracts. Thus, we chose marjoram (*Origanum majorana*) as plant from Salé region, to demonstrate the antimicrobial activity of essential oil.

The essential oil of marjoram is obtained by hydro-distillation in a Clevenger-type apparatus with highest yield leaves up to (2.50%). The inhibitory activity of the essential oil of marjoram is not the same against all tested strains (*Escherichia coli ATCC 25921, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeroginosae* ATCC 27853, *Acinetobacter sp, Staphylococcus aureus* ATCC 25923). The bactericidal activity was remarkable against all strains tested. However, the most important diameter of inhibition of 22 mm is obtained with Escherichia coli. 

## KEYWORDS

Marjoram;
Essential oils;
Antimicrobial activity;
Hydro-distillation;
Pathogens.

## INTRODUCTION

The marjoram (*Origanum majorana*) is an annual plant of the lamiaceae family. It is grown with us for condiment and medicinal reasons. This is a species very close to the oregano, has leaves 1 to 2 cm long, opposite, grayish green, entire oval shape. Its flowers are small, white or purple, arranged in tight clusters in the axils of leaves with two spoon-shaped bracts[1]. This plant works as fresh or dried leaves, alone or in a mixture with other plants to aromatize many culinary preparations. It is a very aromatic plant used in cooking, especially in Mediterranean culinary dishes, its essential oil is known for its antiseptic property[2]. The essential oil of marjoram has a liquid, clear appearance, dark to pale yellow color and a sweet smell, fine, warm and delicate. It is obtained by distillation of its flow-

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ering heads and leaves. It is considered a strong antispasmodic stomach, calms spasms and particularly for those have the stomach and colon spasms, it contributes to wellness of digestive tract[3]. It also has a significant effect on the psycho-sensory system. It is used to reduce the role of the sympathetic system and promote the relaxing and calming action of parasympathetic system[4]. View of its antitoxic, it will be applied locally on the buttons to inactivate the venom inoculated by bite of insect[5]. Among several essential oils that may be useful as antimicrobial agents, marjoram oil (*Origanum majorana*) can have the greatest potential for use in industrial applications[6]. In this context, the aromatic plants, and especially the type of marjoram spices, have emerged as effective compounds to ensure the microbiological safety of food[7]. Marjoram (*Origanum majorana*) is a species plays a leading role among the culinary herbs in global trade. The popularity of marjoram is more and more growing. The result of the recent discovery scientific research indicates antimicrobial properties, marjoram[8].

For reasons of scientific confirmation we are interested in the antibacterial effect of the Moroccan species. This activity will be tested on strains of references and strains from pathological samples.

## MATERIALS AND METHODS

### Material

### Plant material

**Figure 1 : Origanum majorana**

The plant material marjoram (*Origanum majorana*) was collected in June 2013 from the region of salé, samples were drying in darken and well-ventilated area at room temperature for 15 days to facilitate 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.

### Micro-organisms studied

The antibacterial activity was evaluated against different microorganisms showing in TABLE 1.

These bacterial strains were purified by subculture on Mueller-Hinton agar and incubated for 24h in at 37°C; they are the cause of several nosocomials infections (urinary, intestinal, respiratory, etc.)[9, 10].

### Methods

### The extraction of essential oils

The extraction of essential oils of marjoram (*Origanum majorana*) was carried out by hydro-distillation in a Clevenger-type apparatus[11]. Today the hydro-distillation remains the most sought method in the industry for extraction of essential oils. The estimation of the yield of essential oil is based on the weight of total dray matter (vegetative). The obtained essential oils are collected and stored in a refrigerator at 4°Cin dark bottles to protect them from efficacy of the heat and light in the presence of anhydrous sodium sulfate[12]. 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[13]:

**(EOY)Essential oils yield (%) = $W_1$ / $W_2$ X 100**

W1 = net weight of oils from dried leaves (grams)
W2 = total weight of fresh leaves (100grams)

### Microbiological procedure

The used methods are summarized in TABLE 2:

## RESULTS AND DISCUSSION

The essential oil of marjoram obtained is dark to pale yellow color and as wet smell, fine hot and delicate. It has a yield up to 2.50% from the leaves. This oil yield obtained is higher than indicated by

**TABLE 1 : Tested micro-organisms**

| Microbial group | Tested strains | Origin of strain |
|---|---|---|
| Gram negative | *Escherichiacoli*ATCC25921 | UTI |
| | *Klebsiellapneumoniae* | UTI |
| | *Enterobactercloacae* | UTI |
| | *Pseudomonasaeroginosae*ATCC27853 | Skin infection |
| | *Acinetobacter sp* | UTI |
| Gram positive | *Staphylococcusaureus*ATCC25923 | UTI |

