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CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THREE ESSENTIAL OILS FROM PORTUGUESE FLORA

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Abstract- The present work reports on the evaluation of chemical composition and antioxidant and antimicrobial activities of essential oils of three aromatic herbs, growing wild in the south of Portugal, used in traditional food preparations: *Foeniculum vulgare*, *Mentha spicata* and *Rosmarinus officinalis*. The principal components of essential oils were anethole (41.2%) for *F. vulgare*, carvone (41.1%) for *M. spicata* and myrcene (23.7%) for *R. officinalis*. Essential oils showed antioxidant activity either by DPPH radical scavenging method and system β-carotene/acid linoleic method. Antimicrobial activity of essential oils was observed against pathogenic bacteria and yeasts and food spoilage fungi. *F.vulgare* essential oil showed bacterial activity against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Aspergillus niger* and *Fusarium oxysporum* with MICs of 0.25-0.75mg/mL. *M. spicata* oil was active against *E.coli, S.aureus, C.albicans, A. niger* and *F. oxysporum* with MICs ranging between 0.25 and 0.75mg/mL. *R. officinalis* essential oil showed activity against *E.coli* and *C.albicans* with MICs of 0.5-1.0mg/mL.
Having in account the important antioxidant and antimicrobial properties observed in present work, we consider that these essential oils might be useful on pharmaceutical and food industry as natural antibiotic and food preservative.
Key words- *Foeniculum vulgare; Mentha spicata; Rosmarinus officinalis*; essential oil; antioxidant activity; antimicrobial activity.

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Introduction

Many plants contain natural antioxidants that act in metabolic response to the endogenous production of free radicals and other oxidant species. The interest in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage has increased in last decades [1-3]. Aromatic herbs and spices have been used for a long time in Mediterranean cuisine, not only to improve or modify the flavor of foods, but also to avoid its deterioration. In Portugal, people who live in the countryside appreciate and continue to gather and consume wild aromatic plants as flavor corrector, conservatives, fragrances, pigments and insecticides. The antimicrobial properties of some essential oils have been well recognized for many years [4]. The indiscriminate use of antimicrobial drugs as treatment for microbial infectious has developed multiple resistances. Therefore, natural products as essential oils and herbal extracts are nowadays used in medicine and food industry for their antimicrobial activities [5].

In the present work, it was studied some biological properties of essential oils of three wild aromatic plants widely used in Alentejo, a south region of Portugal: *Foeniculum vulgare* Miller (fennel), *Mentha spicata* L. (mint) and *Rosmarinus officinalis* L. (rosemary). The *F. vulgare* belongs to Apiaceae family, *M. spicata* and *R. officinalis* to the Lamiaceae [6]. In traditional medicine, the *F. vulgare* and *M. spicata* are used to normalize light digestive disturbances and in the treatment of infections of the respiratory system and the *R. officinalis* is used in circulatory, rheumatic and digestive problems [6]. To our knowledge, up to now, there are few studies about the selected plants growing in Portugal, in particular at Alentejo. In the present work, and in order to acquire a best known of these plants, the *F. vulgare*, *M. spicata* and *R. officinalis*

essential oils were chemical characterized and their antioxidant and antimicrobial activities were evaluated. The antioxidant activities were determined by two methods, the DPPH free radical scavenging method and the β-carotene/linoleic acid system, for the determination of the inhibitory effect on lipid peroxidation. The evaluation of this biological activity by different methods is due to the chemical complexity of the essential oils characterized by various compounds with different functional groups, polarity and chemical behavior, which can conduct to a different results according to the method used [7, 8]. The antimicrobial activities of essential oils were evaluated against six pathogenic bacterial strains, one pathogenic yeast strain and four saprophytic filamentous fungi by the disc-diffusion method and minimum inhibitory concentrations (MIC). The present work provides a basis for further exploitation and use of these three plants resources on human health and food safety.

