EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AGAINST *E. COLI* ISOLATED FROM RABBITS

Majda B* A.Barchan* A.Aarab* M. Bakkali A. Arakrak* A. Laglaoui***
***Dept. Biol. Facul. Sci.and Tech. Tangier, **Dept. Biol. Facul. Sci. and Tech. Al Hoceima**
Abdelmalek Essaâdi University 416 Tangier, Morocco
Email: benlemlihmajda@yahoo.fr

ABSTRACT

The antibacterial activity of essential oils extracted from *Origanum compactum, Thymus capitatus, Foeniculum vulgare,* and *Rosmarinus officinalis* was assessed with the well diffusion method and a microbroth dilution assay against *E. coli* isolated from the carcasses of rabbits. The chemical composition of these essential oils was also determined by gas chromatography coupled with mass spectrometry (GC/MS). The results of this study indicate that essential oils with high phenol content exert a strong antibacterial activity against *E. coli.* Essential oils of *Origanum compactum* and *Thymus capitatus* containing high amounts of the monoterpenoid phenols thymol and carvacrol (68.99% and 95.25% carvacrol composition, respectively) were particularly effective against *E. coli* with low values of MIC = 0.3125% v/v and MBC = 0.625% v/v to report. The essential oil of *Foeniculum vulgare* also possessed moderate antibacterial activity (MIC = 50 % v/v) with a non-bactericidal effect, while the essential oil of *Rosmarinus officinalis* was ineffective at the concentrations tested.

Keywords: :microproth, phenol content, theymol, essential oils, bactericidal effect

تقييم النشاط المضاد للزيوت الاساسية لبكتريا القولون المعزولة من الفئران

ماجدة برجان عرب باكالي اركرك لاكرلوي

المستخلص

تم تثبيت الفعالية التثبيطية للمستخلصات الزيتية من المصادر النباتية التي شملت *Origanum compactum, Thymus capitatus, Foeniculum vulgare,* and *Rosmarinus officinalis* **باستعمال طريقة الانتشار بالحفر و النمو بالاوساط السائلة تجاه بكتريا القولون المعزولة من الارانب . كذلك تم تحديد المستوى الكيمياوي لهذه المستخلصات الزيتية بتقنية Gc IMS . بينت النتلئج بان المحتوى العالي من من الفينول للمستخلصات الزيتية ذات فعالية تثبيطية تجاه بكتريا القولون , حيث احتوت الزيوت المستخلصة من المكونات العضوية و تايمس كابيتاتس على نسبة عالية من التاريانويد و كاراركقخل 68.99% و 95ز25%رمن الكاراكورول بالتتابع ذات فعالية اساسية تجاه بكتريا القولون و بقيم منخفضة من MIC=0.3125% و MBC= 0.625%' وكان التأثير التثبيطي لزيت فيكنوكالم فالكاري تجاه بكتريا القولون وبدون تأثير قاتل تجاه البكتريا ولم يمتلك الزيت المستخلص رازموميناس اوفشياليز اي تأثير تثبيطي ضد البكتريا لكلا التراكيز المستملة .**

كلمات مفتاحية: مستخلصات زيتية، فينولات بكتريا القولون، تأثير تثبيطي.

Received:16/2/2021, Accepted:20/5/2021

INTRODUCTION

Rabbit colibacillosis is an infectious disease of rabbit caused by an infection of pathogenic *Escherichia coli*. This disease affects animals that have just been weaned resulting in severe diarrhea after infection including some cases that exhibit phases of mucoid diarrhea (24). Death can sometimes precede these symptoms. Antibiotics are often employed by rabbit breeders to treat and prevent the disease, however this approach can be problematic for human health. While antibiotics are capable of eliminating susceptible bacteria, this can have the unintended effect of promoting the proliferation of resistant strains in bacteria and lead to the greater occurrence of infections that are impossible to treat with conventional antibiotics (36). Each year, antibiotics-resistant strains of bacteria cause the death of 25,000 people in Europe and 19,000 in the United States (7). The essential oils of plants have been traditionally used for medicinal purposes and have proven their efficacy in several areas, which suggests one promising alternative to the routine use of antibiotics in animal agriculture. In order to better understand the ability of plants to control infections in rabbit breeding, the essential oils of four medicinal and aromatic plants recognized for their digestive and antispasmodic properties (2 , 9 , `12 , 23) were tested for their bacteriostatic and bactericidal qualities against *E. coli* isolated from the carcasses of dead rabbits. Plants that were featured in this study include: *Foeniculum Vugaris, Origanum compactum, Rosmarinus officinalis*, and *Thymus capitatus.* Essential oils were extracted from the seeds and leaves of *Foeniculum Vugaris* independently.

