*Full Length Research Paper*

# Antioxidant activity, phenols and flavonoids contents and antibacterial activity of some Moroccan medicinal plants against tomato bacterial canker agent

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**Medicinal and aromatic plants (MAP) belonging to 16 species, currently used in southern Moroccan traditional medicine, were evaluated here, firstly, for the action of their aqueous extracts against the bacterial canker agent of tomato *in vitro*. Then the phenolic contents, flavonoids contents and antioxidant capacities of these MAP extracts were estimated. Results obtained show that tested species exhibited biological activity toward the pathogen studied here; the inhibition zone diameter was between 0.5 and 4.88 cm. Furthermore, tested species exhibited a board range of phenolic contents varying from 55.58 ± 5.07 to 3.98 ± 0.16 mg of Cafeic acid equivalents (CAE) per gram of dry weight (DW). The flavonoids contents varied from 19.82 ± 0.65 to 1.74 ± 0.34 mg of Rutine equivalents (RE) per g of DW. Significant and positive linear correlations were found between total phenolic contents (R = 0.87 and $R^2$ = 0.76), flavonoids contents (R = 0.96 and $R^2$ = 0.93) and the biologic activity (IZ diameters) of the aqueous extracts. The antioxidant capacity expressed as Trolox equivalents antioxidant capacity (TEAC) varied from 550.67 to 1.18 mM per 100 g dry weight. Significant and positive linear correlation was found also between antioxidant capacities and both phenolic and flavonoids contents. These results proved that the richness of MAP with phenols and flavonoids was involved in there antibacterial activity and there antioxidant capacity. This finding is useful and can contribute to the development of potent and natural bio pesticides in the future for the control of bacterial canker of tomato as well as other phytopathogens by exploiting MAP compounds accepted by consummators and environmentalists.**

**Key words:** Bacterial canker, tomato, medicinal plants, biological control, flavonoids, Trolox equivalents antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

## INTRODUCTION

The tomato crop is subject to attack by a multitude of pathogenic microorganisms and its intensive culture has generated and amplified the phytosanitary problems. In addition to fungi, viruses and deleterious organisms,

pathogenic bacteria are important factors that reduce the quality and performance of this culture (Gartemann et al., 2003). The bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) (Davis et al., 1984), account among the most important phytosanitary tomato problems in California, Ohio and Morocco (Fatmi, 1989; Fatmi and Schaad, 2002). It is a very contagious and destructive disease in tomato crop both under controlled and field cultures (Utkhede and Koch, 2004). The bacterial canker can cause significant damage which may go up to the destruction of 100% crop (Gitaitis, 1990; Chang et al., 1992). In Morocco, all tomato production areas are contaminated by this pathogen, whose seriousness varies by the following regions (Fatmi, 1989). In the Souss-Massa Draa region, it has become the main cause of the premature death of tomato (Fatmi, 1989). The seeds and infected transplants are the primary sources of primary inoculums of *C. m.* subsp. *michiganensis* (Bryan, 1930; Gleason et al., 1991). Bacteria can survive in the soil (Bryan, 1930; Chang et al., 1992; Strider, 1967; Farley, 1971), in crop residues and multiple alternative hosts (Fatmi and Schaad, 2002). The spread of the disease is ensured by various manipulations like transplantation (Gitaitis, 1990; Gitaitis et al., 1991; Gitaitis and Walcott, 2007), phytosanitary treatments and irrigation systems (Strider, 1969; Chang et al., 1992). The chemical treatments recommended for this disease only reduced the population of the pathogen in the surface of the infected plants (Hausbeck et al., 2000).

