SJIF Impact Factor: 3.458

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 2.
Issue: 5.
Page N. 27-35
Year: 2018

Original Article www.wjahr.com

IN VITRO AND *IN VIVO* STUDY OF ANTI-DERMATOPHYTIC ACTIVITY OF THYMOL, CARVACROL, EUGENOL AND MENTHOL

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Received date: 13 June 2018 **Revised date:** 04 July 2018 **Accepted date:** 25 July 2018

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ABSTRACT

The aim of this work is to test the anti-dermatophytic activity of some Major Compounds (MCs) of Essential oils (EOs) (eugenol, menthol, thymol and carvacrol) against three dermatophytes isolated from clinical cases of dermatophytosis (*Trichophyton violaceum*, *Microsporum canis* and *Trichophyton mentagrophyte*). The anti-dermatophytic activity of MCs was tested *in vitro* by using: the broth dilution method and the agar dilution method in order to determine the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC). The activity was then tested *in vivo* by using an experimental model of induced dermatophytosis in rabbits. Carvacrol and thymol revealed an anti-dermatophytic effect against all tested with (MIC) and (MFC) values between 250 µg/ml and 1000 µg/ml. The sporulation of target dermatophyte species decreased with increasing concentrations of MCs. As the most effective MC, thymol has shown a great effect on the treatment of skin dermatophytosis in rabbits caused by *T. violaceum* and *M.canis*. A significant reduction of the number of colonies sampled from the infected skin was observed at 1000 µg/ml compared to untreated control rabbits. These results demonstrated that thymol and carvacrol are potential sources of natural fungicides and could be used as an alternative to chemical anti-dermatophytic drugs.

KEYWORDS: Dermatophytes, Essential oils, Major compounds, Sporulation, Skin dermatophytosis, Rabbits.

INTRODUCTION

Dermatophytosis has been recognized as a public health problem in many parts of the world and has even reached endemic proportions in certain countries especially in Africa.[1][2] Their incidence has increased mainly in urban environments.[3] In farmed rabbits, dermatophytosis is a common fungal infection causing considerable morbidity and economic losses.[4,5]

Occasionally, it can spread to humans, and it is highly contagious among patients who are immunocompetent.[6]

Dermatophytes species are the most common causative agents including four genera: *Microsporum, Trichophyton, Arthroderma and Epidermophyton,*[7] the most common are: *T. violaceum, T. interdigital, T. mentagrophyte* and *M. canis.*[8,9]

Current medical therapy has many drawbacks and side effects which eventually lead to increasing resistance to conventional antifungal drugs.[10] For this reason, the search for therapeutic alternatives is crucial. Natural products with antifungal properties such as EOs have been considered for that over the past few decades.[11]

Medicinal plants are the richest natural source of bioactive phytochemicals and antioxidants, their monoterpenes are main compounds of their essential oils (Eos).[11] Several studies have focused on the anti-dermatophytic effect of Eos.[12][13] However, none of studies have investigated the effect of every single one of the compounds of these EOs on the growth and the proliferation of dermatophytes.[14][15]

The aim of the present study is to evaluate the anti-dermatophytic activity of four MCs of EOs on the

growth of dermathophytes *in vitro* and to elucidate *in vivo* the efficacy of thymol on an experimental model of rabbit dermatophytosis in order to develop an alternative to conventional drugs against skin dermatophytosis.

MATERIALS AND METHODS

Fungal Strains

The fungal strains used in this work, *T. violaceum, T. mentagrophyte* and *M. canis* were isolated from contaminated rabbits in farms located in Mhaya, Morocco. The confirmation of isolates identification was done using a conventional method based on the colonies' macroscopic characteristics and the microscopic appearance of conidia using the specific Sabouraud medium supplemented with chloramphenicol and cycloheximide (SAC) (Biokar).

Anti-dermatophytic agents

Thymol, carvacrol, menthol and eugenol are natural anti-dermatophytic components. All were purchased from Sigma-Aldrich (Steinhein, Germany). These phenolic major compounds were dispersed in a 0.2% sterile agar suspension.[16]

Culture media

The culture media used were Sabouraud Actidione-Chloramphénicol agar (SACa) (Biokar, France) and Sabouraud Actidione-Chloramphénicol broth (SACb) (Biokar, France). Cycloheximide was added as 0.5 g/l in medium for the inhibition of mould growth.[17][18] The media was used to measure the growth of dermatophytes and determine the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC).

Inoculum preparation

Conidia of dermatophytes were harvested from 10 day-old cultures in SAC by pouring a sterile 0.9% aqueous solution of NaCl onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia cells was adjusted to 10^6 CFU ml^{-1} and was determined by transferring 10µl of the sample suspension of dermatophyte conidia to a Malassez chamber for microscopic examination and counting. Dermatophyte conidia were counted in 10 different fields using standard techniques.[19]

Determination of the anti-dermatophytic effect of MCs in Agar medium

The various concentrations of the MCs (0, 125, 250, 500, 1000 µg/ml) were prepared from a stock suspension and were added to the medium. 20 ml of this mixture was poured in a Petri dish (85mm diameter).

