Chemical composition and antibacterial activity of essential oils of four Moroccan plants against *Allorhizobium vitis*

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Abstract

This study aims to investigate the chemical composition and inhibitory effects of four essential oils from Moroccan medicinal and aromatic plants: *Rosmarinus officinalis* L., *Satureja calamintha* L., *Lavandula stoechas* L. and *Citrus aurantium* L. on *Allorhizobium vitis* strain S4 causal agent of crown gall of grapevine. The identification of compounds was performed by GC-MS analysis. Furthermore, the antibacterial activity of each essential oil was evaluated using aromatogram test. Results showed that the major compounds are cineole (48.12%) and α-Pinene (13.48%) for *R. officinalis*, borneol (29.01%) and 1,8-Cineole (18.18%) for *S. calamintha*, linalool (25.76%) and camphor (21.09%) for *L. stoechas*, linalool (38.81 %) and limonene (37.93 %) for *C. aurantium* essential oil. All essential oils tested exhibit an antibacterial activity *in vitro* against *A. vitis* S4 with a percentage of inhibition and minimal inhibitory concentration values in the range of 7.5-25.88% and 0.15-20.00 mg/ml, respectively.

Keywords: essential oil, chemical composition, antibacterial activity, *Allorizobium vitis*, crown gall, grapevine.

La composition chimique et l'activité antibactérienne des huiles essentielles de quatre plantes aromatiques et médicinales marocaines contre *Allorhizobium vitis*

Résumé

Cette étude vise à étudier la composition chimique et l'activité antibactérienne des huiles essentielles de 4 plantes aromatiques et médicinales marocaines : *Rosmarinus officinalis* L., *Satureja calamintha* L., *Lavandula stoechas* L. et *Citrus aurantium* L. sur l'agent causal de la galle du collet de la vigne *Allorhizobium vitis* la souche S4. L'identification des composés a été réalisée par la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CG-SM). L'activité antibactérienne de chaque huile essentielle a été évaluée à l'aide d'un test d'aromatogramme. Les résultats ont montré que les principaux composés sont le cinéole (46,28%) et le camphre (12,32%) pour *R. officinalis*, le bornéol (23,89%) et le cinéole (18,18%) pour *S. calamintha*, le linalol (25,76%) et le camphre (21,09%) pour *L. stoechas*, limonène (34,56%) et oxyde de linalol (34,24%) pour l'huile essentielle de *C. aurantium*. Toutes les huiles essentielles testées présentent une activité antibactérienne *in vitro* contre *A. vitis* S4 avec un pourcentage d'inhibition (PI) et une concentration minimale inhibitrice (CMI) varient entre 7,5-25,88% et 0,15-20,00 mg/ml, respectivement.

Mots-clés : huile essentielle, plantes aromatiques et médicinales, composition chimique, activité antibactérienne, *Allorizobium vitis*, galle du collet, vigne.

التركيب الكيميائي والنشاط المضاد للبكتيريا للزيوت الأساسية لأربعة نباتات عطرية وطبية مغربية ضد العامل المسبب لمرض التدرن التاجي*لغب* الناتج عن البكتيريا اللوغازبيوم فتيس.

حبادي خولة، صبري ميلود، بنبوعزة عبد اللطيف، فيال لودوفيك، لافير سيلين، كيرزاون إزبيل، بنكيران رشيد وأشباني الحسن

ملخص

تهدف هذه الدراسة إلى دراسة التركيب الكيميائي والنشاط المضاد للبكتيريا الممرضة للزيوت الأساسية لأربعة نباتات عطرية وطبية مغربية: روسمارينوس أوفيسيناليس (إكليل الجبل الطيب)، صاتوريجا كالامينتها (النعناع الجبلي)، لافاندولا ستويچهاس)الخزامى) و اند سيتروس اورانتيوم (البرتقال المر) ا لعامل المسبب لمرض التدرن التاجي الناتج عن البكتيريا اللوغازبيوم فتيس س4. تم تحديد المركبات بواسطة كروماتوغرافيا الغاز المقترنة بمطياف الكتلة *(GC-MS)*. تم تقييم النشاط المضاد للبكتيريا لكل زيت عطري باستخدام اختبار التصوير العطري. اظهرت النتائج ان المركبين الرئيسيين هما السينيول (46.28٪) والكافور (12.32٪) لروسمارينوس أوفيسيناليس، بورنيول (*23.89٪*) وسينول (18.18 ٪) ل ص. چالامينتها و لينالووول (25.76٪) و كافور (21.09٪) ل .*L* ستويچهاس والليمونين (34.56٪) وأكسيد لينا لول (34.24٪) للزيت العطري لنبات س .اورانتيوم. جميع الزيوت الأساسية التي تم اختبارها تظهر نشاطًا مضادًا للبكتيريا في المختبر ضد إ. فيتيس س4 مع نسبة تثبيط وأقل تركيز مثبط يتراوح بين 7.5-25.88٪ و 0.15-20 ، 00 ملغ / مل على التوالي.

الكلمات المفتاحية: الزيوت الأساسية، النباتات العطرية والطبية، التركيب الكيميائي، النشاط المضاد للبكتيريا، ألوريزوبيوم فيتا ،سيلكرم، التدرن التاجي.

Introduction

Crown gall of grapevine, caused by *Allorhizobium vitis* (Mousavi et al. 2014, 2015) previously referred to as *Agrobacterium vitis* (Ophel and Kerr, 1990), is one of the most important bacterial diseases that affect grape production around the world and causes significant losses of crop production every year (Burr and Otten, 1999). The *A. vitis* presents a high degree of natural specialization because it is associated with grapevines and has been only found on grape (Herlache et al. 2001). However, the mechanism of infection used by *A. vitis* has been shown to be very similar to that described for *Agrobacterium tumefaciens* (Smith and Tawnsend, 1904), pathogenic strains with a large range of plant hosts (Burr et al. 1998). The crown gall disease is characterized by a tumor, which is usually formed on a plant stem just above the ground; they obstruct vascular tissue and restrict movement of water and nutrients from the roots to the aerial parts of vine (Schroth et al. 1988). In consequence, loss of plant vigor, reduction in crop yield and in the most serious cases plant death (Poncet et al. 1996).