MATERIALS AND METHODS

Plant and essential oil

Large quantity of seeds from the plant *Foeniculum vulgare* were purchased while leaves of the plant were collected, cleaned and then dried without light in at room temperature. Specimens of *Origanum compactum*, *Rosmarinus officinalis*, and *Thymus capitatus* were collected in northern Morocco during the month of June. Species identification was done by Professor Bakkali, a specialist in botany, in the laboratory of ERGB. The essential oils (EO) of each plant were extracted via steam distillation for 3 hours using a Clevenger-type apparatus. Essential oil yield was determined as a percentage of the weight of the dry plant matter. Chemical analysis of essential oils was done by gas chromatography coupled with mass spectrometry (GC/MS).

Isolation of *E. coli* bacteria

Specimens were collected from the carcasses of rabbits that showed previous signs of diarrhea and bloating. Specimens from the intestinal mucosa and cecum along with their liquid contents were put into a ringer solution. Culture and isolation of *E. coli* was done on MacConkey agar. Petri dishes containing the culture medium were inoculated in depth and incubated at 37 °c for 24 hours. Miniature biochemical tests can conveniently and simultaneously performed on a colony for *E. coli* identification using an API 20 e gallery.

Antibacterial activity

A preliminary assay was performed with the agar diffusion method to compare the antibacterial effects of the essential oils against the performance of the antibiotic Oxytetracycline. The diameters of the resulting inhibition zones were measured in centimeters, including the diameter of the well. The results are expressed as an average by three determinations (+/-) standard deviation. The minimum inhibitory concentration (MIC) is defined as the smallest essential oil concentration capable of producing a total inhibition of growth after an incubation period of 24 to 48 hours (30). The minimum bactericidal concentration (MBC) is defined as the minimum bactericidal concentration of the oil capable of killing the inoculum. The MIC[1] and MBC[2] values were determined by microbroth dilution assay using resazurin as an indicator (21) of bacterial growth.

RESULTS AND DISCUSSION

The yield of each essential oil extracted are summarized in Table 1. The species of plant in order of percentage yield are: Origanum Compactum, seeds of Foeniculum vulgare, Thymus capitatus, leaves of Foeniculum vulgare, and Rosmarinus officinalis.

[1] MIC : Minimum inhibitory concentration
[2] MBC : Minimum bactericidal concentration

Table 1. Essential oils yield from different plant sources

Aromatic and medicinal Plants	Yield (%)
Foeniculum vulgare leaf	1,89
Foeniculum vulgare seed	2,11
Origanum compactum	2,92
Rosmarinus officinalis	0,49
Thymus capitatus	1,96

The chemical composition of essential oils is very complex and diversified. An understanding of their constituents is very important in evaluating their properties and predicting their potential toxicity. The chemical composition of the essential oils of *Foeniculum vulgare, Origanum compactum, Thymus capitatus* and *Rosmarinus officinalis* are shown in Table 2.

Table 2. Chemical composition of essential oil

Component	Retention Time (min)	*Foeniculum vulgare* leaves	*Foeniculum vulgare* seeds	*Origanum compactum*	*Rosmarinus officinalis*	*Thymus capitatus*
				Concentration (%)		
Myrcene	6.55			0,44		
Para cymene	7.19			2,53		
cis-Ocimene	7.73		0,41			
α-Pinene, (-)-	7.75	0,12				
γ terpinne	7.79			3,98		
α-Pinene, (-)-	8.28				18,94	
Linalol	8.51			1,09		
Camphene	8.74				5,38	
α-Phellandrene	9.70				5,46	
Terpinène -4-ol	9.79			0,58		
α-Pinene, (-)-	11.01		2,22			
dl-Limonene	11.01	14,40				
Thymol	11.38			18,67		
Carvacrol	11.55			68,99		
1-8 cineol	11.68				51,62	
Fenchone	12.99		6,14			
Fenchone	13.00	1,32				
β Caryophyllene	13.08			1,08		
α -Campholene Aldehyde	15.63				10,65	
Borneol	16.47				2,59	
Isopulegyl acetate	17.81	0,12				
α-Fenchyl acetate	18.23	0,76				
Trans Anethol	20.07		91,12			
Trans Anethol	20.10	83,29				
Carvacrol	20.69					95,25
Caryophyllene	24.24					1,49
Caryophyllène	24.89				5,36	
Tetradecamethylcycloheptasiloxane	27.26		0,06			
3,5-Diethylphenol	37.95					0,74
1,15-Dihydrohexadecamethyloctasiloxane	38.85		0,05			
6-Acetyl-2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-benzopyran	39.37					0,91

