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Antiradical, Anti-inflammatory and Antifungal Activities of Essential Oils of Two Aromatic Plants: *Apium graveolens* (Apiaceae) and *Thymus vulgaris (Lamiaceae)*

Marguerite Simo Kamdem[1], Modeste Lambert Sameza[2], Pierre Michel Jazet Dongmo[2], Fabrice Fekam Boyom[1], Issakou Bakargna-Via[1], Jean Baptiste Hzounda Fokou[1], Ide Flavie Kenfack Tsague[1], Elisabeth Zeuko'o Menkem[1], Paul Henri Amvam Zollo[3] and Chantal Menut[4]

1. Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé, P.O Box 812, Cameroon

2. Department of Biochemistry, Faculty of Science, University of Douala, Douala, P.O Box 24157, Cameroon

3. ENSAI, University of Ngaoundere, Ngaoundere, P.O Box 455, Cameroon

4. IBMM, Montpellier-UMR 5247, Faculté de Pharmacie, 15 Avenue Charles Flahault, Montpellier 34093, France

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Abstract: The outbreak of oxidative stress, inflammatory diseases and mycoses, constitute an important public health problem. This could be due to the increase of risk factors, side effects and expensive therapeutic molecules available. In the aim to find the potential spring of new therapeutic molecules with efficient and wide spectrum of action, the antiradical, anti-inflammatory and antifungal activities of two essential oils were evaluated. The essential oils were extracted by hydrodistillation. The antiradical activity was evaluated to use DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method and the anti-inflammatory activity was determined using the enzymatic method. The disc diffusion and dilution methods were used to evaluate growth inhibition of three yeasts, three moulds and three dermatophytes. The yields of extraction of *Apium graveolens* and *Thymus vulgaris* essential oils were 0.14% and 0.32% respectively. These essential oils showed antiradical properties with respective SC_{50} of 0.41 and 0.06 g/L for *Apium graveolens* and *Thymus vulgaris*. Only *Thymus vulgaris* presented an anti-inflammatory activity with an IC_{50} of 0.19 g/L. *Cryptococcus neoformans* was the most susceptible fungal strain while *C. albicans* was the most resistant one. The results were compared with the standard antifungal. These results show that these essential oils could be exploited as potential spring of molecules endowed with antiradical, anti-inflammatory and antifungal activities.

Key words: Essential oil, antiradical, anti-inflammatory, antifungal.

1. Introduction

The dysfunctions of oxygen metabolism generate an excess of reactive chemical species known as ROS (reactive oxygen species), and among them are free radicals (like $^{\cdot}OH$, $O_2^{\cdot-}$, RO_2), and non-radical products (like H_2O_2, RO_2H). These species, particularly the radical species, create oxidative damages on biological macromolecules (DNA, lipids and proteins) which can considerably disturb the cell machinery [1]. Chronic stress and malnutrition represents 90% of the causes of immunodepression in subjects without basal pathologies, and are key to the entry into the epithelial and endothelial tissues of fungal species coexisting in the environment [2]. Oxidative stress is involved in numerous pathologies (atherosclerosis, diabetes, neurodegenerative diseases, cancer…) and in the aging process [1]. In order to prevent this, synthetic antioxidants such as BHA (butyl hydroxyanisole) and BHT (butyl hydroxytoluene) are used to stabilize these products for a long time. Due to the known detrimental effects of BHA and BHT, there has been a

Corresponding author: Pierre Michel Jazet Dongmo, Ph.D., research field: biochemistry and natural products. E-mail: mjazet@yahoo.com.

remarkable growth in the demand for natural antioxidants within the past few years. Recently, researchers have focused on natural antioxidants such as essential oils which are used for many purposes including flavoring foods and beverages, aroma additives for cosmetics and household products, and masking agents for unpleasant odors. These essential oils are extracted from aromatic plants which are widely used in traditional medicine to treat many diseases. For example, *Thymus vulgaris* plant is used in traditional medicine of Cameroon against respiratory infections (asthma, cough, whooping cough, bronchitis), rheumatism and the tea are consumed as stimulant by elderly person [3]. Previous reports showed various biological actions of essential oils such as antifungal, antibacterial, antimicrobial, hypolipidemic, antioxidant and anti-inflammatory properties [4-7]. The study aimed to evaluate the antiradical, anti-inflammatory and antifungal potentials of essential oils of leaves of *A. graveolens* and *T. vulgaris* (leaves and stem).

2. Materials and Methods

2.1 Plant Material and Extraction Procedure

The fresh leaves of *A. graveolens* (Celery) and *T. vulgaris* (thyme) were collected in Yaoundé and Dschang in August 2012 respectively. They were identified at the Cameroon National Herbarium in Yaounde with identification numbers 25582/SRF-Cam and 42851-Cam respectively where voucher specimens were deposited. The essential oils tested were extracted by the hydrodistillation method using Clevenger-type apparatus. The recovered oils were dried over anhydrous sodium sulphate and stored at 4 °C.

2.2 Fungal Strains

The fungal strains used in the study were obtained from the "Centre Pasteur" of Yaounde, Cameroon. These strains were three yeasts (*Candida albicans* ATCC24433, *Candida glabrata* CIPA35, *Cryptococcus neoformans* IP 95026), three moulds (*Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*) and three dermatophytes (*Epidermophyton floccosum* E1423, *Microsporum gypseum* E1420, *Trichophyton mentagrophytes* E1425).

2.3 Analysis of Essential Oils

The essential oils obtained were analysed by GC (gas chromatography) and GC/MS (gas chromatography coupled with mass spectrometry).

2.3.1 Gas Chromatography

The oils were analysed on a Varian CP-3380 GC with flame ionization detector fitted with a fused silica capillary column (30 m × 0.25 mm coated with DB5, film thickness 0.25 μm); temperature program 50-200 °C at 5 °C/min, injector temperature 200 °C, detector temperature 200 °C, carrier gas nitrogen (N_2), 1 mL/min. The linear retention indices of the components were determined to use the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking relative response factors into account.

2.3.2 Gas Chromatography Coupled with Mass Spectrometry

GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a quadrupole detector (GC-quadrupole MS system, model 5970). The column temperature was programmed from 70-200 °C at 10 °C/min; injector temperature was 200 °C. Helium was used as the carrier gas at a flow rate of 0.6 mL/min; the mass spectrometer was operated at 70 eV.

