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

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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, thymol, essential oils, bactericidal effect

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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.

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\(^1\) 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* |
|--------------------------------|----------------------|-----------------------------|---------------------------|----------------------|--------------------------|---------------------|
| Myrcene                        | 6.55                 | 0.44                        | 2.53                      |                      |                          |                     |
| Para cymene                    | 7.19                 |                             |                           |                      |                          |                     |
| cis-Ocimene                    | 7.73                 |                             |                           |                      |                          |                     |
| α-Pinene, (-)-                 | 7.75                 | 0.12                        |                           |                      |                          |                     |
| γ terpinne                     | 7.79                 |                             |                           |                      |                          |                     |
| α-Pinene, (-)-                 | 8.28                 |                             |                           |                      |                          |                     |
| Linalol                        | 8.51                 | 1.09                        |                           |                      |                          |                     |
| Camphene                       | 8.74                 |                             |                           |                      |                          |                     |
| α-Phellandrene                 | 9.70                 |                             |                           |                      |                          |                     |
| 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                      |                      | 1.08                     |                     |
| α-Caryophyllene                | 13.08                | 1.08                        |                           |                      |                          |                     |
| α-Campholene                   | 15.63                |                             |                           |                      | 10.65                    |                     |
| Aldehyde                       | 16.47                |                             | 2.59                      |                      |                          |                     |
| Borneol                        | 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-Dihydroxadecamethylcycloheptasiloxane | 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

2 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*
**Table 3. Diameter of inhibition zones of essential oil against E. coli (cm)**

| Bacterial strain | Essential oil | Antibiotic | Oxytetracycline | Leaves | Seeds |
|------------------|---------------|------------|----------------|--------|--------|
| E. coli          | Foeniculum vulgare | Origanum compactum | Rosmarinus officinalis | Thymus capitatus | + Thymus capitatus | + Rosmarinus officinalis |
| 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 (5.1 cm), followed by the essential oil of _Origanum compactum_ (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_ oil against _E. coli_. 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.

**Table 4. Minimum inhibitory concentration and minimum bactéricidal concentration μl/ml**

| Bacterial strain | Essential oil | Leaf | seed |
|------------------|---------------|------|------|
| _E. coli_        | Foeniculum vulgare | 50   | 50   |
|                  | Origanum compactum | 3,125| >50  |
|                  | Rosmarinus officinalis | >50 | >50  |
|                  | Thymus capitatus | >50  | 6,25 |
|                  | + Thymus capitatus | >50  | 6,25 |
|                  | + Rosmarinus officinalis | >50 | >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 to 5% v/v. The essential oil of _Origanum compactum_ exerted strong antibacterial activity against _E. coli_ with small 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 Bkhly 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 14 oxygen-containing terpenes. According to Gulfraz et al., (1) 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 Ouarachi 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 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 comapatum* 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 Comapat* 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 monoterpenes 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 compactum (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.

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