Although, the control of this bacterial disease continues to be difficult, prevention was the first defense line. Given the inefficiency of chemical treatments and their impact on health and the environment, research and development of alternative methods are recommended. The biocontrol is a promising way and much research works have been made worldwide against bacterial canker agent and various plant pathogens and encouraging results were found (Amkraz et al., 2010; Basim et al., 2006; Umesha, 2006; Boudyach et al., 2004; Boudyach et al. 2001; Daferera et al., 2003). With a view to developing effective natural treatments, naturally extracted substances have been obtained from plants as privileged axes of the biological control of plant pathogens both in crop treatments and in post-harvest manages (Talibi et al., 2012; Askarne et al., 2012; Talibi et al., 2011; Blaestra et al., 2009; Taqarort et al., 2008; Ameziane et al., 2007). Daferera et al. (2003) tested the antimicrobial activity of essential oils from aromatic plants against several plant pathogens. The results obtained by these authors showed that *C. m.* subsp. *michiganensis* is very sensitive to essential oils of thyme and oregano. Blaestra et al. (2009) evaluated the antibacterial activity *in vitro* and *in vivo* of the aqueous extracts of two medicinal plants, *Allium sativum* and *Ficus carica*, against several pathogens including the tomato canker agent. The effects obtained were very satisfactory since the extracts of *A. sativum* and *F. carica* helped to control the

bacterial rates ranging, respectively, up to 65 and 38% , in comparison with a standard treatment with copper (Balestra et al., 2009). Also, Kasselaki et al. (2011) have reported a lack of effective seed treatments against pathogenic bacteria. Indeed, compounds based on copper (the only chemical treatments permitted under organic farming standards) provide only partial control.

Goufo et al. (2008) reported the effectiveness of extracts of plants from Camarón to the agent of tomato late blight. Recently, Ravikumar and Garampalli (2013) have shown that aqueous extracts of 13 plants among the 39 they used have an interesting antifungal activity against *Alternaria solani*. These extracts can be used as potential fungicides in organic cultivation of tomato (Ravikumar and Garampalli, 2013). Furthermore, Boulogne et al. (2012) reported in their review that, the potential use of secondary metabolites from plant extracts as antifungal and insecticidal. Indeed, those secondary metabolites were the support of the biological activity of plant extracts (Boulogne et al., 2012).

The objective of this study was to select among 16 medicinal and aromatic plants (MAP) harvested in different regions of Morocco those which aqueous extracts inhibit the growth of *C. m.* subsp. *michiganensis in vitro.* The last step of this study aimed the assessment of the total phenolic contents, the flavonoids contents and the antioxidant capacity of the MAP tested using the classical Folin-Ciocaleteu reagent, the $FeCl_3$ method and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. These data will be helpful for understanding the bioactivity of these MAP and also useful for synthesize potent bio pesticides based on the bioactives molecules implicated (Cai et al., 2004; Surveswaran et al., 2007).

## MATERIALS AND METHODS

### Plant sampling and preparation of extracts

Sixteen Medicinal and aromatic plants (MAP) were collected from several regions of Souss-Massa-Draa, south of Morocco, between March and April 2011 and 2012. The plants harvested were placed in clean plastic bags and numbered. After identification in the laboratory, the samples were air dried in the darkness for a week, followed by a drying in an oven at 35°C for one night. Different parts of the plants were then grinded to powder. Aqueous extracts were prepared as described by Ali-Emmanuel et al. (2002) and Talibi et al. (2011).

Twenty grams (20 g) of each powder were put into suspension in one beaker 250 ml containing 60 ml of distilled water. All were brought to a boil during 5 min. Then, the obtained suspensions were double filtered utilizing the gauze followed by filter paper (Wathmann n°1). The obtained filtrate was dried in an oven at 60°C. The stock solution of each extract corresponded to a concentration of 0.5 g dry extract in 1 ml of distilled water. Extracts were conserved at -4°C in dark medical bottles (30 ml) for further utilization.