Material and Methods

Plant material

F. vulgare, *M. spicata* and *R. officinalis* were collected in autumn at Évora region, Alentejo. All the plants were identified by Professor Marízia Menezes, a staff member of the University of Évora and voucher specimens were kept at the Herbarium of the Department of Biology of the University of Évora.

After harvested, the manual weeding of plants was done in order to obtain only the good parts of plants and eliminate everything that could affect oil purity or interfere in the pharmacological activity of oils. The aerial parts of plants were air-dried in a dark room for 3 days.

Extraction procedure

The essential oils of leaves from the plants studied were obtained by hydrodistillation for 3 h in a modified Clevenger-type apparatus, according to the Portuguese and European Pharmacopoeia method [9]. After extraction, the oils were dried with sodium sulfate and stored at a low temperature.

Chemical characterization of essential oils

Essential oils of each plant were characterized by GC-FID and results were confirmed with GC-MS analysis. Gas-chromatographic analysis was performed on a HP-5890 SERIES II chromatograph equipped with Supelcowax ™ 10 (polyethyleneglycol) column 30 m long_0.25 mm ID_0.25 mm film thickness. Temperature program: 70ºC (3min), 70º- 220ºC (3ºC/min) and 220ºC (5min); column flow rate was 0.6mL He/min; injector and detector temperatures were 250ºC. Injection volume was 0.2mL and split ratio 50:1. Identification of the essential oils components was achieved by comparison of their retention indexes, determined in relation to a homologous series of *n*-alkanes, with those from pure standards or reported in literature [10]. GC-MS analysis were performed with a Trace 2000 Thermo Quest CE Instruments gas chromatograph, detector model Polaris Q, (E.I. Quadrupole). The components were identified by comparison of their mass spectra with those of authentic standards, as well as based on the comparison of their fragmentation patterns in the mass spectra with those from REPLIB, MAINLIB e NISTDEMO libraries and those reported in the literature [11]. The quantification of compounds was performed on the basis of their GC peak areas without corrections for response factors and each oil was analyzed three times. Percent data are shown on Table 1.

Table 1- Chemical composition of essential oils of F. vulgare, M. spicata and R. officinalis

Compounds	Ri [B]	Percentage [A]		
		F. vulgare	*M. spicata*	*R. officinalis*
α-Pinene	1032	4.1	0.9	14.1
Camphene	1066	0.2	0.6	7.4
α-Fenchene	1087	0.6	1.3	-
β-Pinene	1133	0.5	0.9	4
d-3-Carene	1160	-	0.4	0.5
Myrcene	1162	5.2	-	23.7
α-Phellandrene	1170	2.2	-	0.3
α-Terpinene	1180	-	-	0.3
Limonene	1202	24.8	20.1	4.2
1,8-Cineole	1212	0.7	1.5	10.6
Z-β-Ocimene	1235	0.1	0.7	2.8
γ-Terpinene	1248	1.5	0.3	1.5
E-β-Ocimene	1253	0.5	-	-
m-Cimene	1267	-	0.1	-
p-Cimene	1270	1.1	0.2	3.7
α-Terpinolene	1278	0.3	-	0.3
Fenchone	1394	11.9	-	-
Menthone	1461	-	0.1	-
Sabinene hydrate	1452	0.1	-	1.2
Camphor	1496	-	-	12.8
1-Octene-3-ol	1468	-	0.1	0.2
Copaene	1474	0.3	5.6	0.3
Linalool	1557	-	0.4	0.2
γ-Gurjunene	1562	-	0.7	0.7
Bornyl acetate	1576	-	0.3	0.9
β-Caryophyllene	1582	-	4	0.2
Terpinen-4-ol	1586	-	0.6	-
Cedr-8-ene	1600	-	-	0.4
Pulegone	1608	-	0.5	-
Estragol	1625	2.8	-	-
Borneol	1633	-	1.5	1.1
Verbenone	1645	-	-	1.4
Dihydrocarveol	1658	-	1.5	-
Carvone	1664	-	41.1	-
Anethole	1693	41.2	-	-
Muurolene	1617	-	1.1	-
d-Cadinene	1745	-	-	0.7
Caryophyllene oxide	1751	-	0.2	2.0
Cadina-1,4-diene	1755	-	1.8	-
trans-Carveol	1790	-	0.3	-
cis-Calamenene	1817	-	0.9	0.2
Total identified		98.1	87.7	95.7
Grouped components				
Monoterpene hydrocarbons		41.1	25.5	62.8
Monoterpenes oxygenated		12.7	46.3	28.2
Sesquiterpene hydrocarbons		0.3	14.1	2.5
Sesquiterpenes oxygenated		-	0.2	2.0
Benzene derivates		44.0	-	-
Others		-	0.1	0.2