[1] MIC : Minimum inhibitory concentration
[1] MBC : Minimum bactericidal concentration

The essential oils of the leaves and seeds of *Foeniculum vulgare* are predominantly composed of Trans Anethole compound, which has a higher content in the seeds than in the leaves (91.12 and 83.29 % successively). This same case is noted for the Fenchone content with 6.14% in the seeds and 1.32 % in the leaves. The DL-Limonene presents 14.40 % in the essential oil of *Foeniculum vulgare* leaves. The essential oil of *Origanum* *compactum* is predominantly composed of carvacrol (68.99%) and thymol (18.67%). The fundamental component of the essential oil of the *Thymus capitatus* is carvacrol (95,25 %). The essential oil of *Rosmarinus officinalis* consists mainly of 1-8 cineol (51,62%), α-pinene (18,94%), and α-Campholène aldehyde (10,65%). The results of the antibacterial activity of essential oils against *E. coli* are shows in Table 3.

Table 3. Diameter of inhibition zones of essential oil against *E.coli* (cm)

Bacterial strain	Antibiotic	Essential oil						
	Oxy-tetracycline	*Foeniculum vulgare* Leaves	*Foeniculum vulgare* Seeds	*Origanum compactum*	*Rosmarinus officinalis*	*Thymus capitatus*	*Foeniculum vulgare* + *Thymus capitatus*	*Foeniculum vulgare* + *Rosmarinus officinalis*
E.coli	**0,6±0**	**1,1±0,7**	**1,4±0,6**	**4,2±1,1**	**0,6±0**	**5,1 ±0,6**	**2,1±0,6**	**0,9±0,5**

E. coli showed resistance to oxytetracycline with an inhibition diameter of 0,6 cm, while the essential oil of the *Thymus capitatus* showed high antibacterial activity (5,1cm), followed by the essential oil of *Origanum compatum* (4,2 cm). *Rosmarinus officinalis* essential oil appears to be ineffective towards this strain (0,6 cm). Low activity was observed with the essential oils of the leaves and seeds of the *Foeniculum vulgare* (1,1 and 1,4 cm successively). The combination of the two essential oils *Thymus capitatus* and *Foeniculum vulgare* seed was found to be more effective against *E. coli* (2,1 cm) than the antibiotic oxytetracycline (0,6 cm). The minimum inhibitory concentrations (MIC) and bactericidal (MBC) of essential oils against *E. coli* are grouped in table 4.

Tableau 4. Minimum inhibitory concentration and minimum bactéricidal concentration µl/ml

Bacterial strain		Essential oil						
		Foeniculum vulgare (F) Leaf	*Foeniculum vulgare* (F) seed	*Origanum compactum*	*Rosmarinus officinalis*	*Thymus capitatus*	*Foeniculum vulgare* + *Thymus capitatus*	*Foeniculum vulgare* + *Rosmarinus officinalis*
E.coli	**MIC**	**50**	**50**	**3,125**	**>50**	**3,125**	**6,25**	**>50**
	MBC	**>50**	**>50**	**6,25**	**>50**	**6,25**	**6,25**	**>50**