Tumorigenic strains of *A. vitis* can transform genetically plant cell by the oncogenes element called the T-DNA localized on a large tumor-inducing plasmid (pTi) and coded for two groups of genes (Thomashow et al. 1984). The first group encodes for the synthesis of plant hormones (auxin and cytokinin) that cause the proliferation of plant cell and development of galls; and the second group, responsible for production of opines used as carbon, nitrogen and energy sources for *A. vitis* growth (Lacroix and Citovsky, 2013).
The control of pathogenic strain of *A. vitis* in plants is a considerable problem in agriculture practice (Burr, 2004) due to the systemic character of *A. vitis*. In grapevine, the pathogen can be disseminated by asymptomatic propagating plant material which makes difficult to control the disease (Kuzmanovic et al. 2012). Until now, there are no chemical options used to control the crown gall grapevine; the control is limited to the use of general disinfectants (sodium hypochlorite), copper or antibiotics (Armijo et al. 2016; Kawaguchi, 2009; McManus et al. 2002; Burr et al. 1998). These treatments can only kill the pathogens of gall surfaces, and cannot inhibit the development of the systematic *A. vitis* in the vascular tissue (xylem) of the vine (Burr et al. 1996). Consequently, there is an obvious need to search for alternative natural antibacterial agents or biopesticides that can be non-toxic and non-polluting to control crown gall grapevine in agriculture applications (Costa *et al.* 2000).

The use of biological control for the management of crown gall disease have become an important issue in recent years and have been receiving attention (Chen et al. 2007). Several studies have been conducted to develop biological treatment to control tumorigenic strains of *A. vitis* using bacterial antagonists (Habbadi et al. 2017; Bazzi et al.

1999; Burr et al. 1997; Chen et al. 2009) or the extracts of some medicinal and aromatic plants (MAP) (Badawy and Abdelgaleil, 2013; Moghaddam et al. 2014). Many essential oils (EO) extracted from MAP have been tested for their antibacterial activity against phytopathogens bacteria showing great promise in the treatment of pathogens, such as *Erwinia amylovora*, *Agrobacterium tumefaciens* (Badawy and Abdelgaleil, 2013; Castilho et al. 2006; Gormez et al. 2015; Iacobellis et al. 2005; Mikicinski and Sobiczewski, 2012) and *Pseudeumonas savastanoi* pv *savastanoi* (Bouaichi et al. 2015). The aim of this work was to evaluate the antibacterial activity *in vitro* of four Moroccan MAP against *A. vitis* and to analyze the constituents of their EO.

Material and methods

Plant Material and Extraction

Fresh plants of four MAP were collected during the flowering stage from different locations in Morocco during the period between February and July 2017 (Table 1). Plants were dried away from direct sunlight at room temperature. The EO extraction was done by hydrodistillation technique using a Clevenger-type apparatus. The EOs obtained were collected by decantation, dried over anhydrous sodium sulfate, weighed and stored in a dark glass bottle at +4°C. The yields of extraction were calculated as the ratio of mass of the EOs to the one of initial plant.

Table 1: Medicinal and aromatic plants studied

Plant species		Sampling site	Plant part studied
Common name	**Scientific name**		
Calamintha	***Satureja calamintha* (L.) Scheele** Order: Lamiales Family: Lamiaceae	Adrej	Aerial parts
French lavender	***Lavandula stoechas* L.** Order: Lamiales Family: *Lamiaceae*	Meknes	Flowers
Rosemary	***Rosmarinus officinalis* L.** Order: Lamiales Family: *Lamiaceae*	Adrej	Aerial parts
Bigarade orange	***Citrus aurantium* L.** Order: Sapindales Family: *Rutaceae*	Meknes	Fruits

Strains and culture conditions

The bacterial strain used in this study is *A. viti*s strain S4 (sequenced strain) isolated from black raspberry in Hungary (Popoff et al. 1984). *A. vitis* S4 was cultivated on MG medium (Moore et al. 2001) (D-mannitol, 5g/L; L-glutamic acid, 2g/L; KH_2PO_4, 0.5g/L; NaCl, 0.2g/L; $MgSO_4 \times 7H_2O$, 0.2g/L; Yeast extract, 0.5g/L; Agar, 15g/L; pH=7) and incubated, for 24 hours, at 28°C.

Gas chromatography analysis (GC-MS)

The chemical composition of the EOs was analyzed using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-5MS capillary column (30m × 0.25mm i.d., film thickness 0.25µm; Agilent Technologies, USA) and coupled to an HP model 5973 mass selective detector. The oven temperature was initially maintained at 50°C and then increased, by 7°C/min, to 300°C. The injector temperature was 290°C. Purified helium was used as the carrier gas, with a flow rate of 1mL/min, and the split ratio was 60:1. Mass spectra were obtained, in EI mode, at 70eV ionization energy and the mass range was from *m/z* 35 to 400. For each EO, a sample of 10µL was diluted in 990µL of pure hexane, and 1µL was injected for the analysis. The device was managed by a computer system type "HP ChemStation Software" G1701BA, version B.01.00, and the data reworks were carried out with the same software. For each compound, the Kovats retention index (RI) was calculated relative to a standard mix of nalkanes between C8 and C26 (Sigma-Aldrich Co.), analyzed under identical conditions. Identification of the constituents was performed by comparing the RI and MS spectra with those reported in the literature (Adams, 2007) and by computer matching with standard reference databases (NIST98, Wiley275, and CNRS libraries).