The constituents were identified on the basis of the comparison of their retention indices and mass spectra with those given in the literature [8].

2.4 Determination of Antiradical Activity

The antiradical activity of the essential oil was

determined using scavenging 2, DPPH (2-diphenyl-1-picrylhydrazyl) method according to Brand-Williams et *al.* [9], and Cuvelier et al. [10]. This stable free radical scavenger was dissolved in ethanol for a stock solution (0.4 g/L) then a serial dilution was realized. The ethanolic solution of DPPH (1900 µL) was mixed with the methanolic solution of BHT (100 µL) at different concentrations. The essential oil was tested using the same method. The control was made of the DPPH ethanolic solution with methanol. The absorbance was measured at 517 nm during 1 h, at room temperature. The decrease in absorption induced by the test compound was calculated by substracting that of the control. The concentration required for 50% reduction (50% scavenging concentration, SC50) was determined graphically. All the spectrophotometric measurements were performed using a SAFAS UV-mc2 Spectrophotometer, equipped with a multi-cells/ multikinetic measure system and with a thermostated cell-case.

2.5 Determination of Anti-inflammatory Activity

The anti-inflammatory activity was determined to using the enzymatic method; inhibition of 5-Lipoxynase according to Safayhi et al. [11], and Braga et al. [12]. Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1-4 diene structures. The conversion of linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. NDGA (Nordihydroguiaretic acid), a known inhibitor of soybean lipoxygenase, was used as a reference drug. The reaction was initiated by the addition of aliquots (50 µL) of a Soybean lipoxygenase solution (prepared daily in potassium phosphate buffer 0.1 M pH 9 in a sufficient concentration to give an easily measurable initial rate of reaction) to 2.0 mL of sodium linoleate 100 µM in phosphate buffer; the enzymatic reactions were performed in the absence or the presence of the inhibitor and their kinetics were compared. The inhibitors were dissolved in ethanol such that an aliquot of each (10 µL) yielded a final concentration of maximum 300 ppm in each assay The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control (using 10 µL of ethanol instead of 10 µL of the inhibitor-ethanol solution). Each inhibitor concentration was tested in triplicate and the results averaged; the concentration that gave 50% inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration.

2.6 Determination of Antifungal Activities

2.6.1 Disc Diffusion Method

The disc diffusion method was used for the antifungal screening as described by Rubio et al. [13]. The SDA (Sabouraud dextrose agar) medium prepared according to the manufacturer' guides and 10 mL poured into Petri dishes of 55 mm and allowed to rest for solidification. The essential oils were dissolved in 10% DMSO (dimethylsulfoxyde) at different proportion (v/v). The yeast suspensions at 2.5×10^5 CFU/mL were prepared from 48 h old cultures in a saline solution. After the solidification, each Petri dish was seeded with yeast suspension, then sterile paper discs (4.0 mm in diameter) impregnated with 15 µL of essential oils/DMSO were placed at the centre of each dish, and Nystatin was used as positive control. Negative controls were prepared in the same conditions except essential oils. The dishes were incubated in an inverted position at 37 °C for 48 h. The diameters of the inhibition zones were measured in mm for 3 days. All the tests were performed in triplicate.

2.6.2 Minimal Inhibitory Concentration

The MIC (minimal inhibitory concentration) which inhibits the visible growth of fungi was determined by the micro-well dilution method of Tchoumbougnang [14] using SNB (Sabouraud Nutrient Broth). The

essential oils were dissolved in 10% DMSO for *A. graveolens* (80% v/v) and *T. vulgaris (*20% v/v). In the 96-wellplates, 100 µL of SNB were dispensed into each well except first well which had 150 or 180 µL of SNB following each essential oil. A volume of the stock solution of essential oils was added into the first well to give 200 µL as final volume. Then two-fold serial dilutions was done and 20 µL of the inocula were introduced into each well. The final volume in each well was 120 µL. The negative control contained SNB and inoculum. The essential oil concentrations of *A. graveolens* and *T. vulgaris* ranged from 140-0.14 g/L and 56.67-0.06 g/L respectively. Nystatin was used as a positive control with concentration of 2.77-0.0027 g/L. The microplates were covered with a sterile plate sealer and then incubated for 48 h at 37 °C.

2.6.3 Minimal Fungicidal Concentration

For confirmation of the fungistatic or fungicidal activity, all wells showing no visible growth after 48 h were subcultured on to SDA medium and incubated at 37 °C for 48 h. The MFC was recorded as the lowest concentration that did not yield growth.

2.6.4 Anti-Moulds and Anti-Dermatophytes Assays

The preliminary screening and determination of MIC (minimal inhibitory concentration) were done by agar dilution method described by Grover and Moore [15], and De Billerbeck [16], and essential oil was dissolved in DMSO in a proportion of 1/9. The essential oil solution was mixed in the SDA medium with concentrations of 1.275 g/L, 2.550 g/L, and 5.100 g/L for *A. graveolens* and 0.255 g/L; 0.510 g/L; 0.765 g/L (moulds) 0.085 g/L; 0.255 g/L; 0.510 g/L (dermatophyytes) for *T. vulgaris*. The Amphotericin B and Griseofulvin were used as positive controls and negative control was only SDA medium in the dish with inoculum. The 10 mL of the supplemented SDA medium was poured into Petri dishes of 55 mm per dish and allowed to rest for solidification. A mycelia disc of 6 mm in diameter of 4 and 7 days old preculture of *Aspergillus* and *dermatophytes* respectively was inoculated at the center of the dish. The dishes were incubated in an inverted position at 37 °C in the dark during 7 and 15 days for moulds and dermatophytes respectively. The mycelia growth was observed while measuring the diameter according to two perpendicular lines passing across the centre of the dish. For each concentration, three tests were carried out.

2.6.5 Statistical Analysis

Data from three independent replicate trials were subjected to statistical analysis using ANNOVA by Student-Fisher $p < 0.05$ Add software.

