Malaysian Journal of Microbiology, Vol 18(1) 2022, pp. 58-67
DOI: http://dx.doi.org/10.21161/mjm.211239

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Evaluation of antibacterial activity of essential oils extracted from *Thymus satureioides* and *Mentha pulegium* against antibiotic resistance bacteria from raw sheep milk

Fatima Bouazza[1]*, Rachida Hassikou[1], Lamiae Amallah[1], Jihane Ennadir[1] and Khadija Khedid[2]

[1]Laboratory of Botany and Enhancement of Plant and Fungal Resources, Department of Biology, Faculty of Sciences, University Mohammed V-Rabat, Avenue Ibn Battouta, Rabat, Morocco.
[2]Laboratory of Medical Bacteriology, National Institute of Hygiene, Avenue Ibn Batouta, Rabat, Morocco.
Email: fatima.leader2020@gmail.com

Received 20 July 2021; Received in revised form 2 October 2021; Accepted 2 December 2021

ABSTRACT

Aims: This study evaluated *in vitro* resistance and susceptibility of Enterobacteriaceae (*Escherichia coli* and *Klebsiella oxytoca* strains) and Staphylococci strains, isolated from sheep's milk, against antibiotics and essential oils from *Thymus satureioides* and *Mentha pulegium*.
Methodology and results: Antibiotic resistance tests were done using disc diffusion while essential oils were extracted by steam distillation and yields were calculated relative to plant dry matter. Gas chromatography-mass spectrometry (GC-MS) was used to analyze each oil's chemical composition. Amoxicillin + Clavulanic acid (AMC), Cefotaxime (CTX), Cefoxitine (FOX), Nalidixic acid (NA), Gentamicin (CN), Ciprofloxacin (CIP) and Ofloxacin (OFX) were very effective against the *E. coli* strains tested. Half of the strains were resistant to AMC, 60% to Ticarcillin (TIC) and 80% to Tetracycline (TE). *Klebsiella oxytoca* was resistant against AMC, FOX and TIC (100%). Antibiotic-resistant testing on Staphylococci strains indicated *Staphylococcus capitis* and *S. chromogenes* as the most sensitive. *Staphylococcus aureus*, *S. xylosus* and *S. cohnii ureal* exhibited less resistance to Oxacilin (OX), TE, Pristinamycin (PT), Erythromycin (E) and Penicillin (P). *Mentha pulegium* resulted in a higher yield of essential oil of 3.2% oil compared to *T. satureioides* with only 1.85% yield. The monoterpene oxygenated derivatives, monoterpene hydrocarbons and phenols are found in essential oil extracts. *Thymus satureioides* essential oil had high antibacterial activity even at low concentrations (0.2; 0.55 g/mL). The minimal bactericidal concentration (MBC) values indicate that the essential oils from the plants analyzed had bactericidal effects on all strains tested and are similar to the minimal inhibitory concentration (MIC) values.
Conclusion, significance and impact of study: The high antibacterial properties of these medicinal plants, against bacteria isolated from sheep's milk, provide an opportunity to use these medicinal plants in the breeding sector, as additives and preservatives in the dairy industry.

Keywords: Antibiotic resistance, medicinal plants, essential oils, Enterobacteriaceae, Staphylococci, sheep milk

INTRODUCTION

Antibiotics are among the most successful drugs used to cure human and animal infections caused by pathogenic bacteria (WHO, 2020). Their use in livestock farming becomes inevitable as they are essential to treat of disease, disease prevention, modification of physiological functions, improvement of growth and productivity, as well as for ensuring food security (Falowo and Akimoladun, 2019). Unfortunately, the prevalence of antibiotic resistance among foodborne pathogens has increased in recent decades (Olaimat *et al.*, 2018). The number of antibiotic-resistant Gram-negative and Gram-positive bacteria has also increased (Piccirilli *et al.*, 2019) and several studies on Enterobacteriaceae and Staphylococci resistance against antibiotics have been published (Oliva *et al.*, 2018; Singh *et al.*, 2018). According to Mensah *et al.* (2014), in West Africa, only certain contaminants such as contamination by microorganisms (bacteria), pesticide residues and aflatoxin residues are perceived as dangers for the consumer and classified as a major threat to the environment public health (Thornber *et al.*, 2019; Zainab *et al.*, 2020). In Turkey, an earlier study also found beta-lactam antibiotic residues to be prevalent in milk (Yalçin *et al.*, 2020). Evidence shows that resistant strains of pathogens can be transmitted to humans through food (Loayza *et al.*, 2020).

Aromatic and medicinal plants and essential oils are natural products. They are used traditionally as food flavoring and preservatives (Jiofack *et al.*, 2010). Many

*Corresponding author

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

research groups were interested in the benefits of plant extracts especially essential oils. These latter are well-known for their bioactive properties, which include fungicide and bactericide activity, as well as anti-inflammatory, antiviral and antioxidant capabilities (Lakhdar *et al.*, 2015).

In promoting food safety and prudent use of antibiotics to reduce the risks of antibiotic resistance (Caniça et *al*., 2019), the search for a natural alternative to treat herds will be of great help to protect consumers in particular and public health. It is in this context that the present study was conducted, the aim of which was to test the antibiotic resistance of Enterobacteriaceae, and Staphylococci strains isolated from raw milk of Sardi sheep breed, collected manually from six regions in Morocco. It aims to assess *in vitro* antibiotics resistance of *Escherichia coli*, *Klebsiella oxytoca* and five Staphylococci strains isolated from sheep milk using antibiotic discs as well as to evaluate the antibacterial activity of essential oils extracted from *Thymus satureioides* and *Mentha pulegium*.