With the exception of the essential oil of *Rosmarinus officinalis*, all essential oils were able to inhibit the growth of *E. coli* at concentrations ranging from 0,31 à 5% v/v. The essential oil of *Origanum compactum* exerted strong antibacterial activity against *E. coli* with smal values of MIC = 0,3125 % v/v and MBC = 0,625% v/v. This activity is similar to that shown by the essential oil of the *Thymus capitatus*. The two essential oils of *Foeniculum vulgare* leaves and seeds showed the lowest antibacterial effect (MIC = 50% v/v) against this bacterium with no bactericidal action at the concentrations tested. An important activity of the two combined oils of *Foeniculum vulgare* and *Thymus capitatus* both bacteriostatic and bactericidal (MIC = MBC = 6,25%) was demonstrated against *E. coli*. No synergistic effect of these two essential oils was indicated, because their combined activity remains lower than that of *Thymus capitatus* alone (MIC = 3,125 %). The essential oil of *Rosmarinus officinalis* was inactive with respect to the strain tested in this study (MIC > 50 M L/ml). In accordance with the results of the present study, essential oil yield of the *Foeniculum vulgare* (2.11 %) is similar to values published by Stefanini et al. (32) for the Brazilian fennel (2.7%). However, essential oil yield of *Origanum compactum* (2,92%) is slightly higher than values reported by Bkhy et al. (3) (varied from 0,31 to 2,44%). A study by El Ouariachi et al. (13) in February showed that essential oil yield of *Thymus capitatus* (0.5%) was lower than our yield (1.96%). This difference may be due to the harvest period of the plant material. A very low yield was recorded by Moujahed et al. (26) for the Tunisian *Thymus capitatus* (0.25%) and comparable to the yield found in the present work for *Rosmarinus officinalis* (0.43%).This study showed that the essential oils of *Origanum compactum* and *Thymus capitatus* are characterized by high levels of phenol terpenes (oxygenated terpenes) while those of the leaves and seeds of *Foeniculum vulgare* are distinguished by a large percentage of the phenol phenylpropanoidique. The essential oil of *Rosmarinus officinalis* consists of terpenes and o14xygen-containing terpenes. According to Gulfraz et al., () trans-anethole represents the fundamental compound of the essential oil of seeds with a high proportion (70.1%) whereas the presence of fenchone is also noticed by a small percentage (6.9%). Similarly at our results, Džamić et al., 2015 reported that this Libyan essential oil of *Thymus capitatus* (harvested in May) is mainly formed by carvacrol (68,07 %) and thymol (12,27) Other secondary compounds were detected at relatively low levels: p-cymene (3,24%), γ-terpinene (3,09%), p-cymene-7-ol (2,83%). However El Ouariachi et al, (13) found a low percentage in carvacrol (13,4%) and high in P-Cymene (18,9%) for the essential oil of *Thymus capitatus* (harvested in February), the harvest period does not only influence the yield but also the Composition. The essential oil of the Algerian *Thymus capitatus* consists of carvacrol (55%) and γ-terpinene (11%) (34) and that of Tunisia is also composed by carvacrol (70%), accompanied by other constituents β-caryophyllene (8.5%), γ-terpinen (4.3%) and P-cymen (3.8%) (26). Whereas Turkish *Capitatus Coridothymus* is characterized by an average content of carvacrol (35,6%) and P-cymene (21%) and a moderate proportion in thymol (18.6%) and γ-terpinene (12.3%) (15). Bounatirou et al., (6) [1] MIC : Minimum inhibitory concentration

[1] MBC : Minimum bactericidal concentration[1] MIC : Minimum inhibitory concentration

