

# Comparative Study of the Pharmaceutical Activity of two plants of the Moroccan Spontaneous Flora: *Mentha Pulegium* (L) and *Marrubium Vulgare* (L.) (Lamiaceae)

Amal Razik<sup>1</sup>, Farida Adly<sup>1</sup>, Chadi Berhal<sup>3,4</sup>, Mina Moussaid<sup>1,2,4\*</sup>, Abdel Aziz Elamrani<sup>2</sup>, Hassane Moussaid<sup>3</sup>, Nourdinne Bourhim<sup>1</sup>, Mohammed Louffi<sup>1</sup>

<sup>1</sup>Laboratory of Biochemistry, Cellular and Molecular Biology, Department of Biology, Faculty of Science I, Ain Chock, University Hassan II, B.P 5366, Maarif, Casablanca, 20100 Morocco

<sup>2</sup>Laboratory of the Organic Syntheses and Biological Studies, Department of Chemistry, Faculty of Science I, Ain Chock, University Hassan II. B P 5366, Maarif Casablanca, 20100 Morocco

<sup>3</sup>Chemistry laboratory Pharmaceutical, Institute of Pharmacy, Campus of the Plains, Boulevard of the Triumph, 1050, Brussels, Belgium.

<sup>4</sup>Department of phytopathology and urban agriculture, University of Liege, Gembloux, Belgium

## ABSTRACT

The medicinal herbs are a natural source for a large variety of antioxidants. In our work, we conducted a comparative study on the aqueous extract of the sheets of two medicinal herbs which develop under the same natural conditions, The area of Casablanca located west of Morocco, and belong to the same botanical family these two plants are: *Mentha pulegium* (L) and *Marrubium vulgare* (L.) of the family of Lamiaceae. The two plants are largely used in traditional medicine by the local population. Sight their interest we evaluated the quantity of the phenolic compounds and flavonoides total, as well as the bacterial sensitivity which revealed important a disinfectant activity against several disease-causing agents, Moreover, the antioxidant activity of these two plants was led by the use of test VCEAC (Vitamin C Are equivalent Antioxidant Capacity). The results show that these two plants are rich in natural antioxidants, although the sheets of *Mentha pulegium* (L) richer than those of *Marrubium vulgare* (L.).

**Keywords** : Spontaneous Plants; Antibacterial Activity; Antioxidant Capacity are Equivalent; Moroccan Traditional Medicine.

## I. INTRODUCTION

The importance of the Moroccan pharmacopeia is based on two aspects raise, A great botanical diversity an ancestral tradition to know to make containing the medicinal herbs , However, the Moroccan medicinal flora remains ignored until our days, on the 4500 species and under plant species, the counted medicinal species do not exceed the 356 to 600 species. In spite of that, traditional medicine always occupied an important place in the traditions of medication in Morocco and the area of large Casablanca in is a concrete example. The analysis of the Moroccan medicinal bibliography shows that the regional ethnopharmacologic data very

fragmentary and are dispersed, in the same way the knowledge to make is held currently only per few people.

The purpose of the present study, carried out in the circle of large Casablanca is to contribute to the knowledge of some medicinal herbs, to carry out a biological and phytochimic study of two spontaneous plants of the flora in the aforementioned area and to try to trace a safety margin for the therapeutic uses practised by the local population, the fact of being able to insulate, purify and analyze, the extracts of these plants, made it possible to know the composition and to validate the uses of it.

## II. METHODS AND MATERIAL

### *Mentha pulegium (L.)*

also called cosmopolitan mint pouliot of distribution, in Morocco it is rather common, it pushes in the wet places on the rich ground one meets it until 1800 m of altitude. nC' is a plant from 20 to 55 cm height, long-lived by rhizomes, with hairy, strongly aromatic stems and with prickly odor. Its period of flowering extends from June until October.

**Characteristics:** The plant has properties antispasmodic, antalgic - calming and sedative antioxydant, disinfectant - antiviral and antibacterial, para-sympathicotonique powerful (hypotensive, vasodilatatrice, anaphrodisiaque), diuretic, expectorante, and vulnerary.

For the population, area of Casablanca, marjoram is indicated in difficult digestions, the influenzas, coughs, bronchitides. Its infusion in hot milk is very much used to make sleep the children. In local use, it is used in the care of the oral ignitions and the plaices.