MATERIALS AND METHODS

Identification of bacterial isolates

The isolates of Enterobacteriaceae (ten *E. coli* and one *K. oxytoca*) and Staphylococci (five strains) were cultivated on Desoxycholate Lactose (DL) Agar (Oxoid, England culture medium) and Baird Parker Agar culture media (with egg yolk and potassium tellurite). The cell morphology, Gram staining and catalase test (Deb *et al.*, 2020) of all isolates were performed. Cell morphology was observed by phase-contrast microscopy, while identification of Enterobacteriaceae strains was done by determining the biochemical profile using API 20E kit (bioMérieux, France), according to the manufacturer's instructions. Identification of each isolate was obtained using the API Plus software. In this study, *E coli* clinical isolates 1 and 2 were isolated from patients, *E. coli* ATCCS, *E. coli* (EHEC) O157 and *E. coli* ATCC 25922 were used as reference strains obtained from the National Institute of Hygiene in Rabat.

Antibiotic susceptibility testing

Antibiotic susceptibility patterns were evaluated by the solid medium diffusion method using antibiotic discs following CLSI recommendations (CLSI, 2018). The Enterobacteriaceae isolates were subjected to Amoxicillin + Clavulanic Acid (AMC) 30 µg, Cefotaxime (CTX) 30 µg, Cefoxitin (FOX) 30 µg, Trimethoprim + Sulfamethoxazole (SXT) 25 µg, Cephalothin (KF) 30 µg, Amoxicillin (AML) 25 µg, Ticarcillin (TIC) 75 µg, Ceftriaxone (CRO) 30 µg, Nalidixic acid (NA) 30 µg, Gentamycin (CN) 15 µg, Ciprofloxacin (CIP) 5 µg, Tetracycline (TE) 30 µg and Ofloxacin (OFX) 5 µg (OXOID, 2008). On the other hand, Staphylococci isolates were subjected to Oxacillin (OX) 5 µg, Pristinamycin (PT) 15 µg, Erythromycin (E) 15 µg, Teicoplanin (TEC) 30 µg, Penicillin (P) 5 µg, Vancomycin (VA) 30 µg, Ofloxacin (OFX) 5 µg, Lincomycin (MY) 15 µg, Trimethoprim + Sulfamethoxazole (SXT) 25 µg, Gentamicin (CN) 15 µg, Kanamycin (K) 30 µg, Tetracycline (TE) 30 µg, Cefoxitin (FOX) 30 µg and Rifampicin (RD) 30 µg (OXOID, 2008).

A 100 L volume of overnight bacterial suspension (106 CFU/mL) incubated at 30 °C was placed on the surface of Mueller Hinton agar with a turbidity of 0.5 McFarland (108 CFU/mL). After drying the medium for 15 min at 30 °C, sterile antibiotic-containing discs were placed on the agar. After 24-h of incubation at 30 °C, the presence of an inhibitory zone surrounding the discs was observed (OXOID, 2008). The diameter of inhibition zones was measured and quantified using CLSI guidelines for antibiotic breakpoints.

Plant materials

Aerial parts of *T. satureioides* were collected from Imouzzar Idaoutanan in Agadir (southern Morocco) and *M. pulegium* from the Ouazzane regions in June 2014. The species identities were confirmed by Dr. Aafi from the Forestry Research Center at the High Commission for Water and Forests and the Fight against Desertification (HCEFLCD) in Rabat. Specimens of these plants were kept in the herbarium at the same center.

Extraction of essential oil

The essential oils were extracted using the steam distillation method with a Clevenger-type apparatus (Majolo *et al.*, 2016). Three distillations were carried out by boiling for one hour and thirty minutes. A fresh plant weighing about 200 g was put in 1 L of water inside a 2 L flask. Three samples of plants (30 g) were dried in an oven at 60 °C for 48 h to assess and quantify the essential oil yield relative to dry plant matter. After extraction, the essential oils were kept at 4 °C in the dark with anhydrous sodium sulfate (Amarti *et al.*, 2010). The yield of essential oil is the ratio of the weight of the essential oil extracted against the weight of dry plant matter used for the extraction. It is expressed as a percentage and calculated according to the following formula:

$$R = (PHE/Pmv) \times 100$$

R: Yield of essential oil in %
PHE: Weight of essential oil in g
Pmv: Weight of dry plant matter in g

Evaluation of the antibacterial activity of essential oils

Determination of MIC of essential oils against bacterial strains was done by microtiter technique with flat bottom sterile plates (Bio-Rad), as described by Eloff (1998); tetrazolium (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, St. Louis, MO) was used as an indicator of cell viability.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

A total of 100 µL of Brain Heart Infusion (BHI) was deposited in each well and 90 µL of essential oil mixed with Tween 80 or successive dilution of ½ was added into each well. Each well was then inoculated with 10 µL of a microbial suspension (equivalent to 10^6 cells/mL).After incubation at 37 °C for 24 h, 10 µL of the MTT solution, freshly prepared with 0.4 mg/mL of MTT in sterile saline, was added into each well. The plate was incubated again for 10 to 30 min at 37 °C. The wells which showed blue-violet color indicate growth occurred. Bacteria strains that had been tested in a culture medium absent of essential oil extract were prepared in isolated wells as negative controls.

The minimal bactericidal concentration (MBC) was determined by streaking 100 µL of the wells' contents at a concentration greater than or equal to the MIC in the dilution series previously established on nutrient agar. MBC was determined after incubation for 24 h at 37 °C. MBC is the lowest concentration that completely inhibits bacterial growth (Gadisa *et al.*, 2019).

Chemical composition of essential oils

Chromatographic analysis was performed using gas chromatography with electronic pressure control type Hewlett Packard (HP 6890 series) equipped with capillary column HP-5(30 m × 0.25 mm), with the film thickness of 0.25 µm, an FID detector set at 260 °C and fed with a mixture of gases and an H_2/Air split-splitless injector set at 275 °C. The injection mode was split at 1/50 ratio. The gas used was nitrogen with a flow rate of 1.7 mL/min. The column temperature was programmed to increase from 50 to 250 °C at 4 °C/min. The device was controlled using computer system type "HPChemStation" that manages the operation of the device and monitors the chromatographic analysis. Kovats Retention Index (IK) and GC-MS analysis were used to identify each component, utilizing gas chromatography (HP 6890 series) coupled to a mass spectrometer (HP 5973 series). Fragmentation was performed by electron impact at 70 eV. The column used was a HP-5MS capillary column (30 m × 0.25 mm), with film thickness of 0.25 µm. The column temperature was programmed to increase from 50 to 250 °C at 4 °C/min. The carrier gas was helium with a flow rate of 1.5 mL/min. The injection mode was split at a 1/70 ratio. The device was connected to a computer system that manages a library of mass spectra NIST 98 (Amarti *et al.*, 2010).