***UTI: Urinary Tract Infection**

**TABLE 2: Methods used in the study of antimicrobial potency**

| Methods | Used for | Description | Incubation | Play |
|---|---|---|---|---|
| Technical of the aromatogram | Bacteria | Filing of filter paper discs of 6mm diameter before and oil-impregnated essential to surface of the medium(Mueller Hinton Agar), planted in boxes flood[14] | 37 °C/ 24 | Measurement diameter inhibition. |
| Method contact direct | Bacteria | From a stock solution and various dilutions prepared, all set to contact with the medium. The bacteria were sown are a per spot[15] | 37°C/24h | presence or absence of bacterial growth |
| Nature of the antibacterial activity | Bacteria | A sample of the agar disk the surface of Mueller-Hinton agar is made | 37 °C/ 24h | presence or absence of bacterial growth |

**TABLE 3 : The essential oil content of marjoram (***Origanum* ***majorana***)

| Plant | Leaves | Stem | Leaves and stem |
|---|---|---|---|
| Quantity | 100g | 100g | 100g |
| Yield | 2.50% | 0.6% | 1,4% |

Quer (1988)[16].

The author above has shown in a study using the leaves of *Origanum majorana* that the essential oil content after extraction was 1.20%. This variation in performance can be attributed not only to the origin of the plant and the extraction technique, but also to the collection period of the vegetable plant.

The results of antimicrobial activity of the essential oil of marjoram (*Origanum majorana*) are showed in TABLE 4.

The experimental results presented in TABLE 4 show that the essential oil of marjoram (*Origanum majorana*) has a very good activity against all bacteria except *Pseudomonas aeruginosae*, the same activity on Gram-positive and Gram-negative bacteria was noticed. The maximum inhibition of essential oil was against *Escherichia coliATCC 25921* (22mm), *Staphylococcus aureus* (20mm), followed by the other three strains of *Acineto bacter*

(19mm), *Klebsiella pneumoniae* (18mm) et *Enterobacter cloacae* (16mm) these species are sensitive to this EO, while *Pseudomonas aeruginosae* exhibited a resistance to this EO.

According to the inhibition zones generated by the oil of marjoram (*Origanum majorana*), we are confirmed the inhibitory potential of this essential oil by determination of the minimum inhibitory concentration (MIC). The results of minimum inhibitory concentration (MIC) of the marjoram (*Origanum majorana*) essential oil are summarized in TABLE 5.

In fact, the essential oil of marjoram (*Origanum majorana*) showed a significant inhibitory effect against microorganisms studied. All microbial strains were inhibited at a concentration of 1/25(V/V). By comparing the susceptibility of different strains vis-à-vis oil tested, we found that the efficiency of this oil is different from one bacterium to another. *Enterobacter* are more resistance to this EO with an MIC of 1/25(V/V). The MIC of *Klebsiella pneumoniae*, *Acinetobacter, Staphylococcus and Escherichia coli*was the same 1/50 (V/V). The es-

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**TABLE 4 : Antimicrobial activity of the essential oil of marjoram (***Origanum* ***majorana***)

| Bacteria | Diameter of inhibition Zones (mm) |
|---|---|
| *Klebsiella pneumoniae* | 18 mm |
| *Acinetobacter* | 19 mm |
| *Staphylococcus* | 20mm |
| *Pseudomonas* | - |
| *Escherichia coli* | 22 mm |
| *Enterobacter* | 16 mm |

**TABLE 5 : The results of minimum inhibitory concentration (**MIC) for **the essential oil of marjoram (***Origanum* ***majorana***).

| ***Bactéries*** | ***Concentration (V/V)*** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***1/10*** | ***1/25*** | ***1/50*** | ***1/100*** | ***1/200*** | ***1/300*** | ***1/500*** | ***Témoin*** |
| *Klebsiella pneumoniae* | - | - | - | + | + | + | + | + |
| *Acinetobacter* | - | - | - | + | + | + | + | + |
| *Staphylococcus* | - | - | - | + | + | + | + | + |
| *Escherichia coli* | - | - | - | + | + | + | + | + |
| *Enterobacter* | - | - | + | + | + | + | + | + |

**T: witness, - Absence of Growth, + presence of Growth**

**TABLE 6 : Results of minimum bactericidal concentration (MBC) for the essential oil of marjoram (***Origanum* ***majorana***)

| **Bactéries** | ***Klebsiella Pneumoni*** | ***Acineto-bacter*** | ***Staphylo-coccus*** | ***Escherichia coli*** | ***Enterobacter*** |
|---|---|---|---|---|---|
| (CMB)(V/V) | 1/50 | 1/50 | 1/10 | 1/50 | 1/25 |

sential oil of marjoram (*Origanum majorana*) is very active on all strains tested except *Pseudomonas* which was more resistant.