[a] *Normalized peak areas without using correction factors.*
[b] *Retention indices relative to C5-C15 n-alkanes.*

Antioxidant properties

The DPPH free radical scavenging activity of each essential oil was measured from the bleaching of purple colored ethanol solution of the stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH・). Dilutions of the essential oils were prepared with ethanol and 50µL of each dilution was added to tubes containing 50µL of Tween 20 and 1mL of DPPH 0.1mM in ethanol. A control reaction

was prepared using all reagents except the test oil. Butylhydroxytoluene (BHT) was used as standard. The absorbance was measured at 517 nm, after 30 min of incubation at room temperature, in the dark. Tests were carried out in triplicate.

The capability to scavenge the DPPH • radical was calculated according to the following expression:

DPPH scavenging effect (%) = $(A_B-A_A)/A_B$ *100, where A_B is the absorbance of the control reaction and A_A is the test absorbance.

The antioxidant activity based on β-Carotene/linoleic acid method was evaluated by measuring the inhibition of the bleaching of the β-carotene by the peroxides generated during the oxidation of linoleic acid [12]. A β-carotene/linoleic acid solution was prepared by adding 20μL of linoleic acid, 200mg of Tween 40, 25μL of β-carotene solution (2mg/mL in chloroform) and 500μL of chloroform. The chloroform was subsequently removed under vacuum and 25mL of water saturated with oxygen was added. In the test tubes, 1mL of the obtained solution was mixed with 40μL of each oil dilution in ethanol, with the BHT solutions used as standard and with the blank control (ethanol). The absorbance of each solution was measured at 490nm on minute zero and after the tubes, in dark, at room temperature for 48h [12]. Tests were carried out in triplicate. The inhibition of linoleic acid oxidation was calculated as follows: Oxidation Inhibition (%) = $(A_B-A_A)/A_B$*100, where A_B is the difference of the absorbance of control assay at time zero and after 48 h and A_A is the difference of the absorbance of the control assay at time zero and the tested samples after 48h.

Test microorganisms and media

The antibacterial activity was assayed against some human pathogenic strains from to the American Type Culture Collection (ATCC), Maryland, USA: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC10031, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis* ATCC 13076. The antifungal activity was assayed against the pathogenic yeast *Candida albicans* ATCC 10231 and phytopathogenic molds, *Aspergillus niger* and *Fusarium oxysporum*. Molds were isolated from Alentejo's soil and deposited in our laboratory.

Strains were maintained on Nutrient Agar, Sabouraud and Malt Extract Agar slants at 4ºC, for bacteria, yeasts and moulds respectively. All bacterial strains were activated in Nutrient Agar at 37 °C for 24 h, yeast were activated in Sabouraud at 28ºC for 48h and moulds were activated in Malt Extract Agar at 25 °C for 5 days before testing.

Antimicrobial susceptibility testing

The *in vitro* antimicrobial activity of essential oils was tested by the agar disc-diffusion assay and the determination of minimum inhibitory concentrations (MIC), for the sensible microbial strains.