RESULTS

Antibiotic susceptibility test

Antibiogram of Enterobacteriaceae and Staphylococci isolated from sheep milk are shown in Tables 1, 2 and 3. Based on Table 1, the antibiotic susceptibility test performed on *E. coli* strains isolated from raw sheep milk revealed that all test subject strains of *E. coli* are 100% sensitive to Amoxicillin + Clavulanic acid (AMC), Cefotaxime (CTX), Cefoxitin (FOX), Nalidixic acid (NA), Gentamicin (CN), Ciprofloxacin (CIP) and Ofloxacin (OFX). Fifty percent of the strains are resistant to Trimethoprim + Sulfamethoxazole (SXT), 60% of them are resistant to Amoxicillin (AML), Ticarcillin (TIC) and 80% of them are resistant to Tetracycline (TE).

According to the results shown in Table 2, *Klebsiella oxytoca* strain is susceptible to Cefotaxime (CTX), Trimethoprim + Sulfamethoxazole (SXT), Cefalotin (KF), Amoxicillin (AML), Ceftriaxone (CRO), Nalidixic acid (NA), Ciprofloxacin (CIP) and Ofloxacin (OFX) but resistant to Amoxicillin + Clavulanic acid (AMC), Ofloxacin (FOX) and Ticarcillin (TIC).

Antibiotic resistance test performed on *Staphylococcus* sp. isolates revealed that *S. chromogene* and *S. capitis* are the most sensitive to the antibiotics tested (Table 3). While *S. aureus*, *S. xylosus* and *S. cohnii ureal* have shown low resistance to Oxacilin (OX), Tetracycline (TE), Pristinamycin (PT), Erytromycin (E) and Penicilin (P). The maximum percentage resistance in Staphylococci is 20%. The rate of resistance in *Staphylococcus cohnii ureal* to the 14 antibiotics studied is equal to 21.4% (resistant to 3 out of 14 antibiotics).

Table 1: Sensitivity test of *E. coli* strains isolated from raw sheep milk to the antibiotics tested.

Strains	AMC	CTX	FOX	SXT	KF	AML	TIC	CRO	NA	CN	CIP	TE	OFX
E. coli 1	S	S	S	R	I	R	R	S	S	S	S	R	S
E. coli 2	S	S	S	R	I	R	R	S	S	S	S	R	S
E. coli 3	S	S	S	R	S	R	R	S	S	S	S	R	S
E. coli 4	S	S	S	R	R	R	R	R	S	S	S	R	S
E. coli 5	S	S	S	S	S	S	S	R	S	S	S	S	S
E. coli 6	S	S	S	S	I	R	R	S	S	S	S	R	S
E. coli 7	S	S	S	R	R	R	R	S	S	S	S	R	S
E. coli 8	S	S	S	S	I	I	S	S	S	S	S	S	S
E. coli 9	S	S	S	S	S	S	S	S	S	S	S	R	S
E. coli 10	S	S	S	S	S	S	S	I	S	S	S	R	S
% of resistance	0	0	0	50	20	60	60	20	0	0	0	80	0

S: Sensitive; I: Intermediate; R: Resistant

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Sensitivity test of *K. oxytoca* isolated from raw sheep milk to the antibiotics tested.

Strains	AMC	CTX	FOX	SXT	KF	AML	TIC	CRO	NA	CN	CIP	TE	OFX
Klebsiella oxytoca	R	S	R	S	S	S	R	S	S	S	S	I	S
% of resistance	100	0	100	0	0	0	100	0	0	0	0	0	0

S: Sensitive; I: Intermediate; R: Resistant

Table 3: Sensitivity test of *Staphylococcus* sp. isolated from raw sheep milk to the antibiotics tested.

Strains	OX	PT	E	TEC	P	XA	OFX	MY	SXT	CN	K	TE	FOX	RD
S. aureus	R	S	S	S	S	S	S	S	S	S	S	S	S	S
S. xylosus	S	S	S	S	S	S	S	S	S	S	S	R	S	S
S capitis	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. cohnii ureal	S	R	R	S	R	S	S	S	S	S	S	S	S	S
S. chromogenes	S	S	S	S	S	S	S	S	S	S	S	S	S	S
% of resistance	20	20	20	0	20	0	0	0	0	0	0	20	0	0

S: Sensitive; I: Intermediate; R: Resistant

Antibacterial activity of essential oils

The average yield of essential oils was calculated in mL relative to 100 g of dry plant matter. The yields were 1.85% for *T. satureioides* and 3.2% for *M. pulegium*. The results indicated that *M. pulegium* can yield more essential oils than *T. satureioides*. Accordingly, it should be worth considering subject to the good performance of antibacterial activity.

Results of the antibacterial activity test of both essential oils used are summarized in Figures 1 and 2. Note that both essential oils have inhibitory activity against the bacteria tested. The figures show that the essential oils have inhibitory activity on bacterial strains tested with a stronger action by *T. satureioides* essential oil at minimal concentrations (less than 1 µg/mL) on the Enterobacteriaceae and Staphylococci strains.

Escherichia coli (EHEC) O157 is the most sensitive strain to the inhibitory effect of these two essential oils. It was completely inhibited from the minimum concentration of 0.21 µg/mL of *T. satureioides* and 0.9 µg/mL of *M. pulegium* essential oil, whereas *E. coli* ATCC 25922 is the most resistant strain. The *E. coli* ATCC 25922 was only inhibited at a higher concentration of 0.55 µg/mL of *T. satureioides* and 6 µg/mL of *M. pulegium* essential oil (Figure 1).