Antibacterial activity

The determination of the antibacterial activity effect of EO on *A. vitis* was carried out by the disc diffusion method. Sterile Paper disk (5 mm in diameter) were impregnated with 2µL of pure essential oil and transferred into the YPGA medium present in Petri dishes, which had previously seeded by spreading 100µL of *A. vitis* strain S4 suspension adjusted to 10^7 CFU/mL. In the same way sterile distilled water (2 µl) were used as negative control. The filters were placed in Petri dishes, either directly onto the center of the culture medium to evaluate the EO antibacterial activity (aromatogram test), or on the center of the lid to assess the antibacterial activity of the volatile substances (microatmospher test). After incubation at 28°C during 24 hours, the diameter of inhibition zone was measured. And the percentage inhibition was calculated using the following formula (Schultz, 2006):

$$\boldsymbol{Percent\ of\ inhibition}\ \% = [\frac{(\boldsymbol{T1} - \boldsymbol{T2})}{\boldsymbol{T1}}] \times \mathbf{100}$$

T1: diameter of the bacterial load with treatment by sterile distilled water (SDW);
T2: diameter of the bacterial load with treatment by essential oil.

Minimum inhibitory concentration (MIC)

Bacterial growth was analyzed using a Microbiology Bioscreen C Reader (Labsystems®, Helsinki, Finland) according to the manufacturer's instructions. Firstly, a serial of dilution of essential oil of *S. calamintha*, *L. stoechas* and *C. aurantium* were prepared in YPG, supplemented with 0.01% dimethylsulfoxide (DMSO), to obtain EO concentrations ranging from 40mg/mL to 0.075mg/mL (YPG-EO). Bacterial suspensions, from exponential cultures, were inoculated at OD_{600nm} 0.01 in 200µL YPG-EO in Bioscreen honeycomb 100-well sterile plates. Cultures were incubated at 28°C during 3 days, with shaking at medium amplitude. Growth measurements (OD_{600nm}) were taken at 20min intervals. The MIC corresponds to the lowest concentration of EO which resulted in 100% growth inhibition.

Statistical analysis

The significant effect of EOs on growth inhibition of *A. vitis* was evaluated by Analysis of variance (ANOVA1) (factor: treatment), performed with the SPSS 20 statistical software ((IBM Corporation, Somers, NY, USA). The arcsin of the inhibition percentage was used for statistical analysis and was calculated using the formula $Arcsin=\sqrt{(\%I/100)}$, where %I is the rate of bacterial growth inhibition.

Results and discussion

Yield of essential oils

Essential oils obtained by hydrodistillation of the samples (calamintha, rosemary, lavender, and bigarade orange) are light yellow to brown, each with a characteristic odor. Their yields vary with the species. As results, *R. officinalis* has recorded the high yield with 1.65% followed by *L. stoechas* with 0.82%, *S. calamintha* with 0.3% and *C. aurantium* with 0.36% (Fig.1). In comparison with other studies, such as the research work of Boutekedjiret et al. (2003), the yield of rosemary EO obtained by hydrodistillation is 1.2%; they show also that the yield of EO changes by time. Concerning the lavender, the yield of the oil obtained in this work is compatible with study of Msaada et al. (2013) when they show that the yield of *L. stoechas* EO varied from 1.04% to 0.11%. In general, the yield of EO varies considerably from one season to the next, as the age of the bushes and the weather will affect both the quantity and quality of the EO. Moreover, the yield of EO depends also on the species used, the part of plant tested, the stage of plant development, the harvesting area, the harvest time, the climate and the technique of extraction used for isolation of EO (Zrira et al. 2004).

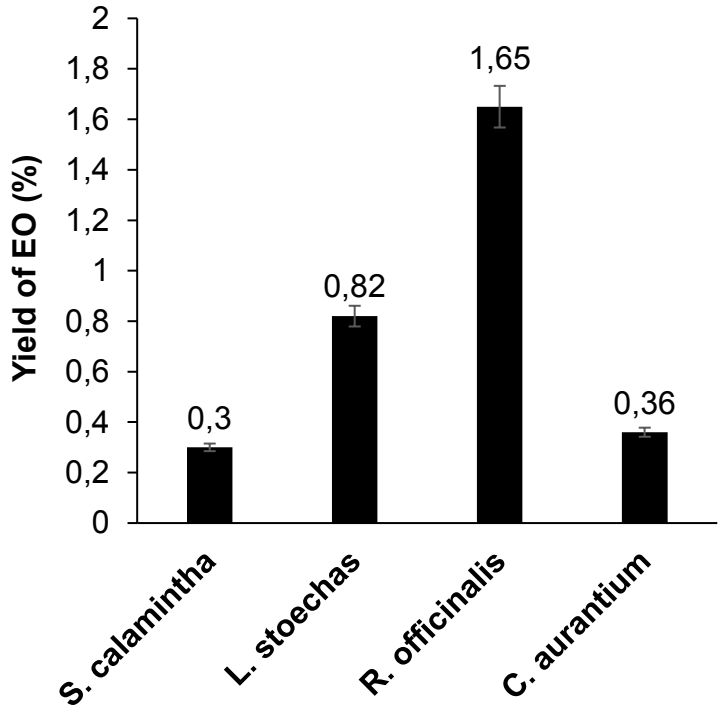

Figure 1: Yield of EO extraction by hydrodistillation

Chemical composition

The main components of the different EO tested in this study are shown in the Table 2 and illustrated in Fig.2. According to gas chromatography (GC-MS) analysis, the studied EO present a different chemical composition containing mainly oxygenated mono and sesquiterpenes, and mono and sesquiterpene hydrocarbons.

For the EO of *R. officinalis*, 19 components were identified, accounting for 100% of the oil. The analysis showed that 1, 8-cineole (48.12%) was the main component in the essential oil of *R. officinalis*. Other major components were identified as α-pinene (13.48%) and camphor (12.7%). The obtained results for this EO are not in accord with that found by Socaci et al. (2008) when they have shown the predominance of α-pinene as a major component of *R. officinalis* essential oil (32.34 - 47.49%) followed by 1.8-cineol (eucalyptol) (15.16 - 18.75%) and camphor (7.70 - 14.66%).