[1] MBC : Minimum bactericidal concentration reported that the composition of the essential oil of the *Thymus capitatus* varies according to the seasons, climatic conditions, geographic location, genotype, soil composition, desiccation procedure, and the of the plant used. According to the study by the major compound identified by Moujahed et al., (26) in this essential oil is 1-8 cineol (44,2%), camphor (12%) and α-pinene (11,6%). These results are similar to our results. The antibacterial activity of essential oils has been demonstrated by several studies (17 , 28), including that of *Origanum compatum* and *Thymus capitatus* (5 , 8), *Foeniculum vulgare,* and *Rosmarinus officinalis* (18, 19, 25). However, in the literature few studies address the effect of essential oils on pathogenic bacteria isolated from farmed animals. The low antibacterial power of the essential oil of *Rosmarinus officinalis* against *E. coli* 011 compared with that of *Origanum Compatum* has already been reported by Mathlouthi et al.(22). The presence of Monoterpene phenols in the two essential oils of *Thymus capitatus* and *Origanum Compactum* seems to be responsible for the important antibacterial activity demonstrated in our work by these two oils. Carvacrol and thymol have proven antibacterial power against a wide range of bacteria (20, 36).The essential oil of *Rosmarinus officinalis* was ineffective against our isolated bacterium despite its capacity in oxygenated monoterpenes (51%). Indeed, Ait-Ouazzou et al., (1) studied the antimicrobial effect of 11 major constituents of essential oils and found that the 1.8 cineole had moderate activity compared with other oxygenated monoterpèniques compounds such as carvacrol, thymol, and linalol. The low antibacterial activity recorded by essential oils of seeds and leaves of *Foeniculum vulgare* has already been demonstrated by the study of Grigore et al., (16). According to this study, the essential oil of *Foeniculum vulgare* (80% d'anéthol and 13% de limonène) has a low antibacterial activity compared to that of *Thymus vulgare*. The De et al. Team (9) found that the anethole isolated from anise is responsible for the antibacterial power of this plant. Phenylpropanoids have a lower antibacterial coefficient than terpene phenols. The low presence of oxygenated monoterpenes such as fenchone and limonene, respectively, in the essential oil of seeds and leaves, may also be responsible for their low antibacterial activity (35). The complex composition of essential oils with all its majority and minority products offers this antibacterial power. Some studies have shown that the mixture of the majority constituents of the essential oil has a low antibacterial activity compared to the whole essential oil (27) which shows that the effect of the compounds quantitatively minority is sometimes not negligible and this

supports the presence of additive or synergistic effect of all the compounds of the essential oil. Mathlouthi et al., (22) showed that the bacterial activity of the essential oil of *Rosmarinus officinalis* against *E. coli* 011 is moderate (MIC = 4, 4mg/ml) compared with that of *Origanum compatum* (MIC = 0.9 mg/ml). According to Sienkiewicz et al., 2013, the essential oil of *Rosmarinus officinalis* has antibacterial activity against clinical strains of *E. coli* isolated from the human abdominal cavity (MIC = 18 M L/ml). In the present study, the potency of the antibacterial action of essential oils varies according to the chemical profile of their majority constituents. The modes of action of essential oils and their main constituents described so far, all seem to affect the wall or cytoplasmic membrane. Indeed, the attack of the bacterial wall by the essential oil and the damage of the plasma membrane causes an increase in permeability, a loss of the cellular constituents and a coagulation of the cytoplasmic content (4). The inhibition of the resultant proton motor force and the alteration of the membrane proteins block the production of the cell energy resulting in the death of the bacterium .In fact, the chemical variability of essential oils suggests the existence of molecules that can act by new cellular mechanisms.

Conclusion

In this work, a relationship between the biochemical families of the active constituents of essential oils and their antibacterial powers has been revealed. Indeed, the essential oils of *Origanum compactum* and *Thymus capitatus* rich in terpene phenols (thymol, carvacrol) demonstrated great antibacterial power, followed by *Foeniculum vulgare* oil rich in phenols phenylpropanoidic (anethole) which revealed Moderate antibacterial activity. The lowest activity is recorded by *Rosmarinus officinalis* oil rich in terpene oxides (1.8 cineole). The essential oil of oregano, thyme, and fennel can be suggested as phytobiotics to prevent, treat colibacillosis and reduce the mortality of rabbit.

REFERENCES

1-Ait-Ouazzou A., L., Espina L., Cherrat M., M., Hassani A., Laglaoui P.Conchello and R., Pagán 2012. Synergistic combination of essential oils from Morocco and physical treatments for microbial inactivation. Innovative Food Science and Emerging Technologies. 16, 283-290

2-Badgujar S. B., V. V.Patel and A. H. Bandivdekar ., 2014. Foeniculum vulgare Mill: A Review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Res Int 2014. http://dx.doi.org/10.1155/2014/842674

3-Bakhy K. O, A. Benlhabib J. Bighelli , F. Casanova and C. Tomi . 2014. Yield and chemical variability of the essential oil isolated from aerial parts of wild Origanum compactum Benth. from Moroccan Western Rif. Am J Essent Oils Nat Prod; 1(4): 1-9

4-Bouhdid, S., S. N. Skali, M. Idaomar, A. Zhiri, D. Baudoux, M. Amensour, and J. AbriniJ., 2008. Antibacterial and antioxidant activities of Origanum compactum essential oil. Afr. J. Biotechnol. 7:1563–1570.