### Antibacterial activity of the MAP extracts

The paper disc diffusion method was used to detect the antibacte-

rial activity of plants extracts as described by Talibi et al., 2011. One suspension of *C. m.* subsp. *michiganensis* JS 07 isolate (Amkraz et al., 2010) was prepared in the sterile physiological water from a young culture of 72 h on the NBY medium. The optical density of bacterial suspension was adjusted to 0.46 at 610 nm to have $10^8$ cfu ml$^{-1}$ (Umesha, 2006). Aliquots (100 µl) of the suspension were speared in the medium surface with sterile glass beads. Cultures prepared were subsequently placed in an incubator at 25°C for 30 min. Sterilized filter paper discs (5 mm in diameter) were soaked with 10 µl of each plant aqueous extract at a concentration of 0.5 g ml$^{-1}$. Discs were placed in the middle of Petri plates containing pathogen dried cultures prepared previously. Distilled water was used as negative control. The plates were incubated at 25°C and observed after 48 to 72 h. At the end of the incubation period, inhibition zones around the paper discs were measured and compared to the controls. All tests were conducted in triplicate.

## Phytochemical screening of medicinal and aromatic plants studied

In order to determine the chemical compounds capable of beings responsible for the antibacterial activity of PAM tested. We were interested in the determination of total phenols, flavonoids and the determination of the antioxidant activity of PAM that showed a significant growth inhibition of *C. m.* subsp. *michiganensis*.

### Extracts preparation

For methanolic extraction, 2 ml of methanol 80% was added to 100 mg of plants' powders in a conical flask, which was kept to room temperature overnight with occasional shaking. The extract was than centrifuged using centrifugation apparatus. The supernatant was recuperated and stored at 4°C until use.

### Determination of total phenol content

The total phenol contents were estimated using the classical Folin-Ciocalteau colorimetric method according to Alemanno et al. (2003). 25 µl of each sample were reacted with 110 µL of Folin-Ciocalteau reagent for 3 min at room temperature. The reaction was then neutralized with 200 µl of saturated sodium carbonate (20%) and 1.9 ml of distilled water. After homogenisation, reaction was allowed to stand for 30 min in the dark at 60°C. Later the absorbance of the resulting blue colour was measured at 750 nm with a spectrophotometer (Janeway 6400). Appropriate dilutions were made before measure. The blank was prepared using 2 ml of methanol. Quantification was done on the basis of a standard curve with Cafeic acid solutions. This calibration curve was done by preparing scale of Cafeic acid concentration from 0.1 to 0.5 mg/ml in methanol 80%. Results were expressed as microgram of Cafeic acid equivalents (CAE) per milligram dry weight (DW). All measures were done in triplicate and values were means ± SD.

### Determination of flavonoids contents

To estimate the flavonoids contents, the method of Singh et al. (2010); Harnafi et al. (2007) was utilized with minor modifications. The FeCl$_3$ reagent was prepared by mixing 400 mg of sodium acetate with 133 mg of FeCl$_3$ powder in 100 ml of 80% methanol. 300 µl of this reagent was added to 600 µl of the sample (plant extract 5% in 80% methanol). After homogenisation, the mixture was left to stand for 30 min in the dark at room temperature. Thereafter, the absorbance was measured at 430 nm with a spectro-

photometer. Dilutions were made before measure if necessary. Total flavonoids contents were calculated as µg of Rutine equivalents from a calibration curve. The calibration curve was prepared with solutions of Rutine at concentrations scale from 0.1 to 0.5 mg/ml in methanol 80%. All measures were done in triplicate and values were means ± SD.

### Determination of antioxidant capacity

The determination of antioxidant activity was performed according to the protocol, slightly modified as described by Rawat et al. (2011). This method is based on the degradation of DPPH radical (2, 2 diphenyl-1-picrylhydrazyl). In the presence of free radical scavengers, DPPH (purple color) is reduced to 2, 2-diphenyl-1-picrylhydrazine (yellow color). The DPPH radical (DPPH$^\bullet$) solution (0.1 mM) was prepared in 80%v/v methanol. The DPPH$^\bullet$ solution (1.9 ml, absorbance of 0.47 ± 0.13 at 517) was added to 100 µl of tested extract. The reaction for scavenging DPPH radicals was carried out at room temperature in the dark for 30 min, and then the reduction in absorbance was recorded at 517 nm. A calibrate Trolox standard curve was also made in the same condition of the experiment with concentration scale of Trolox from 0.25 to 1 mM in 80%v/v methanol. The results were expressed as TEAC units (mM Trolox equivalents per 1 milligram dry weight of sample). All measures were done in triplicate and values were means ± SD.