The volatile compounds profile of *L. stoechas* EO is in the GC-MS chromatogram illustrated Fig.2. A total of 36 components, listed in table 2, were detected by GC-MS analysis. Linalool (25.76%), camphor (21.09%), cineole (12.05%) and borneol (10.14%), were the major compounds. This result was also stated by the work of La Bella et al. (2001) and Msaada et al. (2013) when they have found that the dominant components of *L. stoechas* were linalool, camphor, and cineol with the different area depending to the harvest time, the season and other factors (Zrira et al. 2004).

For *C. aurantium* EO, as shown in table 2, 13 volatile compounds accounting for 98.2 % of total EO were detected. The main compounds are linalool (38.81%), limonene (37.93%), and linalool acetate (8.99%). These results are in agreement with those obtained by Pultrini et al (2006).

The EO of *S. calamintha* was characterized by the presence of 29 compounds; the major compounds are borneol (29.01 %), 1. 8-Cineole (21.91 %) and camphor (16.48 %).
These different results in terms of chemical composition and the relative concentration of each constituent found by different studies carried out on the same plant species can be due to different geographical sources, the genotype, soils and climatic characteristics of the regions where the PAM grow (Mohagheghniapour et al. 2018).

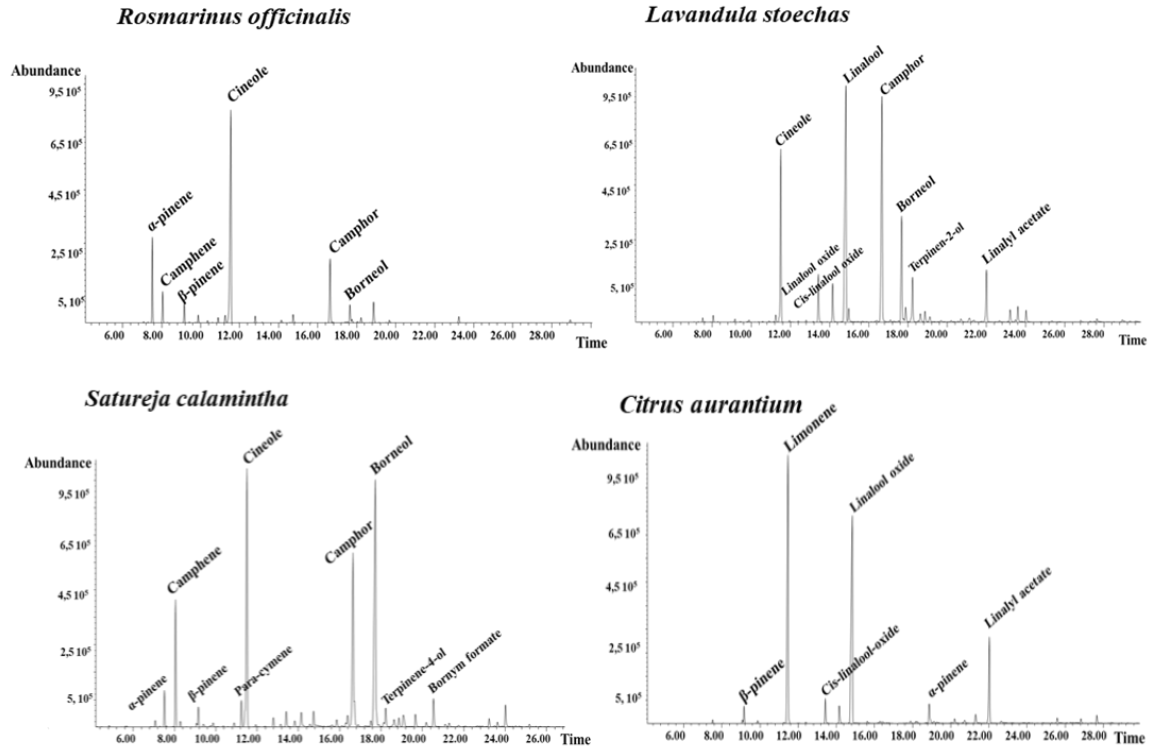

Figure 2: GC-MS chromatograms of the different EOs analyzed in this study (The name of the peaks correspond to the major compounds cited in Table 2).

Table 2: Chemical composition of EO tested in this study

	Compound	RI	% of Essential oil			
			Citrus aurantium	*Lavandula stoechas*	*Satureja calamintha*	*Rosmarinus officinalis*
1	Tricyclene	921	-	-	0,31	-
2	a-Pinene	932	0,30	0,35	1,93	**13,48**
3	Camphene	948	-	0,5	**7,47**	**4,38**
4	Dehydrosabinene	952	-	-	0,29	-
5	Sabinene	971	0,33	-	-	-
6	b- Pinene	976	1,61	0,25	1,11	2,76
7	Myrcene	989	-	0,26	0,3	1,17
8	a-Phellandrene	1006	-	-	-	0,24
9	a-Terpinene	1016	-	-	-	0,69
10	*p*-Cymene	1024	-	-	1,5	1,14
11	Limonene	1029	**37,93**	**-**	0,79	2,3
12	1,8-Cineole	1032	-	**12,05**	**21,91**	**48,12**
13	g-Terpinene	1058	-	0,17	0,52	0,97
14	Cis-linalool-oxide	1070	2,88	3,17	1,28	-
15	Camphenilone	1083	-	-	0,37	-
16	Terpinolene	1084	-	-	-	0,36
17	Trans-Linalool-oxide	1086	2,06	3,67	0,89	-
18	Linalool	1102	**38,81**	**25,76**	1,18	1,48
19	a-Campholenal	1127	-	-	0,41	-
21	Nopinone	1139	-	-	0,26	-
22	Trans-Pinocarveol	1141	-	-	1,31	-
23	Camphor	1149	-	**21,09**	**16,48**	**12,7**
24	Pinocarvone	1162	-	-	0,56	-
25	Borneol	1174	-	**10,14**	**29,01**	3,77
26	Terpinen-4-ol	1181	-	3,42	1,49	0,89
29	a-Terpineol	1195	2,36	1,28	-	3,72
31	Verbenone	1207	-	-	0,75	0,39
32	Trans-Carveol	1219	0,54	0,25	0,29	-
33	Isobornyl formate	1229	-	-	1,75	-
34	Carvone	1244	1,11	-	-	-
35	Linalool acetate	1250	**8,99**	**4,52**	-	-
36	Bornyl acetate	1284	-	-	0,57	0,97
37	Carvacrol	1297	-	1,6	1,15	-
39	Neryl acetate	1358	0,52	-	-	-
40	Geranyl acetate	1378	0,71	-	-	-
41	Trans-Caryophyllene	1419	-	-	-	0,46
44	Spathulenol	1577	-	-	0,82	-
45	Caryophyllene oxide	1582	-	1,31	1,2	-
46	a-Bisabolol	1685	-	-	0,76	-
		Total	**98,2**	**89,79**	**96,7**	**100,0**