### *Marrubium vulgare (L)*

Very widespread plant throughout the year, one finds it on the edges of ways, the meadows dry and the waste grounds, but generally on grounds limestones, its thyme odor distinguishing it from the other plants. employee especially in a fresh state to prepare infusions and decoctions.

It is a hardy perennial of color greying resembling slightly mint, odorous, measuring from 30 to 80 cm according to the places, its sheets very fluffy from 2 to 5 cm have a crushed aspect, flowering extends from April to July the flowers are white (1 to 2 mm), sometimes a little dew,

**Characteristics:** this very widespread plant throughout the year, is especially employed in a fresh state to prepare infusions and decoctions. Managed in the cases of typhus or paludism, it makes fall the fever. One employs it like expectorating at the time of bronchitides and other lung diseases. With strong amount, it is vermifuge. The convalescents or weakened absorb it like reconstituting, tonic and stimulant for the local population, the plant was traditionally employed in the manufacturing of the remedies against cough. It is also used like antidiabetic, only or associated with the

fenugreek, the white lupin, thyme, with the white wormwood and the street, it in fact a true pharmacy is, except in the event of diabetes the decoction, which is very bitter, is edulcorated with honey or raisins

**Method used:** for this work, the inhibition of the bacterial growth by the extracts of our plants, is studied by the method of diffusion in solid medium. With a punch of the wells are dug in the agar of Mueller-Hinton run in limp of Kneaded and sown by a germ-test. The diameters of inhibition are then measured around the wells after a 45 minutes preincubation to room temperature and an incubation with the drying oven with 37°C during 18 hours, and any zone with a diameter higher than 2 mm indicates an antibacterial activity (**Vanden Berghe and Vlietinck, 1991**)

**Preparation of the inoculum:** the method of preparation of the inoculum is that recommended by the French company of microbiology (SFM) (official statement of 2005) which consists in preparing, starting from a culture of 18-24h of the bacterium studied on the agar medium, a suspension in saline solution (NaCl with 0.9%) equivalent to the standard McFARLAND 0.5 (| 108 UFC/ml). Thereafter, 1ml of the suspension of the inoculum, is spread out by flood on the surface of one limps of Petri containing of the agar of Mueller-Hinton, which will be let dry in the septic zone of the Bunsen burner (**SFM, 2005**).

**Stocks of reference:** during our study, several bacterial strains will be studied, for the majority are stocks of reference, i.e., beforehand determined stocks, thus for their culture one carries out only the colouring of Gram to see whether these of Gram ( ) or (-). Other cultures on the other hand, result from wild stock of environmental origin (of CHU de Rabat). For these cultures, an identification of the stock is obligatory, thus the various tests of identification are carried out: api plate 20th, ODC, Mannitol mobility, Oxydase, Catalase (H<sub>2</sub>O<sub>2</sub>), Indol, LDC, ADH, TSI, Coagulase etc, and finally the colouring of Gram (**Cowan and Steel, 1974**).

**Methods of antioxydant test:** Several methods are used to evaluate, in vitro and in vivo, From methodological point of view, the test with free radical DPPH is recommended for compounds containers HS, NH- and OH- groups. It is carried out with room temperature, this making it possible to eliminate any risk from thermal degradation of the molecules thermolabile (Salah and al., 1995; Sharma and Bhat, 2009)

***Test DPPH:*** chemical compound DPPH, was one of the first free radicals used to study the relation antioxidant structure-activity of the phenolic compounds. It has an electron not paired on an atom of the nitrogen bridge. Because of this delocalization, the molecules of the radical do not form dimers, the DPPH remains in its relatively stable monomeric form at ordinary temperature. The delocalization causes also the blue quite characteristic of the solution of DPPH. The measure of the efficiency of an antioxidant is done by measuring the reduction in colouring blue, due to a recombination of radicals DPPH, measurable by spectrophotometry with 515-518 Nm (Sharma and Bhat, 2009).

***B-carotene test:***  $\beta$ -carotene, is a hydrocarbon having 11 double combined connections what explains its orange color, it neutralizes the free radicals effectively and prevents the peroxidation of the fatty-acids in chemical solution, it is also a powerful trapper of oxygen singulet (Pavia and Russell, 1999).