Staphylococcus strains have also shown sensitivity towards the essential oils tested in this study. Concentrations of 0.2 µg/mL of *T. satureioides* essential oil and 2.1 µg/mL of *M. pulegium* essential oil were sufficient to inhibit the growth of the species *Staphylococcus xylosus* (the most sensitive strain of Staphylococci). *Staphylococcus aureus* ATCC 25923 was more resistant and only inhibited at a concentration of 0.35 µg/mL of *T. satureioides* essential oil and 5.5 µg/mL of *M. pulegium* essential oil (Figure 2).

The results of MBCs are shown in Table 4. The inhibitory effect of these two plants' essential oils proves that the oils are bactericidal on all species of Enterobacteriaceae and Staphylococci isolated.

Table 4: Minimum bactericidal concentrations in µg/mL of essential oils tested on Enterobacteriaceae.

Bacterial strains tested	*T. satureioides*		*M. pulegium*	
E. coli clinical isolate 1	0.33	B	4	B
E. coli ATCCS	0.43	B	1	B
E. coli clinical isolate 2	0.36	B	2	B
E. coli (EHEC) O157	0.21	B	0.90	B
E. coli 1	0.51	B	3	B
E. coli 2	0.34	B	3	B
E. coli 3	0.40	B	3	B
E. coli 4	0.25	B	3	B
E. coli 5	0.35	B	4	B
E. coli 6	0.45	B	4	B
E. coli 7	0.45	B	3	B
E. coli 8	0.30	B	5	B
E. coli 9	0.30	B	5	B
E. coli 10	0.50	B	5	B
E. coli ATCC 25922	0.55	B	6	B
Klebsiella oxytoca	0.45	B	2	B
S. aureus	0.25	B	4.50	B
S. xylosus	0.20	B	2.10	B
S. capitis	0.30	B	4.45	B
S. cohnii ureal	0.21	B	2.50	B
S. chromogenes	0.25	B	3.50	B
S. aureus clinical strain	0.30	B	4.50	B
S. aureus ATCC 25923	0.35	B	5.50	B

B: Bactericidal effect

Chemical composition of essential oils

Chromatographic analysis of the essential oils revealed 24 compounds making up 99.55% of *T. satureioides* essential oil and 19 compounds comprising 97.64% of *M. pulegium* essential oil (Table 5). The major constituents of *T. satureioides* essential oil are borneol (25.95%),

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(1) 2022, pp. 58-67
DOI: http://dx.doi.org/10.21161/mjm.211239

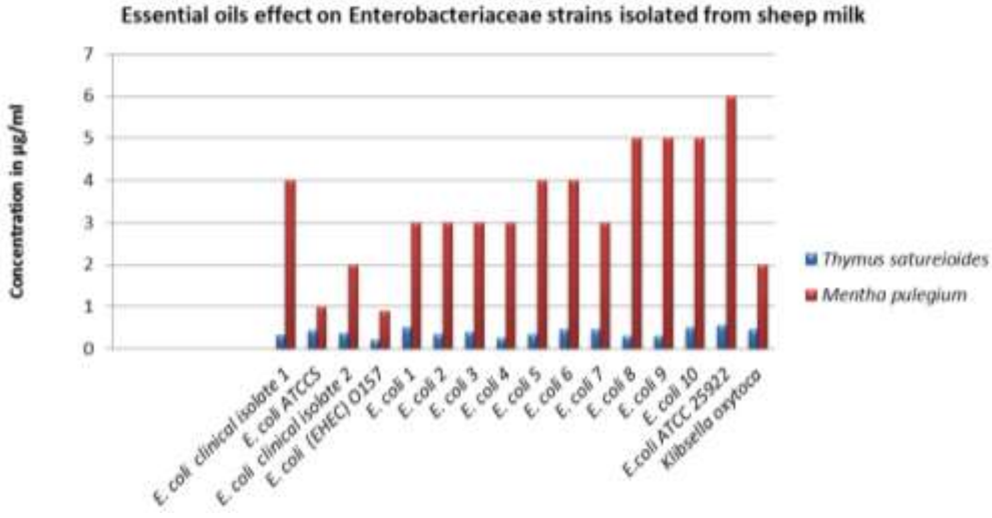

Figure 1: Minimum inhibitory concentrations (MICs) of essential oils on Enterobacteriaceae strains isolated from sheep milk.

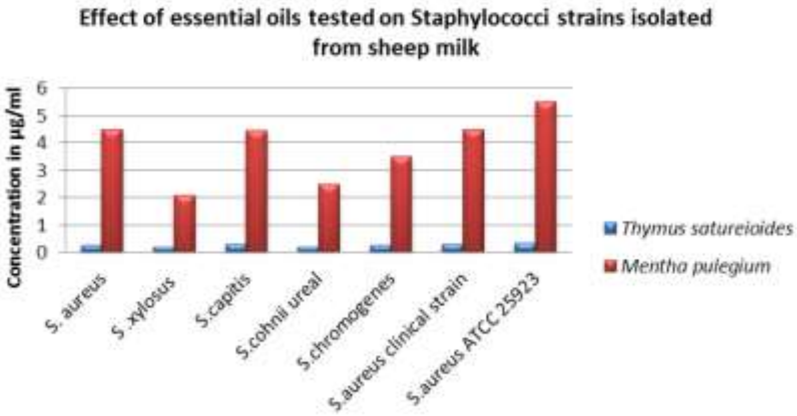

Figure 2: Minimum inhibitory concentrations (MICs) of essential oils on Staphylococci strains isolated from sheep milk.

carvacrol (17.54%) and camphene (10.32%). Other compounds present at lower concentration include α-terpineol (8.75%) and α-pinene (6.04%). The essential oil of *M. pulegium* is characterized by the presence of pulegone as the main constituent making up 80.33% of the oil.