3. Results and Discussion

3.1 Yields of Extraction of Essential Oils

The hydrodistillation of the leaves of *A. graveolens* and *T. vulgaris* gave the yield of 0.14 ± 0.04% and 0.32 ± 0.04% respectively. These results were different to those previously obtained by Nguefack et al. [17] who showed a yield of 0.50% for dry *T. vulgaris* from Dschang region of Cameroon. These variations could be explained by the post-harvest treatment.

3.2 Chemical Composition

The results of the chemical analysis are presented in Table 2.

The essential oils of *A. graveolens* and *T. vulgaris* were made of monoterpenoids (79.9% and 89.8% respectively) with limonene (50.7%) and myrcene (12.5%) in that of *A. graveolens* and thymol (57.9%)

Table 1 Yield of extraction.

Essential oils	*A. graveolens*	*T. vulgaris*
Yield	0.14 ± 0.04%	0.32 ± 0.02%
Color	yellow (light)	yellow

Table 2 Chemical composition of *A. graveolens* and *T. vulgaris* essential oils.

IK	Compounds	Percentage (%) *A. graveolens*	*T. vulgaris*
	Monoterpenes	**79.9**	**89.8**
	Hydrogenatedmonoterpens	**65.7**	**11.4**
935	α-pinene	0.2	-
971	sabinene	0.3	-
978	ß-pinene	0.2	-
987	myrcene	12.5	0.4
1019	p-cymene	1.4	10.3
1035	limonene	50.7	0.5
1041	(E)-ß-ocimene	0.2	-
1056	α –terpinene	-	0.2
1196	ð-terpinene	0.2	-
	Oxygenatedmonoterpens	**14.2**	**78.4**
1055	trans sabinene hydrate	-	2.1
1080	fenchone	0.5	-
1094	linalol	0.1	6.9
1135	camphor	-	2.3
1139	camphor 2	-	1.4
1176	terpinen-4-ol	0.2	2.6
1196	ð-terpineol	0.1	0.3
1222	nerol	-	1.6
1224	thymol methylether	-	0.9
1233	neral	-	0.4
1240	geraniol	0.3	-
1248	ð-carvyl acétate	0.7	-
1279	neryl formate	-	0.4
1289	thymol	11.8	57.9
1352	carvacrol	0.5	1.6
	Sesquiterpens	**12.1**	**5.3**
	Hydrogenatedsesquiterpens	**7.9**	**3.1**
1437	ß-caryophyllene	4.2	2.4
1469	α-humulene	0.3	-
1481	γ-himachalene	0.1	-
1499	germacrene D	-	0.3
1502	ß-bisabolene	2.0	-
1509	ß-selinene	1.3	-
1508	valencene	-	0.2
1522	δ-cadinene	-	0.2
	Oxygenatedsesquiterpens	**4.2**	**2.2**
1529	elemol	-	0.5
1595	caryophyllene oxide	0.4	1.7
1627	humulene oxide	0.2	-
1640	caryophyllen-dienol	0.5	-
1717	2E, 6Z farnesol	2.3	-
1814	Farnesyl acetate	0.8	-
	Aromatic compound	**0.4**	**3.7**
1156	estragol	0.4	-
1348	eugenol	-	3.7
	Aliphatic compounds	**0.8**	
1666	heptadecene	0.3	-
1738	tetradecenol	0.5	-

and p-cymene (10.3%) for *T. vulgaris*. These results were similar to those obtained by Alfreda and Taka [18], with limonène (74.6%) as major compound of the seeds of *A. graveolens* oil. These results were also similar to those obtained by Tchoumbougnang [14], with thymol (27.2; 43.6%) and p-cymene (20.4; 34.8%) as major compounds in the essential oil of *T. vulgaris* harvested from Bafoussam west region of Cameroon. It was also similar to those of Nguefack et al. [17] who showed thymol (27.2%) and P-cymene (23.6%) as major components of essential oils of *T. vulgaris* from Dschang area of Cameroon.

3.3 Antiradical Activities of Essential Oils

The antiradical properties of essential oils are illustrated in Figs 1-3 from which it can be seen that the percentage of free radical capture increased with the concentration of various essential oils in the reaction medium, up to a stationary state. From this, the SC_{50} of the samples were obtained (Table 3). The essential oil of *T. vulgaris* (SC_{50} = 0.06 ± 0.01 g/L) was more effective than that of *A. graveolens* (SC_{50} = 0.41 ± 0.01 g/L). In addition, the antiradical activities of the studied extracts are significantly lower than those of BHT (SC_{50} = 0.0071 ± 0.000 g/L). Indeed, these results are comparable to those of Alfreda and Taka [18] who showed that essential oils of *A. graveolens* have a low antiradical activity because at 0.20 g/L it showed only 55% of scavenging of DPPH radicals. This results were also similar to those of Alitonou [19] where the essential oils were rich in monoterpenes hydrocarbons and/or sesquiterpenes and showed a weak antioxidant activity, but different to those of Yakhlef [20], with SC_{50} = 88.00 ± 2.876 µg/mL from apolar extract of *T. vulgaris*.

Antiradical activities showed by essential oils of these plants would be due to the presence of phenolics compounds like thymol and cavacrol as demonstrated by Sokmen et al. [21] who correlated the high antioxidant activity of essential oil of *Thymus spathulifolius* with their high quantities of thymol (36.5%) and carvacrol (29.8%).

3.4 Anti-Infammatory Properties of Essential Oils

The anti-infammatory property of *T. vulgaris* essential oil with that of nordihydroguaretic acid is presented in Figs. 4 and 5.

These figures help to determine the values of IC_{50} of the samples.