Sensitivity tests of the essential oils were evaluated by the agar disc-diffusion method [13]. A suspension of each tested microorganism was prepared with sterilized physiological saline solution (0.9% w/v), bacterial suspensions were adjusted at 0.5 McFarland standard turbidity and fungal suspensions were prepared with 10^7 CFU/mL, as recommended by the National Committee for Clinical Laboratory Standards [13]. For each tested microorganism, 100 μL of adjusted suspension was spread onto solid media plates, nutrient Agar for Gram-positive and Gram-negative bacteria and Sabouraud Dextrose Agar for yeast and filamentous fungi. Sterile filter paper discs (Ø= 6mm) were located on the inoculated medium by pressing slightly and impregnated with 5 μL of essential oils. Nystatin (33μg) was used as fungal positive control and specific antibiotics disc were used for bacteria, Trimetoprim/Sulfametazole (25μg), Cefalotin (30μg), Amikacin (30μg) and Gentamicin (10μg). A sterilized physiological saline solution (5μL) or the solvent used in each test preparation was used as negative control. All plates after standing at 4 °C for 2 h, to avoid oil volatilization, were incubated at 37 °C for 24 h for bacteria and at 28 °C during 48 h for yeast and 5 days for filamentous fungi. The diameters of the inhibition zones (mm) were measured in millimetres using a 'Fisher-Lilly Antibiotic Zone Reader' (Fisher Scientific Co. USA). Each essential oil was tested in triplicate and repeated three times and results analyzed for statistical significance.

The minimal inhibitory concentrations (MICs) were determined for each essential oil, using the broth macro-dilution method [14] for the sensible microbial strains to the essential oils in the disc diffusion assay. The MIC value was defined as the minimum concentration of an agent that inhibits the growth of a selected organism. Dilutions of each essential oil were prepared in DMSO. Each tube containing the standardized suspension of each tested organism (10^6 CFU/mL) on culture media were added of a different concentration of each essential oil ranging from 31.25 to 1000μg/mL Nutrient Broth (NB) and Sabouraud Dextrose Broth were used for bacterial and fungal assays, respectively. The tubes were incubated, under agitation, at 37ºC for bacteria (24h) and yeast (48h) and at 28ºC for moulds (5 days). The lowest concentrations of the test samples, which did not show any growth of test organisms after macroscopic evaluation, were determined as MICs, which were expressed in μg/mL. All experiments were performed in triplicate.

Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data were performed by analysis of variance (ANOVA *one way*). A probability value at $P<0.05$ was considered statistically significant. Multiple comparisons of means were analyzed by the Tukey test. Statistical analyses were performed with the SPSS® 18.0 Windows *Copyright*$^{\Diamond}$ *, Microsoft Corporation*. Data are presented as mean values ± standard deviation calculated from triplicate determinations.

Results and Discussion

Chemical characterization of essential oils

The chemical composition and the percentage content of the essential oils obtained from the aerial parts of *F. vulgare*, *M. spicata* and *R. officinalis* collected from Alentejo, Portugal was presented in Table 1. The yields of essential oils (w/w) obtained by hydrodistillation, were 0.5% for *F. vulgare,* 0.1% for *M. spicata* and 0.4% for *R. officinalis*. GC-FID and GC-MS analysis of *F. vulgare* essential oil led to identification and quantification of eighteen different components, representing 98.1% of the total oil composition which include monoterpene hydrocarbons (41.1%), oxygenated monoterpenes (12.7%), sesquiterpenes hydrocarbons (0.3%) and benzene derivates (44.0%). Anethole (41.2%), limonene (24.8%), fenchone (11.9%), myrcene (5.2%) and a-pinene (4.1%) are the major constituents of this essential oil. These compounds were also found in the essential oil obtained from the green part of the plant *F. vulgare* as was reported in some studies [15, 16]. A study

reported by Ruberto *et al*. [17] revealed the estragole as the main component, which suggest that the species analyzed by those authors belongs to a different chemotype.