DISCUSSION

The indiscriminate use of antibiotics in animal breeding has contributed to a progressive increase in bacterial resistance to the main classes of antibiotics such as Tetracyclines and Beta-lactams (Hricová *et al.*, 2017). *Escherichia coli* is one of the bacterial species in which the selection of resistance genes has occurred more rapidly over the years following the widespread use of antimicrobials agents (Tadesse *et al.*, 2012). *Staphylococcus*, a serious human pathogen with remarkable adaptability, are the main cause of mastitis in dairy sheep and they are often detected in sheep milk. Staphylococci are responsible for more than 65% of mastitis cases. In ewes, bacterial mastitis is a financially significant problem, especially for dairy production systems (Gelasakis *et al.*, 2015).

The emergence of bacterial resistance to antibiotics has led to changes in the zoo technical sector and reduction in the use of these chemicals, as both metaphylaxis and therapeutic tools. Farms managed with strict regulation of antibiotics usage and organic farms without antibiotics usage have increased along with farms using plant extracts as natural antibiotics (Anses, 2018).

Antibiotic resistance tests carried out on Enterobacteriaceae isolated from milk of Sardi sheep breed in Morocco revealed that Amoxicillin + Clavulanic acid (AMC), Cefotaxime (CTX), Cefoxitin (FOX), Nalidixic acid (NA), Gentamicin (CN), Ciprofloxacin (CIP) and Ofloxacin (OFX) are very effective antibiotics against all *E. coli* strains tested while 50% of the strains are resistant

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(1) 2022, pp. 58-67
DOI: http://dx.doi.org/10.21161/mjm.211239

Table 5: Chemical composition of *T. satureioides* and *M. pulegium* essential oils.

No	IK	Component	% of components	
			T. satureioides	*M. pulegium*
1	919	Tricyclene	0.43	-
2	923	α-thujene	1.18	-
3	930	α-pinene	6.04	0.39
4	945	Camphene	10.32	-
5	952	Cyclohexanone-3-methyl	-	0.28
6	970	Sabinene	0.20	0.45
7	973	β-pinene	1.48	0.16
8	988	Myrcene	1.14	0.99
9	1001	δ-2-carene	-	0.16
10	1018	α-terpinene	1.02	-
11	1019	Limonene	-	1.84
12	1025	o-cymene	4.30	-
13	1029	p-cymene	1.24	-
14	1034	E-β-cymene	-	1.74
15	1054	Menthone	-	0.39
16	1057	γ-terpinene	5.78	-
17	1072	p-mentha-3,8-diene	-	1.39
18	1084	Terpinolene	0.23	-
19	1096	Linalool	2.83	-
20	1164	Borneol	25.98	-
21	-	p-menthene-5-one	-	1.37
22	1173	Menthol	-	0.74
23	1175	terpin-1-ol	1.88	-
24	1189	α-terpineol	8.75	-
25	1194	dihydrocarvone	-	2.57
26	1195	Verbanol	0.46	-
27	1226	Cis carveol	0.48	-
28	1238	R(+)-pulegone	-	80.33
29	1240	Carvone	0.62	-
30	-	Eucarvone	-	3.75
31	1252	pépritone	-	0.97
32	1283	α-Terpin-7-al	0.89	-
32	1288	Thymol	0.23	-
33	1298	Carvacrol	17.54	-
34	1415	Caryophellene	5.98	-
35	1419	Caryophylléne	-	0.95
36	1453	NI	0.55	-
37	1630	γ-eudesmol	-	0.48
38	1649	α-eudemol	-	0.59
		Total	99.55%	97.64%

IK: Kovats retention index
- : Absence

to Amoxicillin, 60% are resistant to Ticarcillin (TIC) and 80% are resistant to Tetracycline (TE). Several studies have reported presence of *E. coli* in cow and sheep milk such as Farougou *et al.* (2011) in Benin, Boudjir and Zehar (2019) and Baazize-Ammi *et al.* (2019) in Algeria. The latter also confirmed that the *E. coli* isolated from milk were resistant to pharmaceutical antibiotics such as AMX and TE. The strains can also resist the effects of penicillin G, oxacillin, MLS, fusidic acid, glycopeptides and oxazolidinones. Saïdani et *al.* (2016) affirmed that although antibiotics help fight bacterial infections, the irindiscriminate usage, sometimes in insufficient doses, leads to the selection of antibiotic-resistant bacteria (Saïdani *et al.*, 2016).

The antibiogram results of the isolated *Staphylococcus* sp. strains revealed that *S. capitis* and *S. chromogenes* strains were most sensitive to the antibiotics tested. While *S. aureus*, *S. xylosus* and *S.cohnii ureal* have shown low resistance to Oxacillin (OX) and Tetracycline (TE), Pristinamycin (PT), Erythromycin (E) and Penicillin (P), respectively. Our results are similar to those reported by Zhang *et al.* (2012) in China and Thaker *et al.* (2013) in India. Therefore, rational use of these antibiotics is imperative because excessive usage in humans and animals accelerate the phenomenon of antibiotic resistance as well as poor infection prevention and control practices (Thaker *et al.*, 2013).

The essential oil yield of *T. satureioides* and *M. pulegium* are 1.85% and 3.2%, respectively. The average yield of these essential oils is relatively low compared to yield from plants used by the industry as source of essential oils. Many factors can influence the yield, content, physicochemical characteristics and chemical composition of essential oils such as plant species, environmental conditions, extraction technique, drying period and medium of harvest, cultural practices and the plant age (Bourkhiss *et al.*, 2011). The results obtained in this study confirm the work of El Ouali Lalami and his colleagues (2013) who obtained a yield of 1.1% essential oil from *T. satureioides* while Elidrissi *et al.* (2013) and Belmalha *et al.* (2015) reported higher values (2.83% and 2.71%, respectively). Similarly, the yield of essential oil extracted from pennyroyal in this study is comparable to that reported by Lakhdar *et al.* (2015) with value of 2.34%.