The determination of the anti-dermatophytic effect of each MC in SAC agar medium was carried out by depositing 10 µl of a suspension of spores (10^6 spores/ml) on the surface of the SACa medium. Three Petri dishes were prepared for each strain and each concentration. Each Petri dish contained three drops of the same strain. After that, the Petri dishes were incubated at 27°C for 10 days.

The growth rate was determined daily by measuring the diameter of the thallus in centimeters for each concentration of MC_S and each strain. The results were compared to the control agar plate. The minimal inhibitory (MIC) was defined as the lowest MCs concentration at which no mycelial growth was visually observed. From these plates, agar discs were aseptically transferred to a new agar plate in order to determine the MFC.

The minimal fungicidal concentration (MFC) was defined as being the lowest anti-dermatophytic agent concentration at which there was a visual absence of growth after 5 days of incubation at 27°C. It was expressed by the percentage of growth inhibition of strain and calculated using the following formula:[20]

$$\mathbf{PI = ((C\text{-}T) / C)) \times 100}$$

Where **C** represents the mycelial growth diameters in control Petri dishes and **T** represents the growth diameters in treated Petri dishes.

Determination of the anti-dermatophytic effect of MCs in the Broth dilution method

The determination of the anti-dermatophytic effect of MCs by macrodilution method in a broth medium was conducted in triplicate using a sterile tube containing 100 µl of conidial suspension of the strain with an inoculum of 10^6 conidia/ml. To a volume of increasing concentrations of MCs (0, 125, 250, 500 and 1000 µg/ml), we added a volume of a SACb medium in order to have a final volume of 5ml in each tube. The mycelial growth was assessed by measuring optical density (OD) at 600 nm using spectrophotometer (J.P. Selecta) after 5 days of incubation at 27°C.

The anti-dermatophytic effect of MCs was expressed by the percentage of inhibition of each strain and calculated using the following formula:

$$\mathbf{PI = ((OC\text{-}\ OT) / OC)) \times 100}$$

Where **OC** represents the optical density of mycelial growth in control and **OT** represents the optical density of mycelial growth in treated tubes.

In order to determine the minimal fungicidal concentration (MFC), 100 µl from the tubes showing no growth were aseptically transferred into sterile tubes containing 4900 µl of a sterile SAC broth medium. The MFC was defined as the lowest anti-dermatophytic agent concentration at which no mycelial growth was visually observed after 5 days of incubation at 27°C.[21]

The dermatophytes sporulation assay

One colony of dermatophyte was taken from each petri dish and transferred into a capped tube containing 2 ml of sterile 0.9% aqueous solution of NaCl. The fungal

suspension was shaken vigorously at 300 rpm for 30 min to dislodge conidia, which were then counted using a Malassez chamber. Each isolate and each MC was tested with increasing concentrations (0, 125, 250, 500 and 1000 µg/ml).

Experimental skin dermatophytosis

This study was performed in accordance with the Principles of Laboratory Animal Care.

Animals

Male and female rabbits (n =32, 5 weeks old; weigh between 0.8 and 1.5 kg) were used in this study. They belong to the New Zealand breed and are supplied by a rabbit breeder. They were kept for one week and were randomized into groups of eight rabbits of the same sex in four cages. The photoperiod was adjusted daily to 12 h of light and 12 h of darkness. The environmental temperature was constantly maintained at 21°C ± 3°C. The rabbits were given food and water *ad libitum.*

Inoculum preparation

Two species of dermatophytes (*M. canis*, *T. violaceum*) were selected to infect the rabbits. The choice of these strains is explained by the fact that these fungi are responsible for dermatophytosis in rabbits.[5]

Skin dermatophytosis in rabbits

Samples were taken from the skin of all rabbits before infection. This action revealed the existence of four types of moulds: *A. niger, A. fumigatus* and *P. chrysogenum* as well as the yeast *C. albicans* and the absence of *T. violaceum* and *M. canis* at day 0.

The rabbits were locally infected every day for three weeks until a mycological lesion by scraping a 14-day culture of *T.violaceum* and *M.canis* on a SACa medium was obtained. Then, Portions (50µl) of fungal suspension containing approximately 10^5- 10^6 conidia were applied with a bent glass rod, on the cutaneous surface of the back and the belly of the previously shaved and scraped animals with a single-use manual razor in order to make the skin more disposed to infection.[22]

Anesthesia

During the shaving period and infection, the rabbits were anesthetized intramuscularly with an anesthetic combination containing: xylazine (5mg/kg body weight) and ketamine (35 mg/kg body weight), the lesion was then covered with a dressing for at least 48 hours. All groups were sampled in the same way to confirm the presence of the dermatophytes and to determine the number of CFU in the skin before the beginning of the treatment.

Skin suspension for treatment

The treatment was done using thymol at a final concentration of 1000 µg/ml. It was dispersed in a viscous solution of 0.2% agar.

Anti-dermatophytic treatment

Animals in the treatment group received treatment with thymol on the infected area for twenty-eight consecutive days. Meanwhile infected and untreated animals only received a locally applied 0.2% agar solution.