Evaluation of the EO antibacterial activity

The antibacterial activity of EOs extracted from four Medicinal and Aromatic Plants (MAPs) was evaluated against *A. vitis* S4 (Fig.3). According to results of aromatogram tests, all the tested EO inhibit the growth of *A. vitis* S4 *in vitro* with differences in the level of activity. The EO of bigarade exhibit an antibacterial activity with a percentage of inhibition equal to 25.88%, french lavander with 16%, calamintha with 13.38%, and rosemary with 7.5%.

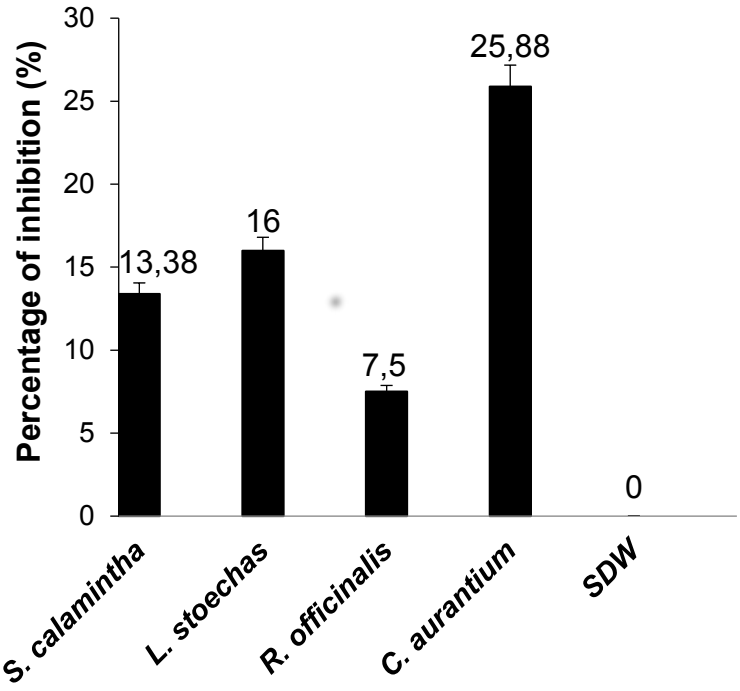

Figure 3: Antibacterial activity of different EOs against *A. vitis* S4 using the aromatogram test.
SDW: Sterile Distilled Water (negative control)

The determination of the MIC was performed for the EO presenting a high percentage of inhibition against *A. vitis* (*C. aurantium*, *S. calamintha* and *L. stoechas*) (Fig.4) by using the micro-dilution method. According to the results, the MIC was determined at 0.3 mg/mL for the EO of *T. vulgaris,* 2.5 mg/mL with *C. aranticum*, 10 mg/mL for *L. stoechas* and 20 mg/mL for *S. calamintha*.

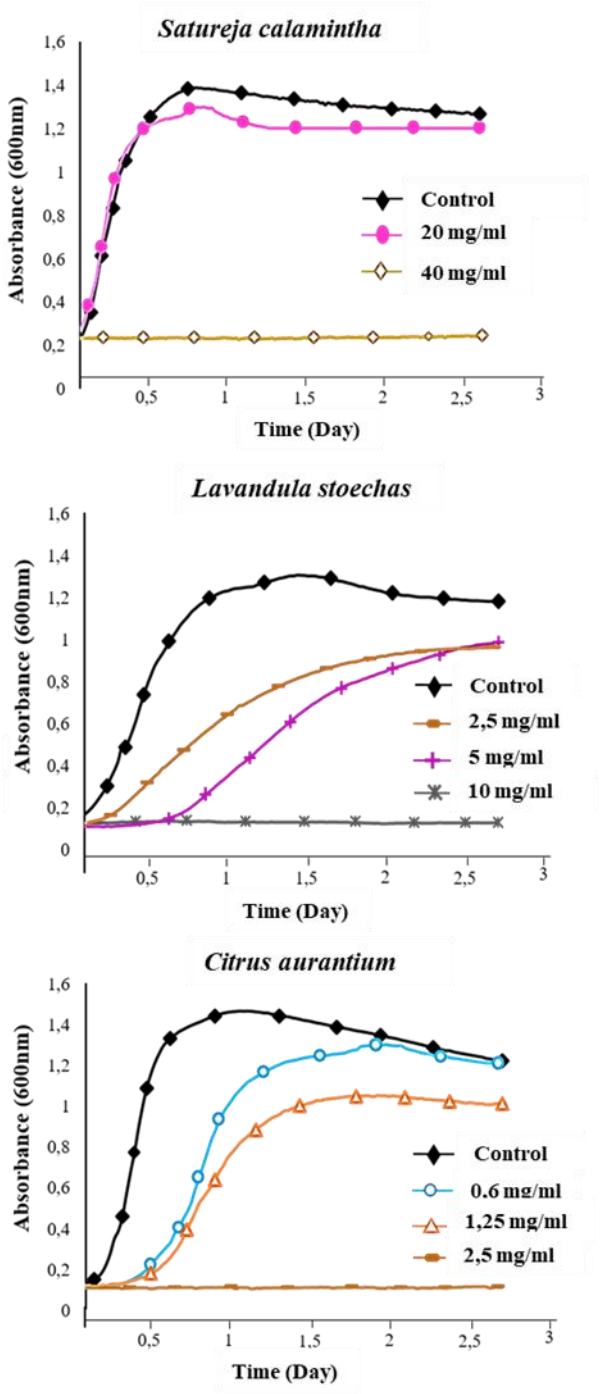

Figure 4: Bacterial growth of *A. vitis* according to different concentrations of essential oil during three days. MIC of *C. aurantium*= 2.5 mg/mL; MIC of *L. stoechas*= 10 mg/mL and MIC of *S. calmintha*= 40 mg/mL.