Chromatographic analysis of essential oils using GC/MS shows that the major components of *T. satureioides* are borneol (25.95%), carvacrol (17.54%) and camphene (10.32%). Compounds or constituents with concentrations below 0.1% were omitted. The results obtained are in agreement with Bellakhdar (1997) and Salhi *et al.* (2018) who reported that the major constituent of essential oil from *T. satureioides* is borneol (with a percentage of 27.0 to 33.0% and 33.0 to 41.8%, respectively), thymol (up to 21.3%), carvacrol (up to 15.23%) with additional compounds found in very small amounts (Salhi *et al.*, 2018). The composition of *T. satureioides* collected in the regions of Midelt reported by Belmalha *et al.* (2015) affirmed that borneol makes up

ISSN (print): 1823-8262, ISSN (online): 2231-7538

20.46% of the oil extract with camphene (7.43%), thymol (6.6%) and carvacrol that made up 23.65% of the oil is the major component. The composition of essential oils in this study was also not similar to those obtained by El Ouali Lalami *et al.* (2013) which confirmed that the most abundant components in *T. satureioides* collected in the regions of Ifrane are p-cymene (27.59%) and thymol (14.09%) (El Ouali Lalami *et al.*, 2013). The variations in essential oil composition seen in this research underscore the importance of the harvesting environment, altitude and climatic conditions in the location where the plants were cultivated.

According to literature, the composition of *T. satureioide s*oil differs from all types of thyme that exist in Morocco. The essential oil from species such as *T. algeriensis* mainly consist of camphor (27.7%) (Amarti *et al.*, 2010). Essential oils extracted from plants such as *T. pallidus*, *T. maroccanus* and *T. zygis* are especially rich in carvacrol (46.1%, 58.5% and 84.6%, respectively), whereas those of the species such as *T. ciliatus*, *T. leptobotrys*, *T. willdenoivii*, *T. villosus* and *T. munbyanus* are thymol-based (44.2%, 49.8%, 59.1%, 61.5% and 70.4%, respectively) (Amarti *et al.*, 2008; El Ajjouri *et al.*, 2008).

Mentha pulegium essential oil is characterized by the presence of pulegone as the main constituent with a content of 80.33%. This result is similar to a study in Morocco by Lakhdar *et al.* (2015) who confirmed that essential oil extracted from *M. pulegium* mainly contains pulegone (71.48%) with other components such as carvone, dihydrocarvone, limonene, octanol-3, p-mentha-3,8-diene, pinocarvone and peperitone present at concentration between 5.66% and 0.07%. The presence of high pulegone content from the *M. pulegium* extract in this study as well as other samples of *M. pulegium* collected around the world indicates that they belong to the pulegone chemotype (Attou, 2017).

Essential oils of plants tested showed a significant activity on selected pathogens including *E. coli* strains, *Staphylococcus* strains and *K. oxytoca*. *Thymus satureioides* essential oil has higher inhibitory activity than *M. pulegium* against the strains tested. This difference in the antimicrobial activity of the two plants can be attributed to their chemical compositions (Lakhdar *et al.*, 2015). In this context, studies have reported the effectiveness of Thyme extract with savory flowers and pennyroyal against bacterial growth or fungal contamination such as those published by El Ouali Lalami *et al.* (2013) and Lakhdar *et al.* (2015).

According to Bellakhdar (1997), the antimicrobial properties of essential oils are related to active components such as phenols. Other works have pointed out that the higher the levels of phenols, the greater the antimicrobial efficacy of essential oils. This applies to the two oils of *T. satureioides* and *M. pulegium* tested in this study. In addition, *T. satureioides* contains molecules of borneol, thymol and carvacrol which have a broad spectrum of antimicrobial activity and are naturally present in the essences in most species of thyme (Mohammedi and Piri, 2014; Hessas and Simoud, 2018).

Studies by the World Health Organization and other researchers (Dorman and Deans, 2000; Amarti *et al.*, 2010; Khaldi, 2018) have also shown that phenols and terpenes possess strong antibacterial and antifungal activity against many species, including *S. aureus*, *E. coli* and *Aspergillus* sp. In his book "The traditional Moroccan pharmacopoeia: Ancient Arab medicine and popular knowledge", Bellakhdar (1997) mentioned that Brussonnet's thyme (*T. satureioides*) is frequently used as a condiment and to preserve milk derivatives such as smen (melted butter).

The plant extracts investigated in this study showed significant antibacterial activity against spoilage bacteria. As such, it is critical for ensuring health security. Aromatic plants, like all medicinal plants researched, provide nutrients and act as preservatives. Additionally, their therapeutic activities are frequently potent, necessitating adequate safeguards.

By comparing the results of antimicrobial properties of natural antibiotics (essential oils) with the chemical antibiotics used in this study, the plant extracts have shown an important effect on the microbiological quality of milk even at very low minimal concentrations (0.2 µg/mL). Our results are in agreement with publications by Moussa *et al.* (2020), which proved that the active ingredients isolated from medicinal plants are very active compared to other chemical antibiotics.

CONCLUSION

Antibiotic resistance in foodborne pathogens is a reality. The existing institutional guideline must be improved and strengthened with regards to dispensing and use of antibiotics and the establishment of a surveillance group to monitor. Scientific and political steps must be taken to eradicate the antibiotic resistance problem. Additionally, plant extracts are less expensive to manufacture and may be easily evaluated for use as a preservative in the dairy business. They have no known adverse impacts on human or animal health. Essential oil mechanisms should be thoroughly studied, and advanced research should be conducted on the synergy of basic compounds and the combination of essential oil extracts in dairy products. Additional research is required to determine the toxicity of these botanical extracts and their potential *in vivo* application as flavoring and preservation agents for milk and its derivatives, as well as to combat microorganisms that cause food poisoning.

ACKNOWLEDGEMENTS

Authors express their gratitude to Dr Bader Satrani, Dr Mohamed Ghanmi and Mr Mohamed Aberchane from the Aromatic and Medicinal Plants Research Unit in the Forest Research Center of Rabat, for their support and valuable advice in completing this study. Our thanks also go to Dr Abderrahman AAFI, a botanist at Rabat's Forest Research Center, for making himself available and helping us identify the medicinal plants we used in our research.