Quantification of infection level and microbiological determination of therapeutic efficacy

The evolution of the lesions was monitored weekly by scraping a sterile cotton swab on the infected skin surface. The cotton swab was then suspended in 1 ml of sterile saline solution (NaCl 0.9%). These samples were diluted to 1/10. 20 µl of each sample and were spread on Petri dishes containing a SAC agar medium. The colonies were counted after 7 to 10 days of incubation at 27°C. The number of viable cells was determined using the drop count method to calculate the log CFU/ml.

Statistical test

The results are presented by the means and their standard error. The data were analyzed by T-test using Sigma Stat 4.0. The significance was verified for the mycelial growth inhibition and microbiological study of therapeutic efficacy of thymol against skin dermatophytosis in rabbits. The significance level chosen for both tests is 5% at $p<0.05$.

RESULTS

Determination of anti-dermatophytic effect of MCs in an agar medium

Table 1: MIC and MFC of the tested MCs on dermatophytes in Agar.

	Thymol (µg/ml)		Carvacrol (µg/ml)		Eugenol (µg/ml)		Menthol (µg/ml)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
M. canis	250	250	125	125	250	500	2000	2000
T.violaceum	250	250	250	250	500	500	2000	2000
T. mentagrophyte	250	500	125	250	500	1000	4000	8000

Values of MIC and MFC of the tested MCs on dermatophytes in an agar medium are shown in table 1. MIC and MFC values ranged between 125 and 250 µg/ml for carvacrol and thymol respectively. These MCs were efficient against the three strains of dermatophytes. For eugenol, the three strains reacted differently and required MIC values between 250 and 500 µg/ml and MFC between 500 and 1000 µg/ml. regarding menthol, all strains showed a MIC and MFC values higher than the previous three compounds.

Table 2: Inhibitory effect of MCs on the growth of *M.canis.*

Major compounds	Mycelial growth inhibition (%)				
	0µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	21.05 ± 0.08	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Carvacrol	0.00 ± 0.00	20.39 ± 0.064	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Eugenol	0.00 ± 0.00	29.60 ± 0.048	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Menthol	0.00 ± 0.00	5.26 ± 0.064	8.55 ± 0.064	9.86 ± 0.064	10.52 ± 0.064

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test.

Table 3: Inhibitory effect of MCs on the growth of *T. violaceum.*

Major compounds	Mycelial growth inhibition (%)				
	0µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	28.67 ± 0.048	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Carvacrol	0.00 ± 0.00	27.97 ± 0.048	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Eugenol	0.00 ± 0.00	52.50 ± 0.08	57.50 ± 0.40	100.00 ± 0.00*	100.00 ± 0.00*
Menthol	0.00 ± 0.00	4.19 ± 0.088	9.79 ± 0.032	15.38 ± 0.064	27.27 ± 0.064

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test

Table 4: Inhibitory effect of MCs on the growth of *T. mentagrophyte.*

Major compounds	Mycelial growth inhibition (%)				
	0µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	28.00 ± 0.00	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Carvacrol	0.00 ± 0.00	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Eugenol	0.00 ± 0.00	25.60 ± 0.066	56.80 ± 0.00	100.00 ± 0.00*	100.00 ± 0.00*
Menthol	0.00 ± 0.00	13.20 ± 0.027	13.20 ± 0.033	18.40 ± 0.044	21.60 ± 0.066

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test

The inhibitory effect of thymol, carvacrol, eugenol and menthol on the mycelial growth of different strains in an agar medium is shown in tables 2, 3 and 4. The most important activity was observed with thymol and carvacrol followed by eugenol and menthol.

For thymol and carvacrol, the inhibition of the mycelial growth of the three strains ranged between 250µg/ml and 1000 µg/ml. for eugenol, a complete mycelial growth inhibition was observed at 250 µg/ml for *M. canis* and 500 µg/ml for *T. violaceum* and *T. mentagrophyte*. Menthol was the least active inhibitor tested, since at the concentration of 500 µg/ml the reduction for *M. canis* of mycelial growth inhibition was only 9.8%.

Determination of anti-dermatophytic effect of MCs in a liquid medium

Table 5: MIC and MFC of tested MCs on dermatophytes in liquid medium.

	Thymol (µg/ml)		Carvacrol (µg/ml)		Eugénol (µg/ml)		Menthol (µg/ml)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
M. canis	125	250	125	250	250	500	500	1000
T. violaceum	125	125	125	125	500	1000	1000	2000
T. mentagrophyte	250	500	250	500	500	1000	2000	4000

Table 5 shows that thymol and carvacrol have a potent effect on all three strains, with MIC values ranging from 125 µg/ml to 250 µg/ml and a MFC values ranging from 125 µg/ml to 500 µg/ml. Eugenol required higher MIC value and MFC value than thymol and carvacrol. Menthol showed the least anti-dermatophytic activity with MIC values between 500 and 2000 µg/ml and MFC values between 1000 and 4000 µg/ml.

Table 6: Inhibitory effect of MCs on the growth of *M.canis* in liquid medium.

Major compounds	Mycelial growth inhibition (%)				
	0 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	99.50 ± 0.07*	99.50 ± 0.00*	99.60 ± 0.00	100.00 ± 0.00*
Carvacrol	0.00 ± 0.00	99.70 ± 0.11*	99.80 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*
Eugenol	0.00 ± 0.00	79.10 ± 0.07	100.00 ± 0.12*	100.00 ± 0.00*	100.00 ± 0.00*
Menthol	0.00 ± 0.00	43.22 ± 0.12	84.80 ± 0.09	99.30 ± 0.11*	100.00 ± 0.00*

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test.