The antiradical activity of the extracts by  $\beta$ -carotene is given by measuring the inhibition of oxidative degradation (discolouration) by the products of oxidation of the linoleic acid. Thus the kinetics of the discolouration of the emulsion in presence or not of antioxidant (negative control in which the sample is replaced by 25 methanol  $\mu$ l) is measured, at initial time ( $T = 0$ ) and successively with  $T = 30$  and  $60$  min, by spectrophotometry with  $470$  Nm (Mansour and Khalil 2000).

### III. DISCUSSION AND CONCLUSION

The present study related to the identification of the various chemical groups, the research of the activity antibacterial and antioxidant, the extracts of two spontaneous plants of the area of Casablanca. From the profitability point of view, the aqueous extracts gave the highest proportions in comparison with the extracts ethanolic, that peuts' to explain by the fact that the ethanol is a not very polar organic solvent thus more volatile than water, this property assure him the aptitude to decrease the powder instead of penetrating inside (Perry and Green, 2007).

The description, in vitro, of the various chemical entities, enabled us to note the presence, the wealth of flavonoïdes, tannins, triterpenes and coumarins at the two desLamiacées plants of the family, etparticulièrement the richesseen sterols and mucilage chezMarrubium vulgare.

The results revealed by in vitro characterization are confirmed by the chromatographic and spectrophometric analyses, and are quoted in the literature.

Indeed, the chromatographic analysis of the extract alcooliquedeMarrubium vulgare (L.) allowed identifierdes bitter principles, in particular marrubiine, acid phenols: caffeic acid, myristic, palmitic, stearic, oleic, linoleic and linoleic, of the mineral matter in the form of salt (of potassium, sodium and iron), of tannins of the saponosides, choline and essential oil in traces (Kurbatova and al., 2003; Giordani and al., 2008).

However, the chromatographic analysis of Mentha pulegium (L.) is related particularly to the chemical composition of its essential oil, this analysis shows the existence of pulégone like principal component, besides acetate of pulégone, menthone, isomenthone, neo-menthol, piperitone,  $\gamma$ -terpinene and  $\beta$ -caryophyllene (Mahboubi and Haghi, 2008)

The pharmaceutical interest of our treated plants, is proven by their biological activity, consequently the study of the bacterial sensitivity shows a broad spectrum of action against pathogenic germs tested.

Determination of the parameters of inhibition (CMI and CMB) allowed to us not only to confirm, quantify and compare the activities, but also to characterize the nature of the effect exerted by an extract on a given micro-organism (Guerin-Faublée and Carret, 1999).

The comparison of the results got by the method of diffusion in solid medium on the one hand and the method of dilution in liquid medium on the other hand, raises that: the values of CMI agree generally with those of the diameters of inhibition, the extracts having induced an important zone of inhibition present smallest CMI on the corresponding stocks. This comparison was carried out according to the method of Fauchere and

Avril (2002), for which a substance is bactericidal when report CMB/CMI is, and bacteriostatic when this report is. If report CMB/CMI is considered, the extract of *Mr. vulgare*, is especially bacteriostatic with regard to *K. pneumoniae* and *S. enteritidis* that it either clear *dessouchesprélevées* *référencesou* of the hospital medium.

To our knowledge, it is the first time that such results are thus clearly shown for these two plants. ((**Moussaid and al., 2011**))

Our work having been realized starting from the aqueous extracts (décoctés) and alcoholics (ethanol), this could thus justify the therapeutic uses of *Marrubium vulgare*, and *Mentha pulegium* in traditional medicine, and which are used as anti-infectious by the majority of the population near which we carried out the investigation. (**Moussaid and al., 2012**)

The antibacterial activity observed, is in addition explained by the results of the chemical analysis of the plants, this analysis reveals the presence of the compounds such as: alkaloids, tannins, saponins and the flavonoïdes, whose antimicrobial properties were already shown (**Cowan, 1999**).

Most these compounds were highlighted in the extracts by order of importance chez *Mentha pulegium* and *Marrubium vulgare*, which makes it possible to as well explain their important activities on the Gram bacteria (-) as on Gram (+).