### Statistical analyses

Data from phenolic contents, favonoids contents and antioxidant capacity were means of triplicates ± SD. The correlation coefficient R and the determination coefficient $R^2$ were calculated using Microsoft Excel 2007.

## RESULTS

### Evaluation of the antibacterial activity of plant extracts

The antibacterial efficacy of tested plants was determined *in vitro* using the agar plate's methods. Table 1 illustrates the summary of results obtained. The most efficient MAP were *Lavandula coronopifolia* (78), *Rubus ulmifolius* (7), *Rosa canina* (43), *Cistus monspliensis* (114), *Pistacia atlantica* (26), *Anvillea radiata* (83), *Cistus crispus* (107), *Lavandula stoechas* (25) and *Ighermia pinifolia* (65) for the aqueous extracts tested here (IZ ≥ 2.59 cm). We can also conclude that the bacterium was more sensitive to extracts containing a large board of molecules suggesting that the antibacterial activity of MAP results in a synergetic activity more than one kind of compounds. However, our results suggest that the plants tested here will be a potent source for natural compounds to combat the bacterial canker agent in tomato crop managements.

### Phytochemical screening of medicinal and aromatic plants studied

Results obtained are summarized in Table 1. If these results are analyzed in a comprehensive manner, we

**Table 1.** Content of total phenols, flavonoids and antioxidant activity of medicinal and aromatic plants selected for their antibacterial activity against the agent of bacterial canker of tomato.

| Tested plants | [Phenols] (mg CAE [b]/g DW) | [Flavonoides] (mg ER [c]/g DW) | IZ [d] (cm) | TEAC [e](mM/100g DW) | Parts used |
|---|---|---|---|---|---|
| *Lavandula coronopifolia* (78) | 55.58 ± 5.07 | 19.51 ± 0.49 | 4.88 | 550.67 | Stem, leaves and flowers |
| *Rubus ulmifolius* (7) | 37.52 ± 6.64 | 19.82 ± 0.65 | 4.05 | 430.50 | Stem and leaves |
| *Rosa canina* (43) | 44.68 ± 0.573 | 17.58 ± 0.98 | 4.04 | 333.73 | Stem and leaves |
| *Cistus monspliensis* (114) | 37.21 ± 0.63 | 19.79 ± 2.07 | 3.86 | 233.27 | Stem, leaves and flowers |
| *Pistacia atlantica* (26) | 62.78 ± 1.51 | 18.38 ± 1.53 | 3.70 | 460.49 | Leaves |
| *Anvillea radiata* (83) | 24.13 ± 5.02 | 15.39 ± 1.43 | 3.55 | 166.89 | Stem, leaves and flowers |
| *Cistus crispus* (107) | 27.62 ± 0.29 | 16.53 ± 0.31 | 3.20 | 118.63 | Stem, leaves and flowers |
| *Lavandula stoechas* (25) | 32.58 ± 4.10 | 6.30 ± 0.26 | 3.07 | 90.80 | Stem, leaves and flowers |
| *Ighermia pinifolia* (65) | 45.41 ± 5.70 | 14.72 ± 0.60 | 2.59 | 73.49 | Stem, leaves and flowers |
| *Artemisea inculta* (89) | 21.99 ± 1.18 | 10.31 ± 1.15 | 2.11 | 35.22 | Stem and leaves |
| *Ceratonia siliqua* (62) | 21.20 ± 0.99 | 7.44 ± 2.26 | 1.58 | 55.38 | Leaves |
| *Fagonia zilloïdes* (93) | 6.55 ± 0.78 | 5.56 ± 1.6 | 1.27 | 11.08 | Leaves |
| *Zygophyllum gaetulum* (104) | 3.98 ± 0.16 | 2.26 ± 1.8 | 1.25 | 1.18 | Stem and leaves |
| *Colocynthis vulgaris* L. (67) | 6.55 ± 0.35 | 2.13 ± 0.72 | 0.82 | 9.82 | Leaves |
| *Sanguisorba minor* (19) | 4.47 ± 0.28 | 1.74 ± 0.34 | 0.68 | 3.64 | Stem and leaves |
| *Reseda alba* (99) | 5.01 ± 0.13 | 1.99 ± 0.28 | 0.50 | 3.59 | Stem and leaves |