REFERENCES

Amarti, F., Satrani, B., Aafi, A., Ghanmi, M., Farah, A., Aberchane, M., El Ajjouri, M., El Antry, S. and Chaouch, A. (2008). Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus capitatus* et de *Thymus bleicherianus* du Maroc. *Phytothérapie* **6, 342.**

Amarti, F., Satrani, B., Ghanmi, M., Farah, A., Aafi, A., Aarab, L., El Ajjouri, M. and Chaouch, A. (2010). Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus algeriensis* Boiss. & Reut. et *Thymus ciliatus* (Desf.) Benth. du Maroc. Biotechnology, *Agronomy, Society and Environment* **14(1), 141-148.**

Anses, (2018). Résapath - Réseau d'épidémiosurveillance de l'antibiorésistance des bactéries pathogènes animals, bilan 2017. Lyon et Ploufragan-Plouzané, France.

Attou, A. (2017). Détermination de la composition chimique des huiles essentielles de quatre plantes aromatiques de l'Ouest Algérien (Région d'Ain Témouchent), etude de leurs activités antioxydante et antimicrobienne. Thèse de Doctorat en Biologie, Universite Abou Bekr Belkaid, Tlemcen.

Baazize-Ammi, D., Gharbi, I., Dechicha, A. S., Kebbal, S. and Guetarni, D. (2019). Qualité bactériologique et sanitaire du lait cru de bovins des circuits direct et indirect dans la région centre de l'Algérie. *Revue Marocaine des Sciences Agronomiques et Vétérinaires* **7(2), 267-272.**

Bellakhdar, J. (1997). Contribution à l'étude de la pharmacopée traditionnelle au Maroc: la situation actuelle, les produits, les sources du savoir (enquête ethnopharmacologique de terrain réalisée de 1969 à 1992). Thèse de Doctorat en Sciences de la vie. Université Paul Verlaine de Metz, France.

Belmalha, S., El Idrissi, M., Amechrouq, A. and Echchgadda, G. (2015). Caracterisation chimique de certaines espèces de thym Marocain du Moyen Atlas (région de Midelt). *Global Journal of Pure and Applied Chemistry Research* **3(2), 43-52.**

Bourkhiss, M., Hnach, M., Lakhlifi, T., Boughdad, A., Farah, A. and Satrani, B. (2011). Effet de l'Age et du Stade Végétatif sur la Teneur et la Composition Chimique des Huiles Essentielles de Thuya de Berbere. *Les Technologies de Laboratoire* **6(23), 65-68.**

Boudjir, I. and Zehar, S. (2019). Evaluation de la qualité physico-chimique et microbiologique du lait de brebis. En vue de l'obtention du Diplôme de Master, University of Bordj Bou Arreridj, Algeria.

Caniça, M., Manageiro, V., Abriouel, H., Moran-Gilad, J. and Franz, C. M. A. P. (2019). Antibiotic resistance in foodborne bacteria. *Trends in Food Science and Technology* **84, 41-44.**

CLSI, Clinical and Laboratory Standards Institute. (2018). Performance Standards for Antimicrobial Susceptibility Tests. 13th Edn. CLSI Standard M02. CLSI, Wayne, PA.

Deb, P., Das, T., Nath, C., Ahad, A. and Chakraborty, P. (2020). Isolation of multidrug-resistant *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp. from dogs in Chattogram Metropolitan Area, Bangladesh. *Journal of Advanced Veterinary and Animal Research* **7(4), 669-677.**

Dorman, H. J. and Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology* **88(2), 308-316.**

El Ajjouri, M., Satrani, B., Ghanmi, M., Aafi, A., Farah, A., Rahouti, M., Amarti F. and Aberchane, M. (2008). Activité antifongique des huiles essentielles de *Thymus bleicherianus* Pomel et *Thymus capitatus* (L.) Hoffm. & Link contre les champignons de pourriture du boisd'œuvre. *Biotechnology, Agronomy, Society and Environment* **12(4), 345-351.**

El Ouali Lalami, A., El-Akhal, F., Ouedrhiri, W., Ouazzani Chahdi, F., Guemmouh, R. and Greche, H. (2013). Composition chimique et activité antibactérienne des huiles essentielles de deux plantes aromatiques du centre nord marocain: *Thymus vulagris* et *Thymus satureioïdis*. *Les Technologies de Laboratoire* **8(31), 27-33.**

Elidrissi, M., Amechrouq, A., Belmalha, S. and Echchgadda, G. (2013). Composition et variabilité chimique des huiles essentielles de deux espèces de thym sauvage du Maroc: *Thymus zygis* L. subsp. Gracilis (Boiss.) R. Morales et *Thymus satureioides* Cosson. *Physical and Chemical News* **69, 76-82.**

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* **64, 711-713.**

Falowo, A. B. and Akimoladun, O. F. (2019). Veterinary drug residues in meat and meat products: Occurrence, detection and implications. *In*: Veterinary Medicine and Pharmaceuticals. IntechOpen Limited, United Kingdom.

Farougou, S., Kpodékon, T. M., Sessou, P., Youssao, I., Boko, C., Yèhouenou, B. and Sohounhloué, D. (2011). Actes: Troisième Colloque des Sciences, Cultures et Technologies de l'UAC-Bénin. Conseil Scientifique de l'Université d'Abomey-Calavi, Bénin. **pp. 323-336.**

Gadisa, E., Weldearegay, G., Desta, K., Tsegaye, G., Hailu, S., Jote, K. and Takele, A. (2019). Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug-resistant bacteria. *BMC Complementary and Alternative Medicine* **19, 24.**

Gelasakis, A. I., Angelidis, A. S., Giannakou, R., Filioussis, G., Kalamaki, M. S. and Arsenos, G. (2015). Bacterial subclinical mastitis and its effect on milk yield in low-input dairy goat herds. *Journal of Dairy Science* **99(5), 3698-3708.**

Hessas, T. and Simoud, S. (2018). Contribution à l'étude de la composition chimique et à l'évaluation de l'activité antimicrobienne de l'huile essentielle de

Thymus sp. Thèse de Doctorat en pharmacie. Université Mouloud Mammeri de Tizi-Ouzou, Algérie.