Table 7: Inhibitory effect of MCs on the growth of *T. violaceum* in liquid medium.

Major compounds	Mycelial growth inhibition (%)				
	0µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	98.70 ± 0.09*	98.90 ± 0.03*	99.10 ± 0.00*	99.36 ± 0.00*
Carvacrol	0.00 ± 0.00	98.30 ± 0.02*	98.90 ± 0.034*	99.14 ± 0.00*	99.39 ± 0.00*
Eugenol	0.00 ± 0.00	70.06 ± 0.047	98.50 ± 0.062*	99.04 ± 0.00*	99.20 ± 0.00*
Menthol	0.00 ± 0.00	20.30 ± 0.033	28.00 ± 0.05	34.39 ± 0.066	98.40 ± 0.00*

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test.

Table 8: Inhibitory effect of MCs on the growth of *T. mentagrophyte* in liquid medium.

Major compounds	Mycelial growth inhibition (%)				
	0µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Thymol	0.00 ± 0.00	96.10 ± 0.032*	98.00 ± 0.04*	98.50 ± 0.00*	100.00 ± 0.00*
Carvacrol	0.00 ± 0.00	96.30 ± 0.063*	98.30 ± 0.00*	98.50 ± 0.00*	100.00 ± 0.00*
Eugenol	0.00 ± 0.00	75.30 ± 0.03	93.40 ± 0.05*	97.30 ± 0.00*	99.90 ± 0.00*
Menthol	0.00 ± 0.00	38.05 ± 0.044	97.80 ± 0.066*	98.20 ± 0.00*	99.00 ± 0.00*

Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test.

The effect of MCs on the growth of dermatophytes in a liquid medium is shown in Tables 6, 7 and 8. The results indicate that the percentage of the mycelial growth inhibition was significantly influenced by the nature of the strains and the concentration of the major compounds used in this experiment.

The results also showed that thymol and carvacrol were the most efficient MCs with a percentage of inhibition higher than 96% at low concentrations (125 µg/ml) for the three strains, followed by eugenol with a percentage of inhibition of 70%. Menthol was the least active MC with a percentage of inhibition of 98.2% at a relatively high concentration (500 µg/ml and 1000 µg/ml).

The dermatophytes sporulation assay in an agar medium

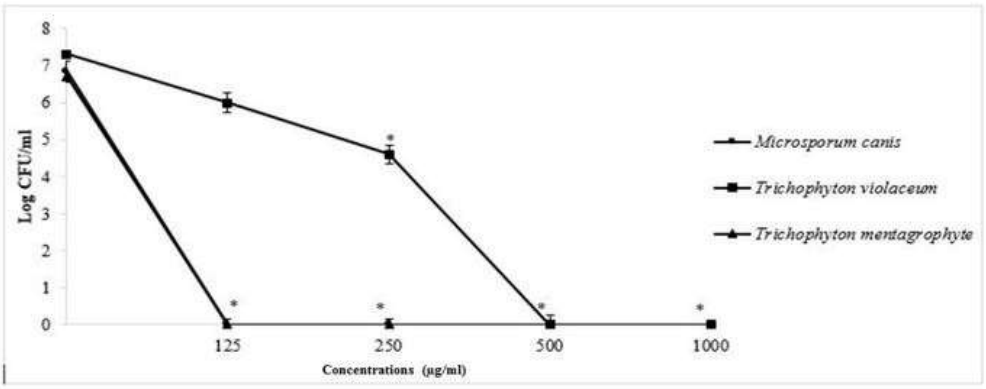

Fig. 1: Effect of thymol on sporulation of dermatophytes in agar medium.

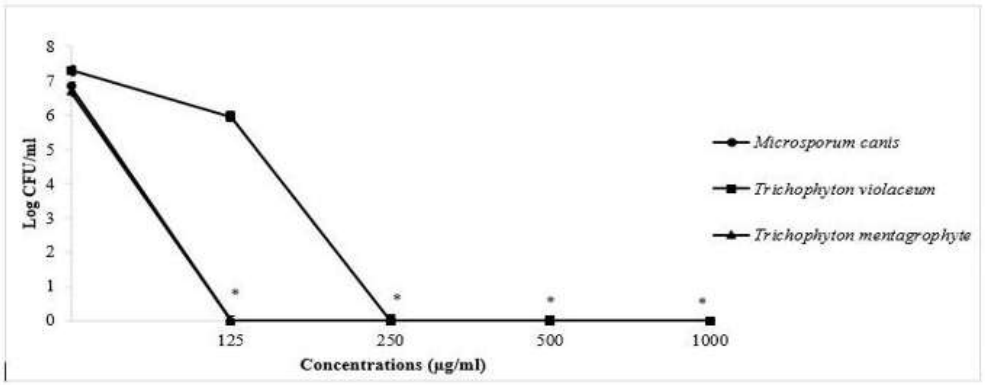

Fig. 2: Effect of carvacrol on sporulation of dermatophytes in agar medium.