The extract sample of *A. graveolens* is not active

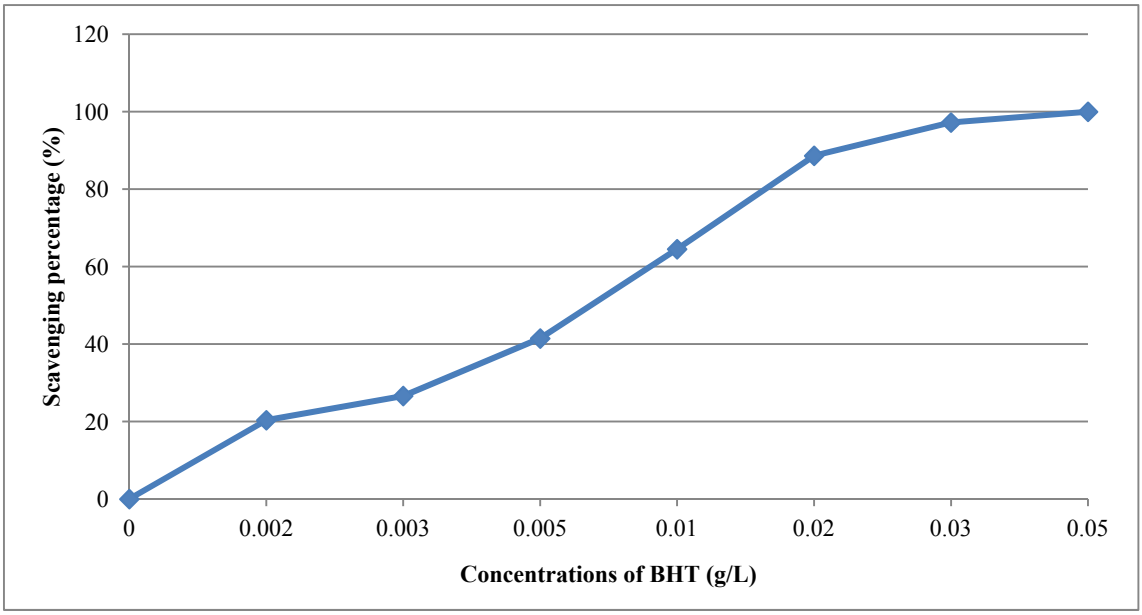

Fig. 1 Antiradical activity of BHT.

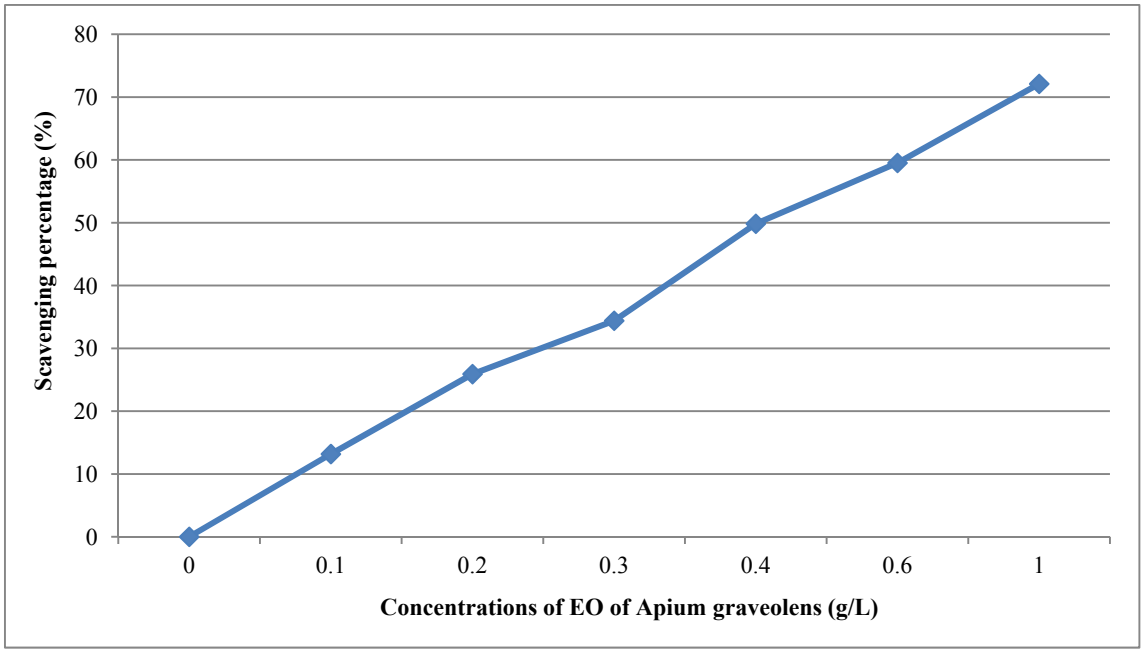

Fig. 2 Antiradical activity of essential oil of *A. graveolens.*

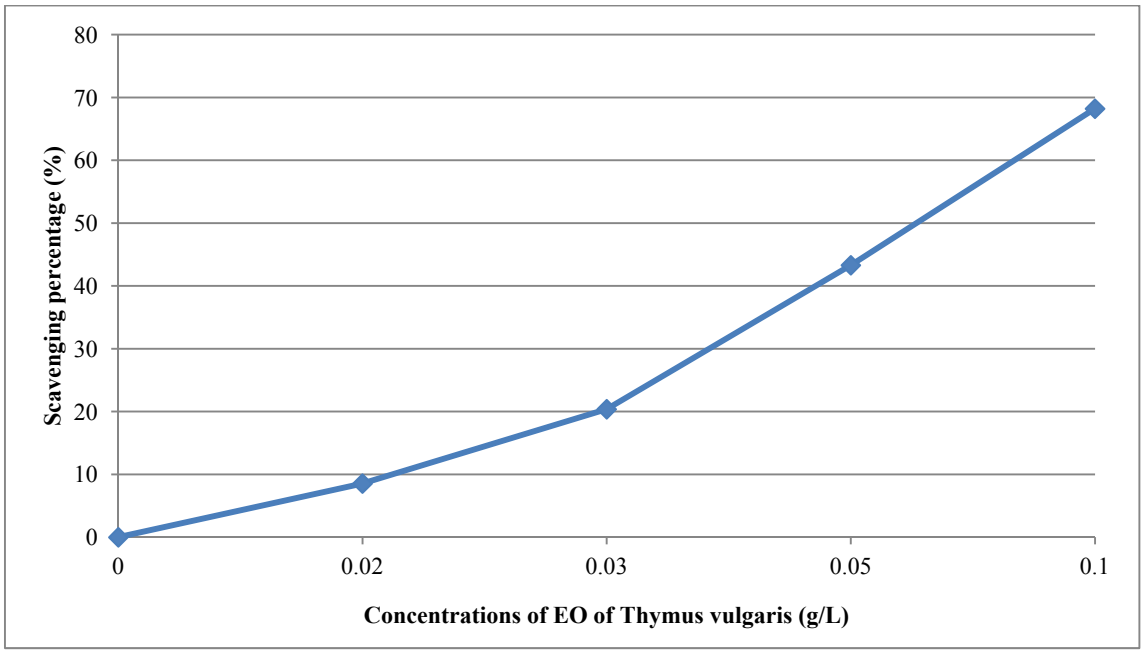

Fig. 3 Antiradical activity of essential oil of *T. vulgaris.*

Table 3 SC_{50} (scavenging concentration 50), EC_{50} (effective concentration 50) and AC (antiradical capacity) values of the studied samples and BHT.