[a]Values are means of three replicates ± SD. [b]Results are expressed in equivalent mg caffeic acid (CAE) per g dry weight (DW) of plants. [c]The results are expressed in mg Rutin equivalents (RE) per gram dry weight (DW) of plant. [d]Diameters of inhibition zones of aqueous extracts in cm. [e]TEAC, Trolox equivalent antioxidant capacity, results are expressed as mM Trolox equivalents per 100 g dry weight.

**Table 2.** Correlation (R) and determination ($R^2$) indices between the content of phenols (Ph), the content of flavonoids (Flav), antioxidant capacity (TEAC) and the antibacterial activity of aqueous extracts (diameters of the inhibition zones, IZ).

| Parameter | Ph/Flav | Ph/IZ | Flav/IZ | Ph/TEAC | Flav/TEAC | TEAC/IZ |
|---|---|---|---|---|---|---|
| R | 0.88852516 | 0.87339293 | 0.96832161 | 0.84607994 | 0.78914914 | 0.86716811 |
| $R^2$ | 0.78947696 | 0.7628152 | 0.93764673 | 0.71585126 | 0.62275636 | 0.75198053 |

noted that the following species: *L. coronopifolia* (78), *R. ulmifolius* (7), *R. canina* (43), *P. atlantica* (26), *A. radiata* (83), *C. crispus* (107), *L. stoechas* (25) and *I. pinifolia* (65) had levels of phenols, flavonoids and anti-oxidant activities much higher than the other tested plants. Furthermore, it is important to note that these parameters change, usually, in proportion to the diameters of the inhibition zones. More precisely, the content of total phenols species tested ranged from 3.98 ± 0.16 to 62.78 ± 1.51 mg of caffeic acid equivalent (CAE) per g dry weight (DW), that of flavonoids ranged from 17.4 ± 3.4 to 19.82 ± 6.5 mg of rutin equivalent (RE) per g of dry weight (DW) and the antioxidant activity ranged from 1.18 to 550.67 mM Trolox equivalent per 100 g of dry weight (DW) (Table 1). Further, a significant positive correlation was observed on one hand, between the phenol content (R = 0.87 and $R^2$ = 0.76), the flavonoid content (R = 0.96 and $R^2$ = 0.93), and the antimicrobial activity of aqueous extracts and secondly between the phenols content, the content of flavonoids and antioxidant activity of aqueous

extracts (Table 2). These results confirm the richness of selected MAP on phenols and flavonoids, which are likely involved in their antibacterial activities and their anti-oxidant capacity. The positive correlation between the antibacterial activity and the antioxidant one is that, more extract had antioxidant capacity, is more interested in terms of biological control. Furthermore, the antioxidant capacity of the samples may explain the activation of the germination already observed in our previous study (Talibi et al., 2011; Amkraz, 2013). This activation of the germination would be due to the action of the antioxidant molecules on the pH of the surrounding medium of seeds. Indeed variations in the pH affect germination of seeds of several plant species (Chachalis and Reddy, 2000).