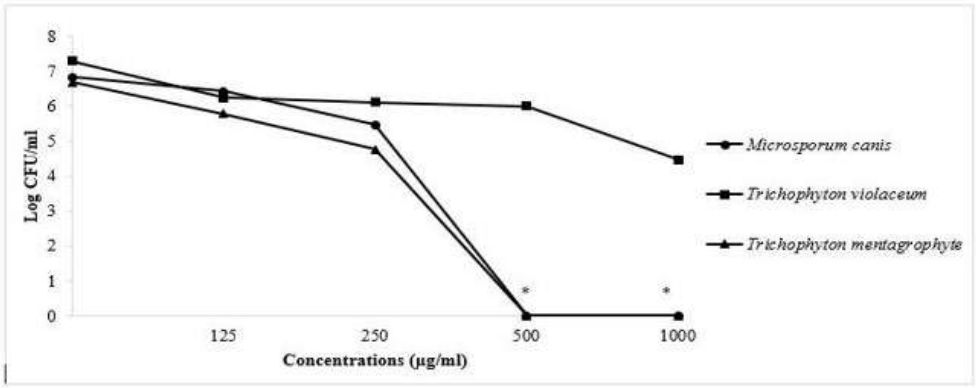

Fig. 3: Effect of eugenol on sporulation of dermatophytes in agar medium.

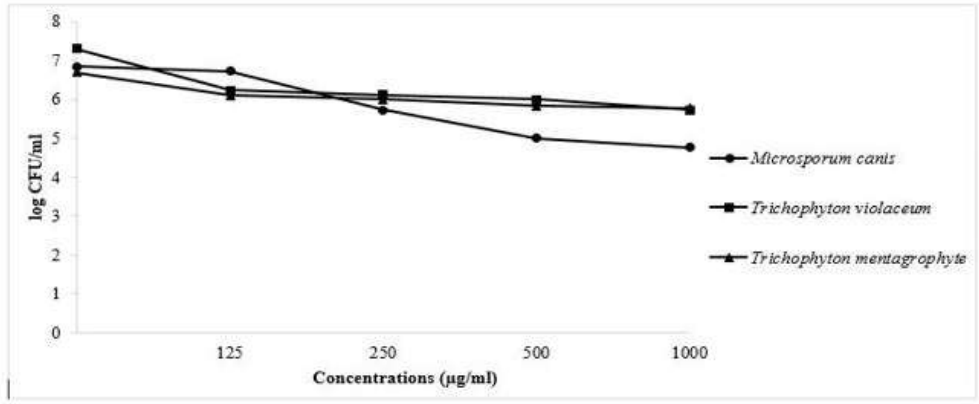

Fig. 4: Effect of menthol on sporulation of dermatophytes in agar medium.

The figures 1, 2, 3 and 4 show that the most effective inhibitors of sporulation of dermatophytes in an agar medium were thymol and carvacrol with an inhibition value of 100% at 125 µg/ml for *T. mentagrophyte* and *M. canis*. For eugenol, a complete inhibition of the sporulation was obtained at 500 µg/ml, while the effect of menthol was the lowest.

Experimental skin dermatophytosis

Table 9: Microbiological study of therapeutic efficacy of thymol against *T. violaceum* and *M. canis* in rabbit's skin dermatophytosis.

	Infection		After 15 days of treatment			After 28 days of treatment		
	Infected animals (%)	Log CFU ± SD	Infected animals (%)	Log CFU ± SD	CFU reduction (%)	Infected animals (%)	Log CFU ± SD	CFU reduction (%)
control	8/8 (100%)	4.30 ±0.26	8/8 (100%)	4.67 ± 0.13	---	8/8 (100%)	4.57±0.17	---
T. violaceum	8/8 (100%)	4.57 ±0.20	8/8 (100%)	4.27 ±0.21*	60.52	2/8 (25%)	3.92±0.19*	77.63
control	8/8 (100%)	4.06 ±0.26	8/8 (100%)	5.23 ± 0.26	---	8/8 (100%)	5.22 ±0.25	---
M. canis	8/8 (100%)	4.83 ±0.09	8/8 (100%)	5.10 ± 0.17	26.98	1/8 (12.5%)	4.22±0.06*	89.88

The rabbits were locally infected every day for three weeks with a suspension containing approximately 10^5- 10^6 conidia of *T.violaceum* and *M. canis*. Thymol was applied locally on the infected area for twenty-eight consecutive days 3 days after the last infection. Data are Means (n=3) ± SD, * indicate significant difference ($p<0.05$) according to t-test.

Table 9 shows the microbial evaluation of the effect of thymol treatment after infection with *M. canis* on the back of rabbits and *T. violaceum* on their belly. Fifteen days after the treatment, animals treated with thymol remained infected but the mean log CFU/swab decreased significantly ($p<0.05$). The percentage of reduction of CFU compared to the control was 26.98% for *M. canis* strain and 60.52% for *T. violaceum.*

After 28 days of treatment, *T. violaceum* and *M. canis* were detected respectively for only 1/8 and 2/8 of animals in thymol-treated group. Moreover, there was a significant percentage decrease of CFU of *T. violaceum* compared with the control (77.63%). An even larger decrease of CFU of *M. canis* (89.88%) was noted when compared with the control.