Hricová, K., Röderová, M., Pudová, V., Hanulík, V., Halová, D., Julínková, P., Dolejská, M., Papoušek, I. and Bardoň, J. (2017). Quinolone-resistant *Escherichia coli* in poultry farming. *Central European Journal of Public Health* **25(2), 163-167.**

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B. A., Mapongmetsem, P. M. and Tsabang, N. (2010). Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *International Journal of Medicine and Medical Sciences* **2(3), 60-79.**

Khaldi, Z. (2018). Elaboration et évaluation biologique de nouveaux matériaux lignocellulosiques antibactériens. Thèse de doctorat de l'Université de Limoges en Chimie des substances naturelles. Université de Limoges, Français.

Lakhdar, L. (2015). Evaluation de l'activite antibactérienne d'huiles essentielles marocaines sur aggregatibacter actinomycetemcomitans: étude in vitro. Thèse de Doctorat en Médecine dentaire. Université Mohamed V, Morocco.

Loayza, F., Graham, J. P. and Trueba, G. (2020). Factors obscuring the role of *E. coli* from domestic animals in the global antimicrobial resistance crisis: An evidence-based review. International *Journal of Environmental Research and Public Health* **17(9), 3061.**

Majolo, C., da Rocha, S. I. B., Chagas, E. C., Chaves, F. C. M. and Bizzo, H. R. (2016). Chemical composition of *Lippia* spp. essential oil and antimicrobial activity against *Aeromonas hydrophila*. *Aquaculture Research* **48(5), 2380-2387.**

Mensah, S. E. P., Koudandé, O. D., Sanders, P., Laurent, M., Mensah, G. A. and Abiola, F. A. (2014). Résidus d'antibiotiques et denrées d'origine animale en Afrique: Risques de santé publique. *Revue Scientifique et Technique* **33(3), 975-986.**

Mohammadi, S. and Piri, K. (2014). Antifungal effects of two medicinal plant native to Iran. *International Journal of Advanced Biological and Biomedical Research* **2(10), 2712-2715.**

Moussa, H., Hriouech, S., Tanghort, M., Chefchaou, H., Mzabi, A., Chami, N. and Remmal, A. (2020). A comparative study of the antifungal activity of a natural product based on essential oils with imazalil and thiabendazole on *Penicillium digitatum* and *Penicillium italicum*. *Plant Cell Biotechnology and Molecular Biology* **21(35&36), 16-23.**

Olaimat, A. N., Al-Holy, M. A., Shahbaz, H. M., Al-Nabulsi, A. A., Abu Ghoush, M. H., Osaili, T. M., Ayyash, M. M. and Holley, R. A. (2018). Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety* **17(5), 1277-1292.**

Oliva, A., Costantini, S., De Angelis, M., Garzoli, S., Božović, M., Mascellino, M. T., Vullo, V. and Ragno, R. (2018). High potency of *Melaleuca alternifolia* essential oil against multi-drug resistant Gram-negative bacteria and methicillin-resistant *Staphylococcus aureus*. *Molecules* **23(10), 2584.**

OXOID. (2008). 2008-2009 Canadian Product Catalogue. Part of Thermo Fisher Scientific. Oxoid Company, Canada.

Piccirilli, A., Brisdelli, F., Aschi, M., Celenza, G., Amicosante, G. and Perilli, M. (2019). Kinetic profile and molecular dynamic studies show that Y229W substitution in an NDM-1/L209F variant restores the hydrolytic activity of the enzyme toward penicillins, cephalosporins, and carbapenems. *Antimicrobial Agents and Chemotherapy* **63(4), e02270-18.**

Saïdani, M., Soudani, A., Dâaloul, M., Ben Chehida, F., Mamlouk, A. and Messadi, L. (2016). Prévalence et antibiorésistance d'*Escherichia coli* dans les mammites bovines au Nord de la Tunisie. *Mediterranean Forum for PhD Students and Young Researchers*, Montpellier, France.

Salhi, N., Fidah, A., Rahouti, M., Ismaili, M. R., Ramzi, H. and Kabouchi, B. (2018). Chemical composition and fungicidal effects of four chemotypes of *Thymus satureioides* Cosson essential oils originated from South-west of Morocco. *Journal of Materials and Environmental Sciences* **9(2), 514-519.**

Singh, A. K., Das, S., Singh, S., Gajamer, V. R., Pradhan, N., Lepcha, Y. D. and Tiwari, H. K. (2018). Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India. *PLoS ONE* **13(6), e0199179.**

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J. and McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases* **18(5), 741-749.**

Thaker, H. C., Brahmbhatt, M. N. and Nayak, J. B. (2013). Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. *Veterinary World* **6, 10-13.**

Thornber, K., Huso, D., Rahman, M. M., Biswas, H., Rahman, M. H., Brum, E. and Tyler, C. R. (2019). Raising awareness of antimicrobial resistance in rural aquaculture practice in Bangladesh through digital communications: A pilot study. *Global Health Action* **12, 1734735.**

WHO, World Health Organization. (2020). Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: Early Implementation 2020. World Health Organization, Geneva.

Yalçin, S, S., Güneş, B. and Yalçin, S. (2020). Incredible pharmaceutical residues in human milk in a cohort study from Şanlıurfa in Turkey. *Environmental Toxicology and Pharmacology* **80, 103502.**

Zainab, S. M., Junaid, M., Xu, N. and Malik, R. N. (2020). Antibiotics and antibiotic-resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Research* **187, 116455.**

Zhang, C. L., Guo X. L., Li, B. Y. and Wang, Y. (2012). Biodegradation of ciprofloxacin in soil. *Journal of Molecular Liquids* **173, 184-186.**