## DISCUSSION

The researches undertaken in our laboratory aimed to

evaluate the antibacterial potential of medicinal and aromatic plants (MAP) from the Souss-Massa Draa valley, against *C. m.* subsp. *michiganensis* and other pthytopathogens both in crop and post-harvest (Talibi et al., 2013; Askarne et al., 2013; Askarne et al., 2012; Talibi et al., 2012a; Talibi et al., 2012b; Talibi et al., 2011; Ameziane et al., 2007). In fact, the aqueous extracts of several MAP (40 species) were studied firstly by measuring their reduction capacity, or even elimination of the inoculums of this pathogen on tomato seeds by Talibi et al. (2011). Effect of these extracts on the seed germination was also evaluated and results obtained confirmed that MAP extracts tested had a positive action on the germination rate. This improved seed germination could be explained by an acceleration of the process of solubilization of seed husks on the one hand and the effect of these extracts on the pH of the medium (Chachalis and Reddy, 2000; Talibi et al., 2011; Amkraz, 2013).

In this paper, we aimed to find eventual relation between the biological activity of the MAP studied previously and their phytochemical composition. To do this, we chose some plants belonging to the three categories defined by Talibi et al. (2011) and Amkraz (2013) based on the inhibition zone diameters obtained against the bacterial canker agent. So, we have chosen 16 MAP for this study. We have firstly repeated the *in vitro* antibacterial activity of these MAP and secondly assessed the relative richness of the MAP with phenolic compounds and we have also assessed their antioxidant activity. This approach could be a promising antibacterial alternative to chemicals whose multiple disadvantages constitute a major constraint for both the consumer and the environment. This is more important because we still do not have, to this day, any commercial cultivars resistant to the bacteria causing bacterial canker in tomato. The results of the preliminary screening showed that aqueous extracts of all tested MAP have antibacterial activity toward the pathogen studied here. The most effective species belong to the following families: Lamiaceae, Rosaceae, Asteraceae, Anacardiaceae and Cistaceae. The results obtained with species belonging to the genus *Lavandula* are remarkable and entirely consistent with those of Daferera et al. (2003) reporting that the *Lavandula* genus includes several species with antimicrobial activity against some plant pathogens. In addition, these authors showed that the essential oil of *Lavandula angustifolia* (600 mg ml$^{-1}$) completely inhibited the growth of *C. m.* subsp. *michiganensis*. According to these authors, the antibacterial activity of this essential oil is probably due to bioactive compounds, especially linaloole and linalyl acetate (Daferera et al., 2003). In addition, Teixeira et al. (2012) confirmed also the high antibacterial potential exercised by a representative of this genus. They have indeed demonstrated that methanolic extracts of *L. stoechas* exhibit significant antibacterial properties toward *Staphylococcus aureus* and

*Staphylococcus epidermidis*. This power is linked to the phenolic and terpene compounds of this plant. According to our results, the second species which significantly inhibited the growth of *C. m.* subsp. *michiganensis in vitro* was *R. ulmifolius*. In fact, Sisti et al. (2008) showed that the dry extract *R. ulmifolius* is of great interest in so far as it has biological activity against a number of human pathogens. In another study, Thiem and Goślińska (2004) had reported that extracts from the leaves of *Rubus chamaemorus* were endowed with antibacterial activity against some Gram negative bacteria.

Furthermore, Arima et al. (2002) showed that rutin (quercetin -3- rutinoside) is responsible for the antimicrobial activity against some Gram negative and Gram positive bacteria. Through our results, the growth inhibitory power of *C. m.* subsp. *michiganensis* exercised by *R. canina* was significant. This result agrees well with that of Montazeri et al. (2011) which showed that the aqueous and methanolic extracts of *R. canina* exhibit antibacterial activity against various pathogens (*S. aureus*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Bacillus subtilis*). Moreover, this result is also consistent with those of Basim and Basim (2004), which showed that the essential oil of *Rosa damascena* is effective against *Erwinia amylovora*, the fire blight pathogen of Rosaceae.