The antioxydant test that we have réalisé in vitro given a notable result, the extracts of our plants are able to reduce radical DPPH, with a better inhibition by decreasing order *Marrubium vulgare* then *Mentha pulegium*. These results revealed that the extracts present anti-ridicalizing powers very important, nevertheless, this activity remains slightly lower than that of the ascorbic acid.

In the same way, the extracts showed a remarkable inhibiting activity of the coupled oxidation of the linoleic acid/ $\beta$ -carotene for the two plants, however this activity significantly remains lower than that of positive control (Propyl gallate).

We notice that the results differ appreciably according to the test used. That could be explained by the specific sensitivity to each test. The use of two different tests enabled us to have a better reading of the anti-ridicalizing activity of our extracts. Indeed we note that the activity of inhibition of the discolouration of  $\beta$ -carotene is lower compared to the results got by the DPPH.

According to the literature, the phenolic compounds present a strong potential antioxydant, made up which were highlighted in the extracts, with a strong concentration at Lamiacées. (**Marvin, 2010**).

#### IV. REFERENCES

- [1] Cowan S. T., Steel K. J. (1974). Manual of identification of medical bacteria. 2nd Éditions, Cambridge University Press, London. 331p.
- [2] Cowan M. M. (1999). Plants products as antimicrobial activity. *Clinicat Microbiology.*, 4: 564-582.
- [3] Fauchere L.L., April J.L. (2002). General and medical bacteriology. Editions Ellipses, Paris. 368 p.
- [4] Giordani R., Hadeif Y., Kaloustian J. (2008). Compositions and antifungal activities of essential oils of some Algerian aromatic plants. *Fitoterapia*, 79, 199- 203.
- [5] Guerin-Faubleé V., Carret G. (1999). The antibiogramme: principles, methodology, interest and limits. *National days GTV-INRA.*, 5-12.
- [6] Kurbatova N-V., Muzychkina R-A., Mukhitdinov N. M., Parshina G-N. (2003). Comparative phytochemical investigation of the composition and content of biologically active substances in *Marrubium vulgare* and *M. alternidens*, *Chemistry of Natural Compounds.*, 39: 501- 502.
- [7] Mahboubi M., Haggi G. (2008). Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil, *Journal of Ethnopharmacology.*, 119: 325-327.
- [8] Mansour EH., Khalil AH. (2000). Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chem.*, 69: 135-41.
- [9] Marvin E., (2010). Anti-oxidants, Controversies and perspectives: How can the failure of clinical

- studies using anti-oxidants be explained?  
*Médecine & Nutrition* ,46: 19-29
- [10] Moussaid M., Elamrani A., Berahal C., Moussaid H., Bourhim N. , Benaissa M. (2011). Evaluation of the Antioxidant Potential of Some Morocco Medicinal Plants, *Global Journal of Pharmacology*., 5 (3): 153-158.
- [11] Moussaid M., Elamrani A., Berahal C., Moussaid H., Bourhim N., Benaissa M. (2012). Comparative evaluation of phytochemical and antimicrobial activity between two plants from the Lamiaceae family: *Marrubium vulgare* (L.) and *Origanum majorana* (L.), *International Journal of Natural Products Research*., 1 (1): 11-13
- [12] Pavia SA., Russell RM. (1999). Beta-carotene and other carotenoids as antioxidants. *Journal of the American College of Nutrition*., 18:426–33.
- [13] Perry RH., Green DW. (2007). *Perry's chemical engineers' handbook*, 8eme Éditions, McGraw-Hill, 2640 p.
- [14] SFM. (2005), *Bulletin of the French company of Microbiology*. 19: 191-193:  
<http://www.sfm.asso.fr/>
- [15] Salah N., Miller N.J., Paganga G., Tijburg L., Bolwell, G.P., Rice-Evans C.A. (1995), Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics*. 332 : 339-346.
- [16] Sharma Om P., Bhat T.K. (2009). DPPH antioxidant assay revisited. *Food chemistry*., 113: 1202
- [17] Sharma Om P., Bhat T.K. (2009). DPPH antioxidant assay revisited. *Food chemistry*., 113: 1202
- [18] Vanden Berghe D-A., Vlietinck A-J. (1991). Screening methods for antibacterial and antiviral agents from higher plants. In: Hostettmann K. Ed., *Methods in plant biochemistry*, London, Academic Press., 6: 47-69.