Samples	SC_{50} (g/L)	EC_{50} g/mol de DPPH	AC (x 10^{-3}) mol
BHT	0.007 ± 0.000^{a}	7.35 ± 0.000^{a}	136.13 ± 11.71^{a}
A. graveolens	0.41 ± 0.01^{b}	482.75 ± 0.02^{b}	2.07 ± 0.67^{b}
T. vulgaris	0.06 ± 0.01^{c}	62.97 ± 0.01^{c}	15.88 ± 5.37^{c}

a, b indicate the statistical analysis, in the same column the activity of three compounds is different ($P \leq 0.05$).

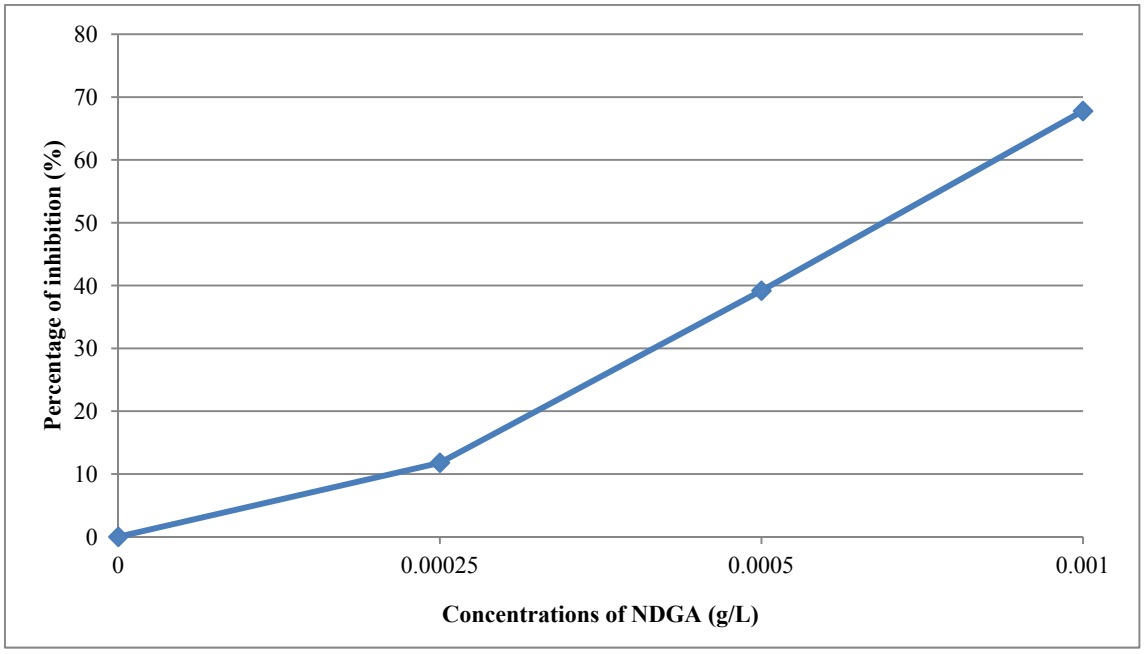

Fig. 4 Anti-inflammatory activity of NDGA.

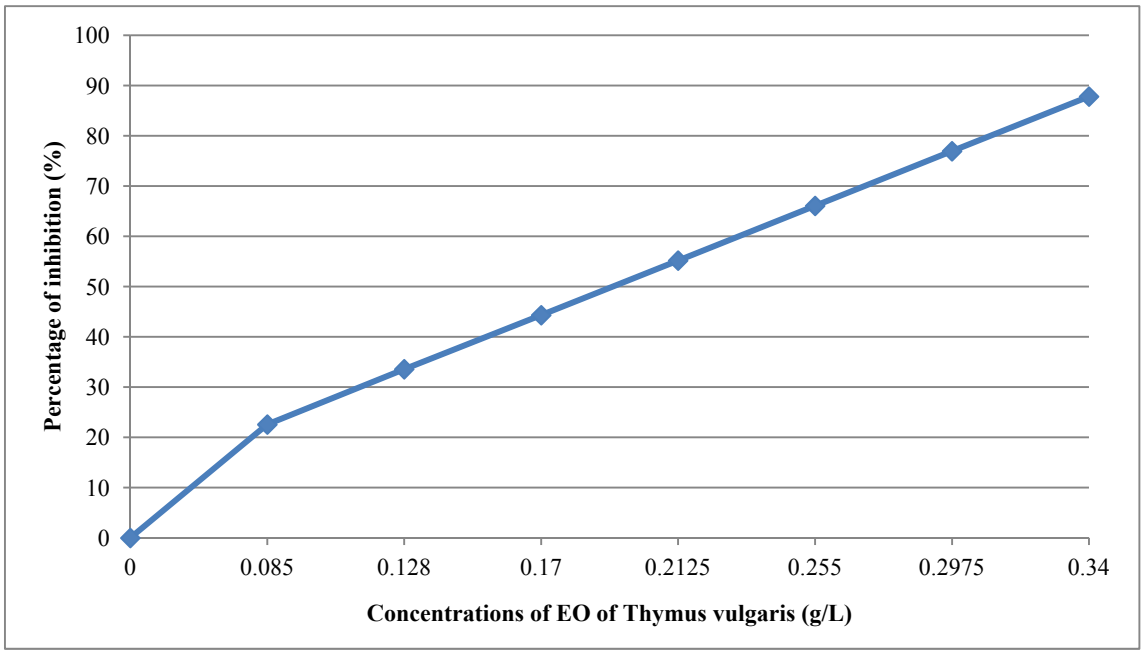

Fig. 5 Anti-inflammatory activity of essential oil of *T. vulgaris*.