In previous studies, the specific phenolic components of the genus Rosa such as catechins and procyanidins has been shown to be effective antioxidants. As regards the inhibitory effect toward *C. m.* subsp. *michiganensis* exercised by *A. radiata*, the results correlate well with those of other authors. Indeed, El Hassany et al. (2004) showed the existence of a new terpene compound, obtained by extraction with chloroform, which is the main component involved in the antibacterial activity. This compound has a strong inhibition against *B. cereus*, *Streptococcus*, *Proteus vulgaris*, *Enterococcus faecalis*, *E. coli* and *Pseudomonas aeruginosa*. We also obtained a significant anti *C. m.* subsp. *michiganensis* activity with extracts of *P. atlantica*. This is in perfect agreement with the results of Hosseini et al. (2013) that showed the high inhibitory potential of *Streptococcus mutans* by the aqueous extracts and diethyl ether of this plant. Regarding the genus *Cistus*, we showed that *C. crispus* and *C. monspeliensis* induced a significant inhibition of the proliferation of *C. m.* subsp. *michiganensis*.

These results are in agreement with most of the work involving representatives of this kind. Indeed, Bouamama et al. (2006) reported that the sheets of *C. villosus* and *C. monspeliensis* have antibacterial activity against some Gram-negative bacteria responsible for certain human diseases. This activity is linked to the richness of this species in polyphenols which are known for their antibacterial property.

Furthermore, it has been shown repeatedly that the aqueous and methanolic extracts of *C. villosus* induce very high antimicrobial activity against the major pathogenic

of citrus in postharvest (Ameziane et al., 2007). Talibi et al. (2012a, b) also reported that aqueous extracts of *C. monspeliensis*, *C. villosus* and *C. crispus* from southern Morocco, reduce mycelial growth of *Geotrichum candidum*. This significant reduction is of the order of 100, 98 and 52%, respectively.

The content of total phenols varied between 3.98 and 62.78 mg of EAC (caffeic acid equivalents) per g dry weight (DW). This increase in the concentration of total phenols in some samples was also highlighted by Surveswaran et al. (2007). These authors reported levels ranging from 0.6 to 356.3 mg Gallic acid equivalents (GAE) per g dry weight in extracts of 113 Indian medicinal plants. In the same context, Bahri-sahloul et al. (2009) have shown, in the analysis of 8 MAP samples, which the amount of these secondary metabolites varies between 0.45 and 16.38 mg GAE per g of DW. This relative wealth of some MAP with polyphenols was also confirmed by Ebrahimzadeh et al. (2010) whose analysis of the samples revealed a total phenol content between 35.4 and 90 mg GAE per g dry weight. Finally, it is essential to clarify that a significant fluctuation, 52.8 to 16.65 mg GAE per g of DW was also discovered during the analysis of leaves of the same species, *Cinnamomum osmophleum*, harvested from eleven different locations (Wu et al., 2013).

As regards to the dosage of flavonoids, the results obtained revealed that the content of the compounds tested in these plants varies between 1.74 and 19.82 mg RE per g of DW. The great variability in rates of flavonoids at the MAP analyzed was reported by El Allagui et al. (2007). According to this team, the level of these compounds in the five studied samples varies between 2.90 and 36.20 mg Quercetin equivalent per gram of dry weight. Ebrahimzadeh et al. (2010) also showed that the plants they studied have relatively high contents in flavonoids, ranging from 22.80 to 48.20 mg Quercetin equivalent per g of DW. According to Bahri - Sahloul et al. (2009), the rate of flavonoids on the samples tested varies between 3.178 and 7.536 mg RE per g of DW. These results are of great interest because they reflect significant changes in concentrations of these metabolites according to locality, variety and even according to the maturity of the plant, and that for the same organ (flowers). In addition, a remarkable variability in levels of these compounds were identified in the comparative study of the same MAP samples; subjected to various types of extraction or fractionation protocols (Li et al., 2010; Montazeri et al., 2011).