The antibacterial activity of these EO against others phytopathogens bacteria has already been reported. The effectiveness of EO of bigarade, was tested against many human pathogenic microorganisms that present a resistance for several antibiotic such as, *Acinetobacter baumanii*, *Candida albicans*, *Enterobacter spp*. and *Pseudomonas aeruginosa* (Ouedrhiri et al. 2015; Hammer et al. 1999) and against phytopathogens bacteria such as *Erwinia herbicola* and *Pseudomonas putida* (Pandey et al. 2012). Mohammad et al (2019) have also indicated the antibacterial activity and the effectiveness of the *C. aurantium* EO extracted from small green branches with limonene as the main constituent against *Agrobacterium tumefaciens*, *Dickeya solani* and *Erwinia amylovora*.

The EO of lavender which gave a percentage of inhibition of S4 equal to 16%, it showed an antimicrobial activity against different microorganisms (Cavanagh and Wilkinson, 2002). It was tested against tomato late blight disease agent, *Phytophtora infestans* (Soylu et al. 2005). Furthermore, Pattnaik et al. (1997) found that linalool, a major compound of lavender oil, among 18 bacteria tested, it could inhibit 17 bacteria (Gram negative and positive).

In the previous researches, borneol, 1,8-cineole, linalool, α-pinene, limonene and camphor has been reported to have an antimicrobial activity against several phytopathogens and human pathogens. In the study work of Tabanca et al. (2001), they show that the borenol have an inhibitory effect on Gram- and Gram+ pathogenic microorganisms. Hendry et al. (2009) have noted that the 1,8- cineole exhibit an antimicrobial activity *Staphylococcus aureus* (methicillin-resistant), *Escherichia coli* and *Candida albicans*, and biofilm cultures of *Pseudomonas aeruginosa*. For the compound α-pinene, Dai et al. (2013) and Da Sila et al. (2012) explain that the use of this compound presents a high antimicrobial activity alone and in synergy with the other minor compounds of the tested EOs. Park et al. (2012) demonstrate that the linalool exhibited strong antimicrobial activity against periodontopathic and cariogenic bacteria. However, their concentration should be kept below 0.4 mg/ml if they are to be used as components of toothpaste or gargling solution. Moreover, other compounds with antimicrobial activity against periodontopathic and cariogenic bacteria should be used in combination. For he limonene compound, Han and Chen (2019) confirmed in their study that this compound present antimicrobial susceptibility and mechanism against *Listeria monocytogenes* targeting the cell membrane permeability. It has been demonstrated also that the camphor ha natural antimicrobial function against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, and *Bacillus thuringiensis*; characterized specifically by a high antifungal activity (Pen *et al.,* 2012).

The antibacterial activity can be correlated to a number of terpenoids and phenolic compounds presents in the EOs. Several compounds have been characterized by their antibacterial activity. Among the compounds having a great success in the inhibition of bacterial growth there are the thymol and carvacrol. In our pervious study (Habbadi et al., 2017), these two compounds (carvacrol and thymol), which are present in oregano and thyme EOs, show a very height activity against *A. vitis in vitro* and *in planta* in comparison with the essential oils tested in this research work. They are the most documented active components of EO in literature (Burt S. 2004).

In general, the antimicrobial activity of EOs depends on their chemical constituents and the virulence of the phytopathogen agent. However, the antimicrobial activity and mechanism of action of each constituent as antimicrobial has not been fully elucidated. This is complicated by the fact that there are a large number of chemical compounds present in Eos and also the degree of sensitivity of the bacterial tested strain ((Dorman and Deans, 2000; Kalemba and Kunicka, 2003; Zomorodian et al., 2012). It is also important that thee EOs had the same effectiveness on Gram positive bacteria as well on Gram negative bacteria. However, Gram negative bacteria appear to be less sensitive to their action (Burt, 2004; Zomorodian et al., 2012) due to the specific structure of their cell wall (Burt, 2004).

The major compounds presented strongly in EOs may demonstrate the difference in biological activities between EOs, but it is necessary to signal that other compounds in lower amount may also contribute to improve the activity provided by each EO. Thus, one may take into consideration that the inherent activity of an oil can be due to the synergistic effects and interaction between different constituents present in the EO (Dorman and Deans. 2000).

Conclusion

In this study, the chemical composition and antibacterial activity against *A. vitis* S4 of the EO of four MAPs from different locations in Morocco were investigated. The results demonstrate the effectiveness of thyme EO against *A. vitis* S4. Our results suggest that the EO of *T. vulgaris* may act as an alternative to synthetic bactericides to control the causal agent of crown gall of grapevine. Therefore, the MAPs can be a reliable source of bioactive organic substances that can be used as a less expensive alternative to control phytopathogens microorganisms and in the same time more respectful of human health and environment.

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References

Adam K., Sivropoulou A., Kokkini S., Lanaras T., Arsenakis M. (1998). Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry. Vol. 46(5). p.1739-1745.

Adams R.P. (2007). Identification of essential oil components by gas chromatograph/mass spectrometry. 4th ed. Allured Publishing Corporation, Carol Stream, USA.