Table 4 IC_{50} values of the studied samples.

Samle	IC_{50} (g/L)
ANDG	0.0007 ± 0.000^{a}
A. graveolens	-
T. vulgaris	0.192 ± 0.011^{b}

a, b indicate the statistical analysis, in the same column the activity of two compounds is different ($P \leq 0.05$).

and the sample of *Thymus vulgaris* is less active than NDGA.

3.5 Antifungal Activity

3.5.1 Anti-Yeast Activities

The results of the disc diffusion testing of essential oils are listed in Tables 5 and 6.

The Table 6 shows that the inhibition zone diameter of the essential oils tested varies from 0 to 13.87 mm. The strongest activity was shown against *C. albicans* with 13.0 and 13.87 mm inhibition zone. The NC (negative controls) showed no inhibiting effect. All isolates were found sensitive to Nystatin (positive control).

The results in Table 7 indicate that their MIC varied, respectively, from 8.87 to 70 g/L for essential oil of *A. graveolens* and from 0.22 to 0.89 g/L for essential oil of *T. vulgaris*. *C. albicans* was shown to be resistant at the highest concentration tested (70 g/L) for essential oil of *A. graveolens* and *C. glabrata* for essential oil of *T. vulgaris* at 0.89 g/L. These essential oils were fungicidal at the same concentrations.

3.5.2 Anti-Mould Activities

The Figs. 6 and 7 show that for two essential oils at the three concentrations tested, the inhibition varies from 0 to 100%. At 0.255 g/L, no inhibitions on *A. fumigatus* was observed with the *T. vulgaris* essential oil. Whereas at 0.51 and 5.1 g/L, 100% of inhibition was observed on *A. flavus* and *A. fumigatus*.

Table 5 Diameter of inhibition zone (mm) from essential oil of *A. graveolens*.

Conc AG (g/L)	*C. albicans*	*C. glabrata*	*Cr. neoformans*
Nyst	15.62 ± 0.17	7.75 ± 0.70	13.50 ± 0.70
170	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
250	6.87 ± 1.23	5.25 ± 1.76	7.75 ± 0.35
340	8.62 ± 0.88	7.37 ± 0.53	8.62 ± 0.88
420	13.87 ± 0.53	7.50 ± 0.70	10.62 ± 1.23
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

AG: *A. graveolens;* Nyst: Nystatin; NC: Negative control.

Table 6 Diameter of inhibition zone (mm) from essential oil of *T. vulgaris*.

Conc TV (g/L)	*C. albicans*	*C. glabrata*	*Cr. neoformans*
Nyst	15.62 ± 0.17	7.75 ± 0.70	13.50 ± 0.70
42.50	4.25 ± 1.06	0.00 ± 0.00	0.00 ± 0.00
85.00	6.62 ± 2.29	3.75 ± 0.35	6.00 ± 1.41
212.50	8.25 ± 1.6	4.50 ± 0.0	7.50 ± 0.70
255.00	13.00 ± 1.41	7.50 ± 2.12	9.00 ± 1.41
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

AG: *A. graveolens;* Nyst: Nystatine; NC: Negative control.

Table 7 MIC and MFC of essential oils.

Parameter	*C. albicans*		*C. glabrata*		*Cr. neoformans*	
	AG	TV	AG	TV	AG	TV
MIC (g/L)	70.00	0.55	17.50	0.44	4.37	0.11
MFC (g/L)	70.00	0.55	35.00	0.89	8.75	0.22
MFC/MIC	1.00	1.00	2.00	2.00	2.00	2.00

AG: *A. Graveolens;* TV: *T. Vulgaris.*

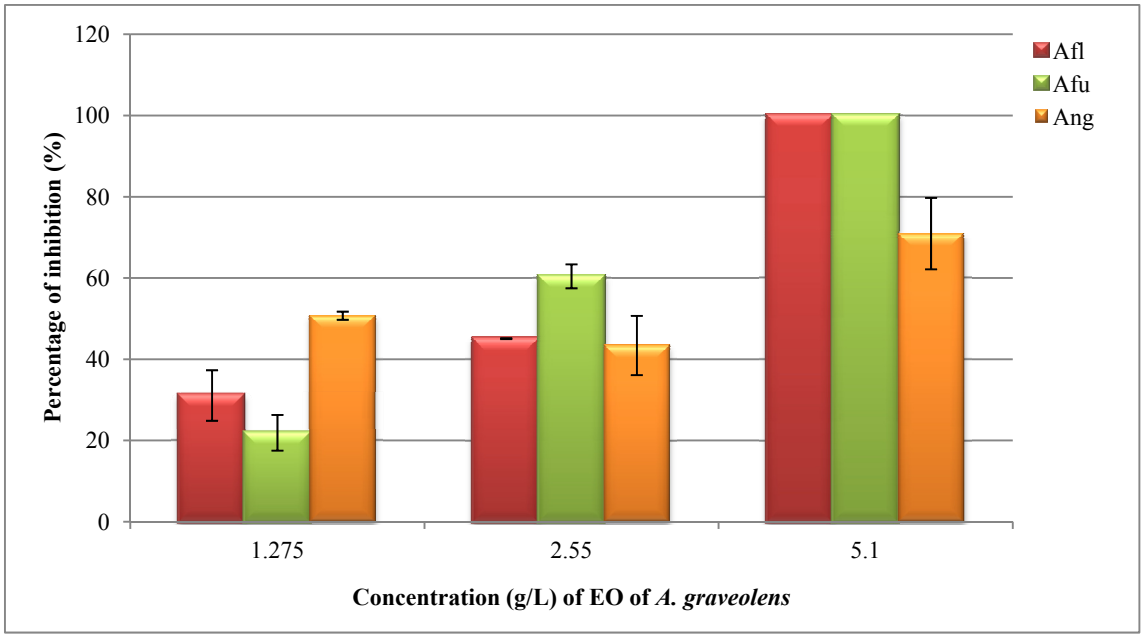

Fig. 6 Percentage of inhibition of essential oil of *A. graveolens*.