5-Bouhdid S, J. Abrinin, A. Zhiri , M. J Espuny and A. Munresa . 2009. Investigation of functional and morphological changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Origanum compactum essential oil. Journal of Applied Microbiology.; 106: 1558-1568

6-Bounatirou S, S. Smiti, MG Miguel, M.N.Rejeb M.Neffati M, M.M.Costa , L. Faleiro AC. Figueiredo JG., Barroso JG and L.G. Pedro . 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. Et Link. Food Chemistry. 105: 146-155

7-Carlet J., V. Jarlier , S. Stephan Harbarth , A. Voss , H. Goossens and D. Pittet . 2012. Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action Antimicrobial Resistance and Infection Control. 1:11. http://www.aricjournal.com/content/1/1/11

8-Chedia A., H. Ghazghazi , S. Dallali , S. Houssine , H. Brahim and M. Abderazzak 2013 Comparison of Chemical Composition, Antioxidant and Antimicrobial Activities of Thymus capitatus L. essential Oils from two Tunisian localities (Sousse and Bizerte). International Journal of Agronomy and Plant Production. 4, 1772-1781

9-De M., A. K. De ., R. Mukhopdhyay M., Miro and A. B. Anerjee . 2001. Actividad antimicrobiana de Illicium verum Hook. f. Antimicrobial actions of Illicium verum Hook. Ars Pharmaceutica, 42:3-4; 209-220

10-Djeddi, S., N. Bouchenah, I. Settar, . and H.D. Skaltsa . 2007. Composition and antimicrobial activity of the essential oil of Rosmarinus officinalis from Algeria. Chemistry of Natural Compounds 43(4): 487-490

11-Džamić A.M , B.J.Nikolić , A.A. Giweli , D.S.Mitić-Ćulafić , M.D.Soković , MS. Ristić , JB. Knežević-Vukčević and PD.Marin . 2015. Lybian Thymus capitatus essential oil: antioxidant, antimicrobial, cytotoxic and colon pathogen adhesion-inhibition properties. Journal of Applied Microbiology ; 119(2):389-99

12-Ennabili A, N. Gharnit and EL. El Hamdouni . 2000. Inventory and social interest of medicinal, aromatic and Honey-plants from Mokrisset region (NW of Morocco). Ediciones Universidad de Salamanca 19: 57-74.

13-El Ouariachi E.M., J. Paolini , A. Bouyanzer , P. Tomi ., B. Hammouti , R. Salghi , L. Majidi and J. Costa ., 2011. Chemical Composition and Antioxidant Activity of Essential Oils and Solvent Extracts of Thymus capitatus (L.) Hoffmanns and link from Morocco. Journal of Medicinal Plants Research, 5: 5773-5778

14-Gulfraz M., S. Mehmood, N. Minhas , N. Jabeen , R. Kausar , K. Jabeen , and G. Arshad . 2008. Composition and antimicrobial properties of essential oil of Foeniculum vulgare. African Journal of Biotechnology 7 : 4364-4368

15-Goren AC, G. Bilsela, M. Bilsela , H. Demira and E.E. Kocabas ., 2003. Analysis of essential oil of Coridothymus capitatus (L.) and its antibacterial and antifungal activity. Z. Naturfo. B., 58: 687-690

16-Grigore A., S. Colceru-Mihul, S. Paraschivi, Nita , R. Christof , R. Iuksel and M. Ichim ., 2012. Chemical analysis and antimicrobial activity of indigenous medicinal species volatile oils. Romanian Biotechnological Letters 17, No.5

17-Hammer K. A., C. F. Carson . and T.Y. Riley . 1999. Antimicrobial activity of essential oils and other plant extracts. Journal Of Applied Microbiologiy, 86, 985-990

18-Issabeagloo E., P. Kermanizadeh , M. Taghizadieh and K. Foroughi , 2012. Antimicrobial effects of rosemary (*Rosmarinus officinalis* L.) essential oils against Staphylococcus spp. African Journal of Microbiology Research . 6(23), 5039-5042

19-Javed S., S. Mushtaq , L. Khokhar , R. Ahmad and M.S. Haider . 2012. Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria. African Journal of Biotechnology 11 : 16065-16070