Ahmad A., Khan A., Akhtar F., Yousuf S., Xess I., Khan L.A., Manzoor N. (2011). Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. European Journal of Clinical Microbiology. Vol. 30. p. 41-50.

Armijo G., Schlechter R., Agurto M., Munoz D., Nunez C., Arce-Johnson P. (2016). Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios. Frontiers in Plant Science. doi: 10.3389/fpls.2016.00382.

Badawy M.E.I., Abdelgaleil S.A.M. (2013). Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Industrial Crops Products*. Vol.* 52. p. 776-782.

Bazzi C., Alexandrova M., Stefani E., Anaclerio., Burr T.J. (1999). Biological control of *Agrobacterium vitis* using non-tumorigenic agrobacteria. Vitis. Vol. 38 (1). p. 31-35.

Boruga O., Jianu C., Misca C., Golet I., Horhat F.G. (2014). Thymus vulgaris essential oil: chemical composition and antimicrobial activity. Journal of Medicine and Life. Vol. 7(3). p. 56-60.

Bouaichi A., Benkirane R., Habbadi K., Benbouazza A., Achbani EL.H. (2015). Antibacterial activity of the essential oils from medicinal plants against the growth of *Pseudomonas savastanoi* pv. *savastanoi* causal agent of olive knot. Journal of Agriculture and Veterinary Sciences. Vol. 8(12). p. 41-45.

Boutekedjiret C., Bentahar F., Belabbes R., Bessiere J.M. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour and Fragrance Journal. Vol. 18. p. 481-484.

Burr T.J. (2004). Grape crown gall biology and strategies for control. *Foundation Plant Services*, *Grape Program Newsletter*, *University of California-Davis*. p. 16-18.

Burr T.J., Bazzi C., Sul S., Otten L. (1998). Crown gall of grape: biology of *Agrobacterium vitis* and the development of disease control strategies. Plant Disease. Vol. 82 (12). p. 1288-1297.

Burr T.J., L Otten. (1999). Crown gall of grape: biology and disease management. Annual Review of Phytopathology. Vol. 37. p. 53-80.
Burr T.J., Reid C.L., Splittstoesser D.F., Yoshimura M. (1996). Effect of heat treatment on grape bud mortality and survival of *Agrobacterium vitis in vitro* and in dormant grape cuttings. American Journal of Enology and Viticulture*. Vol.* 47(2). p. 119-123.
Burr T.J., Reid C.L., Taglicti E., Bazzi C., Süle S. (1997). Biological control of grape crown gall by strain F2/5 is not associated with agrocin production or competition for attachment site on grape cells. Phytopathology. Vol. 87. p. 706-711.
Castilho P., Liu K., Rodrigues A.I., Feio S., Tomo F., Casanova J. (2007). Composition and antimicrobial activity of the essential oil of *Clinopodium ascendens* (Jordan) Sampaio from Madeira. Flavour and Fragrance Journal. Vol. 22. p. 139-144.
Chen F., Guo Y.B., Wang J.H., Li J.Y., Wang H.M. (2007). Biological control of grape gall by *Rahnella aquatilis* HX2. Plant Disease. Vol. 91. p. 957-963.
Chen X.H., Scholz R., Borriss M., Junge H., Mogel G., Kunz S., Borriss R. (2009). Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. Journal of Biotechnology. Vol. 140. p. 38-44.
Costa T.R., Fernandes O.F.L., Santos S.C., Oliveira C.M.A., Lião L.M., Ferri P.H. (2000). Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. Journal of Ethnopharmacology. Vol. 72. p. 111-117.
Dorman H.J.D., Deans G.S. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology. Vol. 88. p. 308-316.
Gormez A., Bozari S., Yanmis D., Gulluce M., Sahin F., Agar G. (2015). Chemical composition and antibacterial activity of essential oils of two species of lamiaceae against phytopathogenic Bacteria. Polish Journal of Microbiology. Vol. 64(2). p. 121-127.
Habbadi K., Benkirane R., Benbouazza A., Bouaichi A., Maafa I., Chapulliot D., Achbani El.H. (2017). Biological Control of Grapevine Crown Gall Caused by *Allorhizobium vitis* using Bacterial Antagonists. International Journal of Science and Research. Vol. 6(6). p. 1390-1397.
Hammer k., Carson C., riley T. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. Vol. 86. p. 985-990.
Herlach T.C., Zhang H.S., Ried C.L., Carle S.A., Zheng D., Basaran P., Thaker M., Burr A.T., Burr T.J. (2001). Mutations that affect *Agrobacterium vitis*-induced grape necrosis also alter its ability to cause a hypersensitive response on tobacco. Phytopathology. Vol. 91. p. 966-972.
Iacobellis N.S., Lo Cantore P., Capasso F., Senatore F. (2005). Antibacterial activity of *Cuminum cyminum* L, and *Carum carvi* L, essential oils. Journal of Agricultural and Food Chemistry. Vol. 53(1). p. 57-61.
Karami-osboo R., Khodaverdi M., Aliakbari F. (2010). Antibacterial effect of effective compounds of Satureja hortensis and Thymus vulgaris essential oil against *Erwinia amylovora*. Journal of Agricultural Science and Technology. Vol. 12. p. 35-45.
Kawaguchi A. (2009). Studies on the diagnosis and biological control of grapevine crown gall and phylogenetic analysis of tumorigenic *Rhizobium vitis. Journal of General Plant Pathology*. Vol. 75. p. 462.
Kuzmanovic N., Gasic K., Ivanovic M., Prokic A., Obradovic A. (2012). Identification of *Agrobacterium vitis* as a causal agent of grapevine crown gall in Serbia. *Archive of Biological Sciences*. Vol. 64(4). p. 1487-1494.