DISCUSSION

1- Choice of anti-fungal agents

Essential oils are generally complex mixtures of monoterpenes and sesquiterpenes.[23] They are known for their anti-dermatophytic activity.[24] However, in some cases, it is very difficult to determine the active ingredient of the EO and the efficient dose to apply while preventing the risk of toxicity.[25]

The four MCs used in this study were chosen based on scientific reports on their reputation as strong anti-fungal agents.[26][27][28] As far as we know, this is the first time that MCs are used to control the growth of dermatophytes *in vitro*, all previous studies focused on the use of EOs and not their MCs.

2- Determination of MIC and MFC in an agar and in a liquid medium

The main aim of this study was to evaluate *in vitro* the anti-dermatophytic activity of some MCs using both broth and agar dilution methods using an improved method of MC dispersion.[29][18][27] reported that the dilution method is the best among several methods widely used.

The results in an agar medium show that carvacrol and thymol have the lowest MIC values for the three strains of dermatophytes tested. Thymol recorded MIC values of 250 µg/ml and MFC values between 250 and 500 µg/ml, meanwhile carvacrol had MIC values and MFC values between 125 and 250 µg/ml. These MIC and MFC values are much lower than the values described by other authors.[30][31][32]

We have also determined the MIC and MFC values in a liquid medium. The results obtained showed that the MIC values of thymol and carvacrol ranged between 125 and 250 µg/ml while eugenol MIC values ranged between 250 and 500 µg/ml. These MIC and MFC values are well below the values described by.[33][34][35] Although there are many studies that evaluate the anti-dermatophytic activity of thyme essential oil that is rich in thymol and carvacrol responsible for the anti-dermatophytic effect,[36] but their antifungal activity was lower than that obtained by the major compounds because it is difficult to attribute the activity of complex mixtures.[37]

These results show that the method in a liquid medium is more efficient and that the anti-dermatophytic power of the four compounds tested was observed at low concentrations compared to the dispersion method in the agar. These results are consistent with the work of[38][39] who showed that the inhibitory effects of the four major compounds on the mycelium growth of pathogenic dermatophytes depended on the type of dermatophytes used more precisely the size of conidia inoculum,[40][41] the method used and the use of detergents or solvents that influence the anti-dermatophytic activity.[29]

The results of the MIC and MFC obtained on an agar and a liquid medium show that thymol and carvacrol have an anti-fungal dose-dependent activity with values much lower than those described by other studies.

3- Effect of MC on sporulation of the dermatophytes

While testing MCs anti-dermatophytic effect in an agar medium, we have noticed a color change of the thalli formed in the treated petri dishes compared to the thalli formed in the control ones. In this context, we have demonstrated a direct effect of MCs on the sporulation process by counting the number of spores after 5 days of incubation.

The results obtained showed that there is a significant decrease in the number of spores as the concentration of MCs increases. Thymol and carvacrol had the greatest effect to prevent sporulation especially that of *T. mentagrophyte* and *M. canis* strains at a lower concentration than the MIC values. Our results are match those obtained by[38] who tested the action of thyme and rosemary EOs on dermatophytes. However, the concentrations they used to inhibit the sporulation are higher than ours.

4- Skin dermatophytosis

The results obtained in this study prompted us to look for an application of MCs for treating dermatophytosis. We therefore decided to test the anti-dermatophytosis effect of thymol in rabbits, since it is the MC that proved to be the most effective. To our knowledge, this is the first time MCs are used for this purpose, all existing data shows the anti-dermatophytic effect of EOs only.

In the present study we demonstrated a significant clinical efficacy of thymol against *M. canis* and *T.violaceum* dermatophytosis.

The results obtained after 28 days of *in vivo* treatment indicate that thymol yielded a total recovery of seven subjects out of eight for animals infected with *M. canis*, while eight out of eight control subjects showed skin redness and lesion severity. Our results are in concordance with [13] who reported *in vivo* the use of an active EOs mixture to treat cats infected with *M. canis.*

Three days after the last treatment of thymol, there was no recurrence of the dermatophytosis. The cure rate demonstrated in the present study was comparable to that reported in previous studies with a shorter and similar treatment duration.[42][43][44]

The results of this test show that our experimental conditions were sufficient enough to cause a lasting dermatophytosis and to evaluate the effectiveness of the treatment used. Our results suggest also that thymol may be considered as a potential drug with better efficacy for the treatment of dermatophytosis. Its potent efficacy in the rabbit model suggests that it may be of value to investigate the possibility of treating humans suffering from dermatophytosis, especially when the infection is refractory to known antifungal drugs.

CONCLUSION

This work is a fundamental contribution toward determining the anti-dermatophytic action of MCs and the development of an innovative preparation with local application for a preliminary clinical study of dermatophytosis treatment.