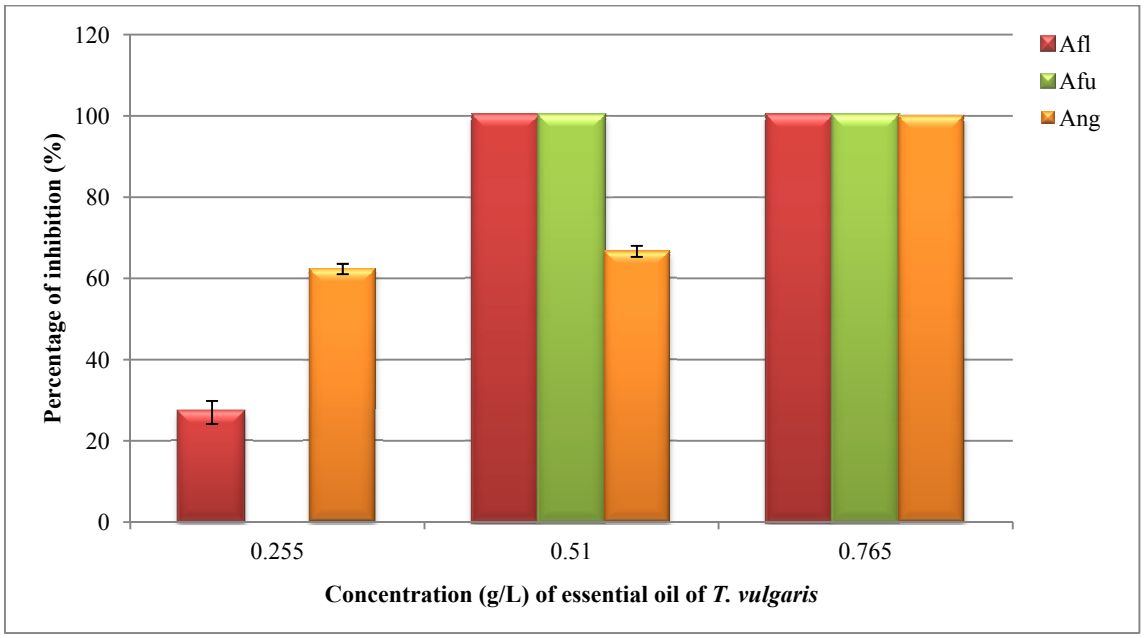

Fig. 7 Percentage of inhibition of essential oil of *T. vulgaris*.

Afl: Apergillusflavus; Afu: Apergillusfumigatus; Ang: Apergillusniger.

The results of MIC on moulds are presented in Table 8. The value varies between 4.68 to 6.80 g/L for essential oil of *A. graveolens* and 0.47 to 0.55 g/L for essential oil of *T. vulgaris*. The essential oil of *T. vulgaris* showed the best activity than *A. graveolens*. The strongest activity was shown against *A. fumigatus* with 0.47 and 4.68 g/L MIC values. They also showed moderate activities against *A. niger* with 0.55 and 6.80 g/L MIC values for essential oils of *T. vulgaris* and *A. graveolens* respectively. All isolates were found sensitive to Amphotericin B (positive control).

3.5.3 Anti-dermatophyte Activities

The Figs 8 and 9 show that the percentage inhibition of dermatophytes varies from 16 to 100%.

Table 8 MIC and MFC of essential oils.

Parameter	*A. flavus*		*A. fumigatus*		*A. niger*	
	AG	TV	AG	TV	AG	TV
MIC (g/L)	4.68	0.49	4.68	0.47	6.80	0.55
MFC (g/L)	5.10	0.49	5.10	0.47	6.80	0.55
MFC/MIC	1.09	1.00	1.09	1.00	1.00	1.00

AG: *A. Graveolens;* TV: *T. vulgaris.*

At 0.255 g/L and 2.55 g/L, 100% of inhibitions on *T. mentagrophytes* was observed with the *T. vulgaris* essential oil whereas the *A. graveolens* essential oil at 0.51 and 5.1 g/L 100% of inhibitions on the three species.

The MIC of dermatophytes is presented in Table 9. It varies between 1.28 to 3.40 g/L for essential oil of *A. graveolens* and 0.26 to 0.43 g/L for *T. vulgaris* essential oil. The essential oil of *T. vulgaris* showed the best activity against *T. mentagrophytes* at 0.26 g/L than that of *A. graveolens*. It also showed moderate activities against *E. flocosum* with 0.43 and 3.40 g/L for essential oils of *T. vulgaris* and *A. graveolens* respectively. All isolates were found sensitive to Amphotericin B and Griseofulvin.

The essential oil of *A. graveolens* is rich in limonene (50.7%) and myrcene (12.5%) whereas thymol (57.9%) and p-cymene (10.3%) were present in that of *T. vulgaris*. This composition is similar to previous reports by Alfreda and Taka [18] and Nguefack et al. [17]. The presence of thymol in these essential oils is among a group of chemicals known as monoterpenes which are the strongest activity against fungus [22]. All the concentrations of the essential oils inhibited the fungal species with varying degree of sensitivity. The yeast species were more resistant than the moulds. *C. neoformans* was the most susceptible yeast strain and *C. albicans* was more resistant. The most susceptible mould strain was *A. fumigatus* and the more resistant was *A. niger*. The *T. vulgaris* oil showed best antifungal activity than *A. graveolens* oil. *E. flocosum* was more resistant than other dermatophytes and these results corroborate for those of Ouraïni et al. [23] who studied of fungitoxic