20-Lambert R.J.W., P.N. Skandamis , P.J. Coote and G.J.E.Nychas ., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91, 453-462

21-Mann C and J. Markham . 1998 A new method for determining theminimum inhibitory concentration of essential oils. J Appl Micro-biol 84:538–44

22-Mathlouthi N., T. I. Bouzaienne, F. Oueslati, M. Recoquillay, M. Hamdi, M. Urdaci and R. Bergaoui., 2012. In vitro antimicrobial activities and effects on growth performance Use of rosemary, oregano, and a commercial blend of essential oils in broiler chicken. Journal of Animal Science. 90:813-823

23-Megdiche-ksouri Saada M., B. Soumaya , M. Snoussi , Y. Zaoulali and R. Ksouri , 2015. Potential use of wild Thymus algeriensis and Thymus capitatus as source of antioxidant and antimicrobial agents Journal of New Sciences, Agriculture and Bi otechnology, 23(4), 1046-1056

24-Milon A., E. Oswald and J. De Rycke,1999. Rabbit EPEC: a model for the study of enteropathogenic Escherichia Coli . Vet res 30, 203-219

25-Mounchid K., F. Bourjilat , N. Dersi , T. ,Aboussaouira , A. Rachidai ,A. Tantaoui-, A. Elaraki and M. Alaoui-Ismaili . 2005. The susceptibility of *Escherichia coli* strains to essential oils of Rormarinus officinalis and Eucalyptus globules. African Journal of Biotechnology Vol. 4 (10), pp. 1175-1176. http://www.academicjournals.org/AJB

26-Moujahed N., Y. Bouaziz , A. Ben Jannet and Z. Ghrabi . 2011. Nutritive value and essential oils characterization of Rosmarinus

officinalis and Thymus capitatus from the central region of Tunisia. Options Méditterranéennes, 99, 245-249
27-Mourey, A.; and N.Canillac, . 2002. Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control, 13 : 289-29.
28-Prabuseenivasan S., M. Jayakumar and S. Ignacimuthu ., 2006. In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 6:39. http://www.biomedcentral.com/1472-6882/6/39
29-Prescott L. M, J. P. Harley and D. A. Klein . 1995. Microbiologie. De Boeck ed. pp: 1014
30-Remmal A., T.Bouchikhi , K. Rhayour and M. Ettayebi . 1993. Improved methode for the determination of antimicrobial activity of essential oils in agar medium. J. Essent . Oil Res. 5: 179-184
31-Shahat A.A., A.Y. Ibrahim , S.F. Hendawy , E.A. Omer . F.M., Hammouda , F.H. Abdel-Rahman and M.A. Saleh .2011. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. Molecules, 16: 1366-1377.
32-Sienkiewicz M., M. Łysakowska, M. Pastuszka , W. Bienias and E. Kowalczyk . 2013. The Potential of Use Basil and Rosemary Essential Oils as Effective Antibacterial Agent. Molecules. 18, 9334 - 9351
33-Stefanini, M. B., L. C. Ming, M. O. M. Marques , R. Facanali , M.A.A. Meireles, , L.S.Moura, J.A., Marchese, and L.A. Sousa, . 2006. Essential oil constituents of different organs of fennel (Foeniculum vulgare var. vulgare). Rev. Bras. Pl. Med. 8, 193–198
34-Tani Z.B., M. A. Khelil , M. Bendahou and M. Mestari . 2009. Action des huiles essentielles de trois plantes aromatiques de l'Ouest algerien sur la mite Tineola Bisselliella. Rev. Ivoir. Sci. Technol., 14: 175 – 186
35-Vimal M., P.P. Vijaya , P. Mumtaj and M.S. Seema Farhath . 2013. Antibacterial activity of selected compounds of essential oils from indigenous Plants. J. Chem. Pharm. Res., 5(1):248-253
36-Wassenaar T.M., 2005. – The use of antimicrobial agents in veterinary medicine and implications for human health. Crit. Rev.Microbiol., 31, 155–169.
37-Xu J. F., Zhou , B.P. Ji , R.S. Pei and N. Xu . 2008. The antibacterial mechanism of carvacrol and thymol against Escherichia coli. Letters in Applied Microbiology 47, 174–179.