La Bella S., Tuttolomondo T., Dugo G., Ruberto G., Leto C., Napoli E.M., Potorti A.G., Fede M.R., Virga G., Leone R., D'Anna E., Licata M. (2015). Composition and variability of the essential oil of the flowers of Lavandula stoechas from various geographical sources. Natural Product Communications. Vol. 10(11). p. 2001-4.
Lacroix B., Citovsky V. (2013). Crown gall tumors. Brenner's Encyclopedia of Genetics. Vol. 2(2). p. 236-239.
Mahboubi M., Haghi G. (2008). Antibacterial activity and chemical composition of *Mentha pulegium* L. essential oil. Journal of Ethnopharmacology. Vol. 119(2). p. 325-327.
McManus P.S., Stockwell V.O., Sundin G.W., Jones A.L. (2002). Antibiotic use in plant agriculture. Annuak Review of Phytopathology. Vol. 40. p. 443-465.
Mikicinski A., Sobiczewski S. (2012). Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. Journal of Plant Protection Research. Vol. 52(4). p. 467-471.
Moghaddam M., Alymanesh M.R., Mehdizadech L., Mirzaei H., Ghasemi P.A. (2014). Chemical composition and antibacterial activity of essential oil of *Ocimum ciliatum*, as a new source of methyl chavicol, against ten phytopathogens. Industrial Crops Products. *Vol.* 59. p. 144-148.
Mohagheghniapour A., Saharkhiz M.J., Golmakani M.T. (2018). Variations in chemical compositions of essential oil from sour orange (*Citrus aurantium* L.) blossoms by different isolation methods. Sustainable Chemistry and Pharmacy. Vol. 10. p. 118–124.
Moore L.W., Bouzar H., Burr T.J. (2001). *Agrobacterium*. In: Schaad N.W., Jones J.B, Chun W. (eds). Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed., p.: 17-35. APS Press, St. Paul, MN, USA.
Mousavi S.A., Osterman J., Wahlberg N., Nesme X., Lavire C., Vial L., Paulin L., de Lajudie P. (2014). Phylogeny of the *Rhizobium-Allorhizobium-Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. Systematic and Applied Microbiology. Vol. 37. p. 208-215.
Mousavi S.A., Willems A., Nesme X., de Lajudie P., Lindstrom K. (2015). Revised phylogeny of Rhizobiaceae: proposal of the delineation of *Pararhizobium* gen. nov., and 13 new species combinations. Systematic and Applied Microbiology. Vol. 38. p. 84-90.
Msaada K., Salem N., Tammar S., Hammami M., Saharkhiz M.J., Debiche N., Limam F., Marzouk B. (2013). Essential oil composition of *Lavandula dentate*, *L. stoechas* and *L. multifidi* cultivated in Tunisia. Journal of Essential Oil Bearing Plants. Vol.15(6). p. 1030-1039.
Nostro A., Papalia T. (2012). Antimicrobial activity of carvacrol: current progress and future prospectives. Recent Patents on Anti-Infective Drug Discovery. Vol. 7. p. 28-35.
Ophel K., Kerr A. (1990). *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. International Journal of Systematic and Evolutionary Microbiology. Vol. 40. p. 236-241.
Ouedrhiri O., Bouhdid S., Balouiri M., El ouali Lalami A., Moja S., Ouazzani Chadi F., Greche H. (2015). Chemical composition of Citrus aurantium L. leaves and zest essential oils, their antioxidant, antibacterial single and combined activity. Journal of Chemical and Pharmaceutical Research. Vol. 7(1). p. 78-84.
Pandey A.K., Singh P., Palni U.T., Tripathi N.N. (2012). *In-vitro* antibacterial activities of the essential oils of aromatic plants against *Erwinia herbicola* (Lohins) and *Pseudomonas putida* (Kris Hamilton). Journal of the Serbian Chemical Society. Vol. 77(3). p. 313-323.

Pattnaik S., Subramanyam V.R., Papaji M., Kole C.R. (1997). Antibacterial and antifungal activity of aromatic constituants of essential oil. Microbios. Vol. 89. p. 39-46.

Poncet C., Antonini C., Bettachini A., Hericher D., Pionnat S., Simonini L., Dessaux Y., Nesme X. (1996). Impact of the crown gall disease on vigour and yield of rose trees. Acta Horticulturae. doi: 10.17660/ActaHortic.1996.424.39

Popoff M.Y., Kersters K., Kiredjian M., Miras I., Coynault C. (1984). Position taxonomique de souches d'*Agrobacterium* d'origine hospitalière. Annales de l'Institut Pasteur/Virologie. Vol. 135. p. 427-442.

Pultrini A.D.M., Galindo L.A., Costa M. (2005). Effects of the essential oil from *Citrus auranticium* L. in experimental anxiety models in mice. Life Science. Vol. 78(15). p. 1720-1725.

Sbayou H., Oubrim N., Bouchrif B., Ababou B., Boukachabi K., Amghar S. (2014). Chemical composition and antibacterial activity of essential oil of *Origanum compactum* against foodborne bacteria. International Journal of Engineering & Technology. Vol. 3. p. 3562-3567.

Schroth M.N., McCain A.H., Foott J.H., Huisman O.C. (1988). Reduction in yield and vigor of grapevine caused by crown gall disease. Plant Disease. Vol. 72. p. 241-246.

Schultz D.L. (2006). Biology 155 General Biology I Laboratory Supplement. p. 78.

Smith E.F., Townsend C.O. (1907). A plant-tumor of bacterial origin. Science (New York). Vol. 25. p. 671-673.

Soylu E.M., Soylu S., Kurt S. (2005). Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophtora infestans*. Mycopathologia. Vol. 161(2). p.119-128.

Thomashow L.S., Reeves S., Thomashow M.F. (1984). Crown gall oncogenesis: evidence that a T-DNA gene from the *Agrobacterium* Ti plasmid pTiA6 encodes an enzyme that catalyzes synthesis of indoleacetic acid. Proceedings of the National Academy of Sciences. Vol. 81. p. 5071-5075.

Zrira S., Bessiere J.M., Menut C., Elamrani A., Benjilali B. (2004). Chemical composition of the essential oil of nine eucalyptus species growing in Morocco. Flavour and Fragrance Journal. Vol. 192. p. 172-175.