Following these results, the essential oil of marjoram has shown very interesting antibacterial characteristics against the micro-organisms tested. The results of minimum bactericidal concentration (MBC) of the marjoram (*Origanum majorana*) essential oil are summarized in TABLE 6.

The bactericidal activity appears highly variable against gram-negative bacteria compared with gram-positive bacteria.

## CONCLUSION

The essential oil of marjoram obtained is dark to pale yellow color and as wet smell, fine hot and delicate. The essential oil of marjoram is obtained by hydro-distillation in a Clevenger-type apparatus with high yield (2.50%). The antimicrobial activity of the essential oil is very important with respect to all studied strains (*Escherichia coli* ATCC 25921, *Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter sp, Staphylococcus aureus* ATCC 25923). The result was not the same with Pseudomonas aeroginosae. This last showed complete resistance bioactive substances present in our extraction product.

The findings observed in essential oil level of the marjoram coming from the Moroccan city "Salé" allows us to declare the possibility of its use as a natural preservative, it can be recommended used for food, phytosanitary, cosmetic and pharmaceutical industries. In addition to its ease of culture, its attractive yield, marjoram could be developed as part of a pushed valuation policy.

## REFERENCES

**[1]** R.R.Vera; et Chane J.Ming; Chemical composition of the essential oil of marjoram (Origanum majorana L.) from Reunion Island, Food Chemistry, **66**, 143±145 **(1999)**.

**[2]** T.E.Furia, 8c N.Bellanca; Enaroli's handbook of favor ingredients, The Chemical Rubber Co., Cleveland, OH, **(1971)**.

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**[3]** S.Burt; Essential oils: Their antibacterial properties and potential applications in foods—a review, Int.J.Food Microbiol., **94**, 223-253 **(2004)**.

**[4]** J.Kim, M.R.Marshsll, C.Wei; Antibacterial activity of some essential oil components against five foodborne pathogens, J.Agric.Food Chem., **43**, **(1995).**

**[5]** D.G.Williams; The chemistry of essential oils, Micelle Press, England, **(1997)**.

**[6]** N.B.Ezzeddine, M.M.Abdelkafi, R.Ben Aissa, M.M.Chaabouni; Antibacterial screening of (Origanum majorana L.), oil from Tunisia, Journal of Essential Oil Research, **13**, 295-297 **(2001)**.

**[7]** E.Ipek, H.Zeytinogla, S.Okay, B.A.Tuylu, M.Kurkeuoglu, K.H.C.Baser, **(2005)**.

**[8]** Genotoxicity and antigenotoxicity of Origanum oil and carvacrol evaluated by Ames Salmonella / microsomal test, Food Chemistry, **93**, 551, 556.

**[9]** M.D.Cristiani, M.Arrigo, G.Mandalari, F.Castelli, M.G.Sarpietro, D.Micieli; Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity, Journal of Agricultural and Food Chemistry, **55**, 6300-630 **(2007)**.

**[10]** C.H.Lu, W.N.Chang, Y.C.Chuang, H.W.Chang; *J.Hosp.Infect*., **40**, 27 **(1998)**.

**[11]** S.C.Chang, W.C.Hsieh, C.Liu; *Diagn.Microbiol.Infect*.Dis., **36**, 107 **(2000)**.

**[12]** J.F.Clevenger; "Apparatus for the determination of volatile oil", *J.Am.Pharm.Assoc*., **17(4)**, 346-351 **(1928)**.

**[13]** E.F.K.Denny; "Field distillation for herbaceous oils", *2nd Edition., Denny, McKenzie Associates, Lilydale, Tasmania, Australia,* **(1991)**.

**[14]** AFNOR, "Recueil des normes françaises "huiles essentielles" AFNOR, Paris, 57 **(1986)**.

**[15]** J.N.Joffin, G.Leyral; "Microbiologie technique". Tome 1 : Dictionnaire des techniques, *4ème Edition, Centre régional de documentation pédagogique d'Aquitaine*, France, ISBN 2 86617 515 8, **(2006)**.

**[16]** P.Courvallin, F.Goldstein, A.Philippon, J.Sirot; "L'antibiogramme", *MPC-VIDEOM*, Paris, France, **(1985)**.

**[17]** D.R.P.F.Quer; Plantas Medicinales-Labiadas—Mayorana, 11th Edition, Editorial Labor S.A., Barcelona, 696, **(1988)**.