ACKNOWLEDGEMENT

This work is a partial fulfillment of Imane Jabeur's PhD thesis, which is deeply grateful to Miss Imane Remmal & Mrs Helene Mock for their assistance in checking English of the manuscript.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Nweze E I, Okafor J I. Prevalence of dermatophytic fungal infections in children: a recent study in Anambra State, Nigeria. Mycopathologia, 2005; 160(3), 239-243.
2. Nweze E I. Dermatophytosis in Western Africa: a review. Pak J Biol Sci, 2010; 13(13): 649-56.
3. Ginter-Hanselmayer G, Weger W, Ilkit M, Smolle J. Epidemiology of *tinea capitis* in Europe: current state and changing patterns. Mycoses, 2007; 50(s2): 6-13.
4. Cafarchia C, Camarda A, Coccioli C, Figueredo L A, Circella E, Danesi P, Otranto D. Epidemiology and risk factors for dermatophytoses in rabbit farms. Med Mycol, 2010; 48(7): 975-980.
5. Cafarchia C, Weigl S, Figueredo L A, Otranto D. Molecular identification and phylogenesis of dermatophytes isolated from rabbit farms and rabbit farm workers. Vet Microbiol, 2012; 154(3-4): 395-402.
6. Ansari S, Hedayati M T, Zomorodian K, Pakshir K, Badali H, Rafiei A, Seyedmousavi S. Molecular characterization and *in vitro* antifungal susceptibility of 316 clinical isolates of dermatophytes in Iran. Mycopathologia, 2016; 181(1-2): 89-95.
7. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev, 1995; 8: 240 – 259.
8. Ahmad M, Gupta S, Gupte S. A clinico-mycological study of onychomycosis. Egypt. Dermatol. Online J, 2010; 6(4).
9. Bassiri-Jahromi S, Khaksar A A. Non dermatophytic moulds as a causative agent of onychomycosis in Tehran. Indian J. Dermatol, 2010; 55(2): 140.
10. Yamada T, Maeda M, Alshahni M M, Tanaka R, Yaguchi T, Bontems O, Salamin K, Fratti M, Monod M. Terbinafine resistance of *Trichophyton* clinical isolates caused by specific point mutations in the squalene epoxidase gene. Antimicrob. Agents Chemother, 2017; 61(7): e00115-17.
11. Lopes G, Pinto E, Salgueiro L (2017). Natural products: an alternative to conventional therapy for dermatophytosis. Mycopathologia, 2000; 182(1-2): 143-167.
12. Fontenelle R O, Morais S M, Brito E H, Brilhante R S, Cordeiro R A, Lima Y C, Rocha M F. Alkylphenol activity against *Candida spp.* and *Microsporum canis*: a focus on the antifungal activity of thymol, eugenol and O-methyl derivatives. Molecules, 2011; 16(8): 6422-6431.
13. Mugnaini L, Nardoni S, Pinto L, Pistelli L, Leonardi M, Pisseri F, Mancianti, F. *In vitro* and *in vivo* antifungal activity of some essential oils against feline isolates of *Microsporum canis.* J. Mycol. Med, 2012; 22(2): 179-184.
14. Cavaleiro C, Pinto E, Goncalves M J, Salgueiro L. Antifungal activity of Juniperus essential oils against dermatophyte, *Aspergillus* and *Candida* strains. J. Appl. Microbiol, 2006; 100(6): 1333-1338.
15. Dias N, Dias M C, Cavaleiro C, Sousa M C, Lima N, Machado M. Oxygenated monoterpenes-rich volatile oils as potential antifungal agents for dermatophytes. Nat. Prod. Res, 2017; 31(4): 460-464.
16. Ellabib M S, Khalifa Z M. Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. Ann Saudi Med, 2001; 21(3/4): 193-195.
17. Georg L K. Use of a cycloheximide medium for isolation of dermatophytes from clinical materials. AMA Arch Derm Syphilol, 1953; 67(4): 355-361.
18. Remmal A, Bouchikhi T, Tantaoui-Elaraki A, Ettayebi M. Inhibition of antibacterial activity of essential oils by Tween 80 and ethanol in liquid medium. J. Pharm. Belg, 1993b; 48: 352-356.
19. Ryley J F, Meade R, Hazelhurst J, Robinson T E. Methods in coccidiosis research: separation of oocysts from faeces. Parasitology, 1976; 73(3): 311-326.
20. Ali-Shtayeh M S, Abu Ghdeib S I .Antifungal activity of plant extracts against dermatophytes. Mycoses, 1999; 42(11-12): 665-672.
21. Rodriguez-Tudela J L, Arendrup M C, Arikan S, Barchiesi F, Bille J, Chryssanthou E, Fegeler W. EUCAST DEFINITIVE DOCUMENT E. DEF 9.1: Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds. Clin Microbiol Infect, 2008; 14: 982-4.