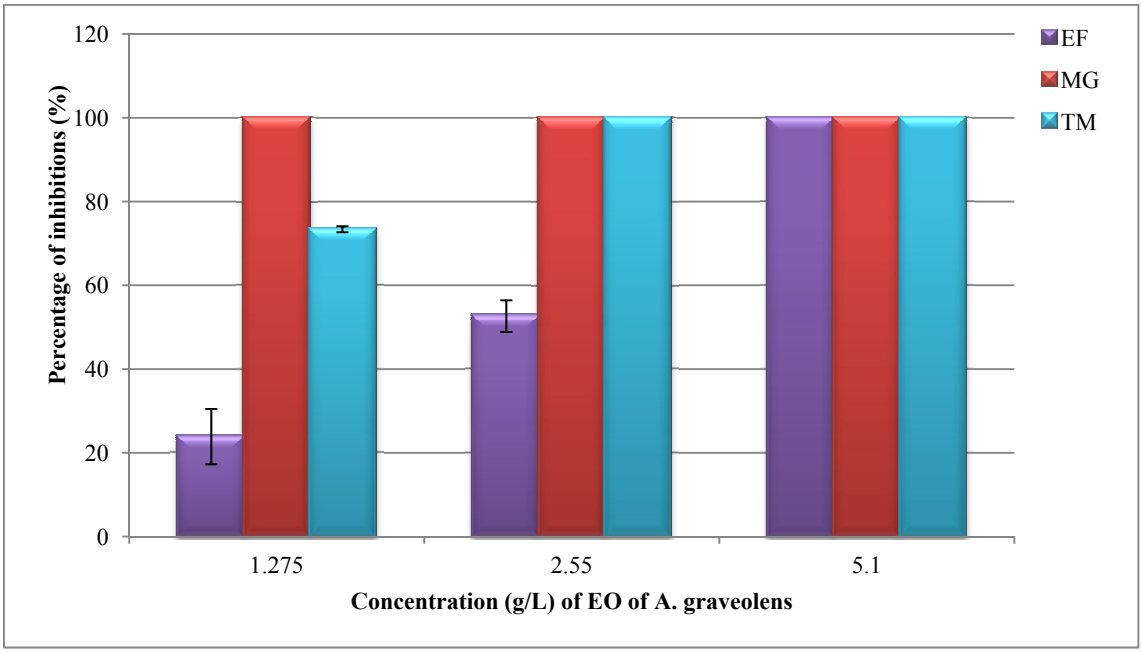

Fig. 8 Percentage of inhibition of essential oil of *A. graveolens*.

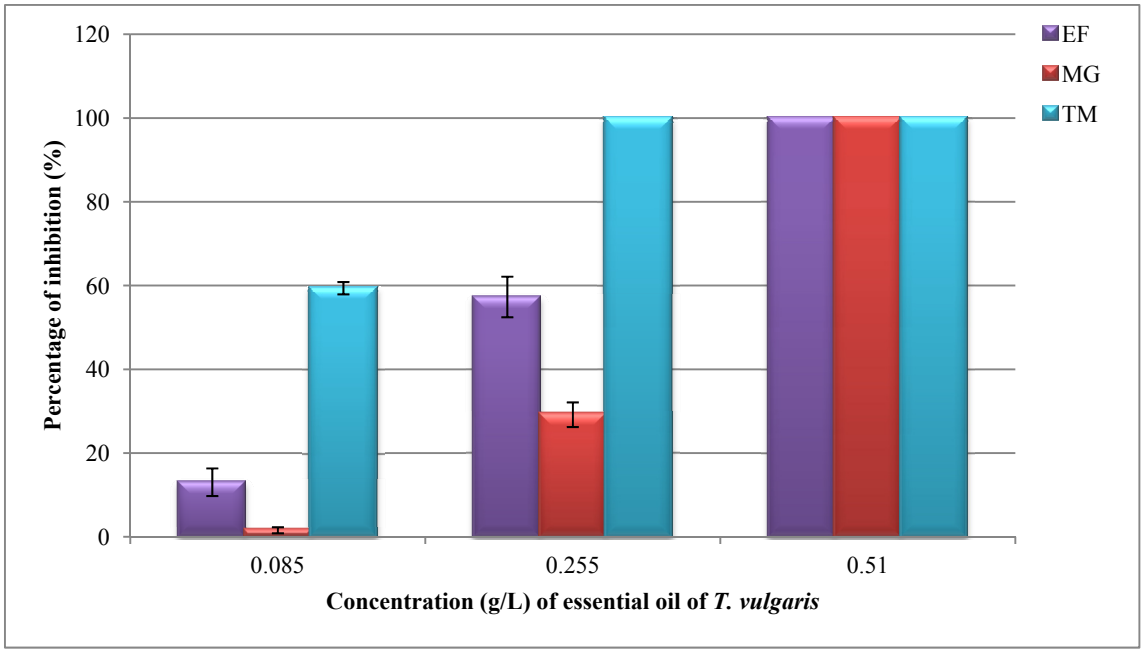

Fig. 9 Percentage of inhibition of essential oil of *T. vulgaris*.

EF: Epidermophytonflocosum; MG: Microsporumgypseum; TM: Trichophytonmentagrophytes.

Table 9 MIC and MFC of essential oils.

Parameter	*E. flocosum*		*M. gypseum*		*T. mentagrophytes*	
	AG	TV	AG	TV	AG	TV
MIC (g/L)	3.40	0.43	1.28	0.43	2.13	0.26
MFC (g/L)	3.40	0.51	1.28	0.51	2.55	0.43
MFC/MIC	1.00	1.20	1.00	`1.20	1.20	1.65

AG: *A. Graveolens;* TV: *T. vulgaris.*

activity of essential oils of aromatic and medicinal plants such as thyme (*Thymus saturejoïdes L.*), European pennyroyal (*Menthe pulegium L.*) and rosemary (*Rosmarinus officinalis L.*) on the germination, mycelial growth and sporulation of dermatophytes responsible for different cases of mycoses among humans. These two plants have been previously observed to possess antifungal activity [24]. The presence of biologically active compounds (thymol, carvacrol) in the essential oils could be correlated to its antifungal activity. In fact, the poor antifungal activity of *A. graveolens* oil could be due to lowest quantity of thymol than limonene which may not play the major role in the antifungal activity [7]. The best activity of *T. vulgaris* oil could be due to the thymol which is responsible for the highest antifungal activity among terpenoids [25, 26].

4. Conclusion

The essential oils of *A. graveolens* and *T. vulgaris* constitute a source of natural antioxidants, anti-infammatory and antifungal. They are therefore beneficial for traditional medicine in the prevention and cure of some diseases. In addition, these plants could be used as food supplements in the protection against some emergent diseases.

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