The antioxidant activity of the 16 MAP studied here, expressed in mM TEAC per 100 g DW varies between 1.18 and 550.67 mM TEAC per 100 g of DW. This result is quite comparable to those described by other authors (Fu et al., 2010 etc). Indeed, Surveswaran et al. (2007) showed after extensive sampling and analysis of 133 species of medicinal and aromatic Indian plants that anti-oxidant activity varies between 0.16 and 500.70 mM TEAC

per 100 g of DW. The substantial variability in the antioxidant activity between the different samples is probably linked to the presence of a different combination of chemical compounds having the antioxidant potential; this is not only determined by the nature and the concentration of each compound but linked also to the synergism that can establish certain components relative to others. A comparative analysis, case by case, for our best samples to representatives of the same kind described in the literature test was conducted. However, we encountered some heterogeneity in obtained results that perfectly explains the diversity of extrinsic factors (geography, climate) and intrinsic (variety, maturation stage, organ analyzed, method of extraction and assay technique) which the plant samples are submitted and inducing heterogeneous phytochemical profiles. Despite this, we must remember that at least six of our samples are remarkable because of their high levels of phenols, flavonoids but also by their significant antioxidant potential. This is even more interesting when we have only tested the aqueous extracts. Indeed, the methanol, ethyl or flavored extracts using other organic solvents reveal even greater potential as has been repeatedly demonstrated by several authors (Panizzi et al., 2002; Fu et al., 2012; Oliveira et al., 2012). The major advantage is that our samples is also reflected in the strong correlation both between the phenolic content (R = 0.84) and flavonoids (R = 0.789) and antioxidant activity and secondly between the phenols content (R = 0.87) but mainly flavonoids (R = 0.96) and antibacterial activity. These correlations agree well with the results of other teams for other models plant / pathogen (Cai et al., 2004; Bahri-Sahloul et al., 2009; Fu et al., 2010; Surveswaran et al., 2010). Furthermore, phenols appear to be more involved in the antioxidant activity, while anti-microbial activity appears more related to flavonoids (Bahri-Sahloul et al., 2009; Fu et al., 2010; Surveswaran et al., 2010).

Finally, we must note that all MAP samples tested here are available in the Souss-Massa Draa valley southern Morocco and grow very easily. In addition, the extraction method adopted is very simple using the most accessible and least expensive solvent, namely water (Talibi et al., 2011; Amkraz, 2013).

## Conclusions and perspectives

The application of micro-organisms and/or natural substances for control or suppression of pest pathogens populations has recently been imposed by government guidelines to minimize or even eliminate the use of chemical pesticides seen concerns of the general public on the one hand, to the problem of resistance to chemical pesticides (Martineza et al., 2013) and secondly, to the adverse effects of chemical residues on both the envi-ronment and the human health (Whipps and Lumsden, 2001). Thus, several biopesticides have recently been

made (Hynes and Boyetchko, 2006). The research started in this framework allows us to conclude that southern Morocco and especially the region of Souss Massa Draa, is rich in MAP with significant antibacterial activity against *C. michiganensis* subsp. *michiganensis*. The best results are obtained with plants belonging to the genera: *Lavandula*, *Rubus*, *Rosa*, *Cistus*, *Pistacia* and *Anvillea*. A positive correlation was found between the antibacterial activity and the content of flavonoids and phenols. These results are promising and may contribute to the future development of natural biopesticides for the control of bacterial canker of tomato. The development of a reliable method of seed treatment with MAP, or their bioactive compounds to eliminate or reduce the initial inoculums of the pathogen on the seed is a very interesting application. The success of this process will help to get free seeds of this pathogen. This certification is essential to trade tomato seeds internationally. Further studies are needed to complete and confirm these results in green house level in order to estimate the power of the selected plants to prevent and control the bacterial canker in tomato. Indeed, the possibility of combining biological methods remains an important factor in promoting the use of biological control. This approach can provide powerful and reliable tools for biological control against the major pest problems in crops. Indeed, Bardin et al. (2008) reported that separated plant extract made of *Reynoutria sachalinensis* (Milsana) and mushroom *Microdochium dimerum* formulations exhibit high efficiency in the respective control of powdery mildew and *Botrytis cinerea* affecting tomato crops in greenhouse. These authors showed that the two treatments are compatible because the combination does not alter their respective efficiency.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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