22. Flores F C, Beck R C, da Silva C D B. Essential oils for treatment for onychomycosis: a mini-review. Mycopathologia, 2016; 181(1-2): 9-15.
23. Isman M B. Plant essential oils for pest and disease management. Crop prot, 19(8-10): 603-608.
24. Saunte D M, Simmel F, Frimodt-Moller N, Stolle L B, Svejgaard E L, Haedersdal M, Arendrup M C. *In vivo* efficacy and pharmacokinetics of voriconazole in an animal model of dermatophytosis. Antimicrob Agents Chemother, 2007; 51(9): 3317-3321.
25. Zuzarte M, Gonçalves M J, Canhoto J, Salgueiro L. Antidermatophytic activity of essential oils. Science against microbial pathogens: communicating current research and technological advances, 2011; 2: 1167e78.
26. Bennis S, Chami F, Chami N, Bouchikhi T, Remmal A. Surface alteration of Saccharomyces cerevisiae induced by thymol and eugenol. Lett Appl Microbiol, 2004; 38(6): 454-458.
27. Bouddine L, Louaste B, Achahbar S, Chami N, Chami F, Remmal A. Comparative study of the antifungal activity of some essential oils and their major phenolic components against Aspergillus niger using three different methods. AJB, 2012; 11(76): 14083-14087.
28. Oukhouia M, Sennouni C.I, Jabeur I, Hamdani H, Remmal A. *In-vitro* Study of Anti-Fusarium Effect of Thymol, Carvacrol, Eugenol and Menthol. J. Plant.Pathol. Microbiol, 2017; 8(10): 423.
29. Remmal A, Tantaoui-Elaraki A, Bouchikhi T, Rhayour K, Ettayebi M. Improved method for the determination of antimicrobial activity of essential oils in agar medium. J. Essent. Oil Res, 1993a; 5: 1179–1184.
30. Mugnaini L, Nardoni S, Pistelli L, Leonardi M, Giuliotti L, Benvenuti M N, Pisseri F, Mancianti F. A herbal antifungal formulation of *Thymus serpillum*, *Origanum vulgare* and *Rosmarinus officinalis* for treating ovine dermatophytosis due to *Trichophyton mentagrophyte*. Mycoses, 2013; 56(3): 333-337.
31. Nardoni S, Tortorano A, Mugnaini L, Profili G, Pistelli L, Giovanelli S, Pisseri F, Papini R, Mancianti F. Susceptibility of *Microsporum canis* arthrospores to a mixture of chemically defined essential oils: a perspective for environmental decontamination. Z. Naturforsch, 2015; 70(1-2): 15-24.
32. Cavaleiro C, Salgueiro L, Gonçalves M J, Hrimpeng K, Pinto J, Pinto E. Antifungal activity of the essential oil of Angelica major against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. J. Nat. Med, 2015; 69(2): 241-248.
33. Pinto E, Pina-Vaz C, Salgueiro L, Gonçalves M J, Costa-de-Oliveira S, Cavaleiro C, Martinez-de-Oliveira J. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J .Med. Microbiol, 2006; 55(10): 1367-1373.
34. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. J .Med. Microbiol, 2009; 58(11): 1454-1462.
35. Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Martinez-de-Oliveira J. Antifungal activity of *Thymus* oils and their major compound. J. Eur. Acad. Dermatol. Venereol, 2004; 18(1): 73-78.
36. Bassolé I H N, Juliani H R. Essential oils in combination and their antimicrobial properties. Molecules, 2012; 17(4): 3989-4006.
37. Vale-Silva L A, Gonçalves M J, Cavaleiro C, Salgueiro L, Pinto E. Antifungal activity of the essential oil of *Thymus x viciosoi* against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. Planta Med, 2010; 76(09): 882-888.
38. Ouraïni D, Agoumi A, Ismaïli-Alaoui M, Alaoui, K, Cherrah Y, Amrani M, Belabbas M A. Étude de l'activité des huiles essentielles de plantes aromatiques à propriétés antifongiques sur les différentes étapes du développement des dermatophytes Study of the activity on the various stages of development of dermatophytes of essential oils from aromatic Plants with antifungal properties. Phytothérapie, 2005; 3(4): 147-157.
39. Suhr K I, Nielsen P V. Anti-dermatophytic activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J Appl Microbiol, 2003; 94(4): 665-674.
40. Pujol I, Guarro J, Sala J, Riba M D. Effects of incubation temperature, inoculum size, and time of reading on broth microdilution susceptibility test results for amphotericin B against Fusarium. Antimicrob Agents Chemother, 1997; 41: 808-811.
41. Sabulal B, George V, Dan M, Pradeep N S. Chemical composition and antimicrobial activities of the essential oils from the *rhizomes* of four *Hedychium* species from South India. J. Essent. Oil Res, 2007; 19(1): 93-97.
42. Saunte D M, Hasselby J P, Brillowska-Dabrowska A, Frimodt-Møller N, Svejgaard E L, Linnemann D, Arendrup M C. Experimental guinea pig model of dermatophytosis: a simple and useful tool for the evaluation of new diagnostics and antifungals. Medical mycology, 2008; 46(4): 303-313.
43. Soković M, Glamočlija J, Ćirić A, Kataranovski D, Marin P D, Vukojević J, Brkić D. Anti-dermatophytic activity of the essential oil of *Thymus vulgaris L.* and thymol on experimentally induced dermatomycoses. Drug Dev Ind Pharm, 2008; 34(12): 1388-1393.
44. Prasad C S, Shukla R, Kumar A, Dubey N K. *In vitro* and *in vivo* antifungal activity of essential oils of *Cymbopogon martini* and *Chenopodium ambrosioides* and their synergism against dermatophytes. Mycoses, 2010; 53(2): 123-129.