Thirty components were identified in *M. spicata* essential oil, which constitute 87.7% of the total composition. Oxygenated monoterpenes (46.3%), monoterpene hydrocarbons (25.5%) and sesquiterpenes hydrocarbons (14.1%) were found to be the major groups of constituents, the main one being the oxygenated monoterpene carvone (41.1%) followed by the monoterpene hydrocarbon limonene (20.1%). The carvone-scented *M. spicata* plants are widespread throughout the world as we can observed through the referred by others studies about the mint oil obtained from plants collected in different geographic areas [8, 18-20]. The *R. officinalis* essential oil revealed as major compounds: myrcene (23.7%), α-pinene (14.1%), camphor (12.8%) and 1,8-cineole (10.6%) among the thirty two components identified, which represents about 95.7% of the total composition and the dominant groups are monoterpene hydrocarbons (62.8%) and oxygenated monoterpenes (28.2%). The compounds referred above were also identified in essential oils of the same plant species according to the others studies but the concentrations of each component is different according the region [2, 21- 24]. The difference in the percentage of constituents may be attributed to genetic, seasonal and geographical reasons.

Antioxidant properties

As shown in Table 2, the antioxidant properties revealed by the essential oils analyzed were different for the three essential oils, in all concentrations tested (1, 2 and 4mg/mL). The antioxidant activity of *M. spicata*, *R. officinalis* and *F. vulgare* essential oils for the highest concentration tested (4mg/mL), measured by the DPPH method, was 31.45%, 29.56% and 26.64% and by the β-carotene/linoleic acid method was 14.89%, 13.00% and 17.26%, respectively. The antioxidant activity observed, in both methods, increases with the concentration of the essential oils and for some concentrations, results were significantly different ($P<0.05$) for DPPH method and for β-carotene/linoleic acid method (Table 2). Results showed that the free radical scavenging capacity was significantly higher than the ability to inhibit the oxidation of linoleic acid ($P<0.05$).

Table 2- Antioxidant activity of essential oils and comparison to the BHT

Sample	DPPH Scavenging Activity (%)			Inhibition of Linoleic Acid Oxidation (%)		
Essential Oils	1 mg/mL	2 mg/mL	4 mg/mL	1 mg/mL	2 mg/mL	4 mg/mL
M. spicata	26.5±0.4 ^{a}	26.9±0.7 ^{a}	31.5±1.2 ^{b}	7.8±1.9 ^{g}	13.0±1.3 ^{h}	14.9±1.9 ^{h}
F. vulgare	26.3±1.8 ^{a}	26.5±1.6 ^{a}	26.6±1.6 ^{a}	15.1±1.2 ^{h}	16.8±1.5 ^{h}	17.3±1.4 ^{h}
R. officinalis	25.5±0.7 ^{c}	28.0±1.3 ^{c,d}	29.6±1.8 ^{d}	4.5±1.3 ^{i}	8.3±1.7 ^{g}	13.0±1.2 ^{h}
BHT (Control)	78.1±3.8 ^{e}	88.6±1.6 ^{f}	90.8±0.4 ^{f}	45.9±1.6 ^{j}	71.6±1.4 ^{l}	88.7±1.9 ^{m}

Values are mean ± standard deviation of three different experiments.

Different letters in superscript indicate significant differences ($P<0.05$).

The results obtained, confirm which has been related by several authors about the importance of essentials oils as natural antioxidants and its role in the protection of human health [1, 12]. This capacity of essential oils is due to the presence in its complex composition of some components, mainly phenolic compounds. Some compounds as phenylpropanoids, monoterpenes and oxygenated sesquiterpenes are compounds that hold oxidation inhibition capacity [17]. Compounds of those chemical groups were found in the essential oils studied in present work, namely the phenylpropanoid methyleugenol and the monoterpenes limonene, α-terpinolene, γ-terpinene, 1,8-cineole, carvone, so the observed activity may be attributed to the presence of those compounds, that achieve the percentage value of 54.6 in *M. spicata*, 18.1 in *R. officinalis* and 16.7 in *F. vulgare* essential oils compositions, which seems to show a direct correlation with the antioxidant capacity revealed by these oils.

Antimicrobial properties

Results of the antimicrobial screening have shown that the three studied essential oils were effective against the majority of tested bacterial and fungal strains (Table 3). The essential oil of *F. vulgare* shows activity against all pathogenic bacterial strains tested. The inhibition zone diameter was 9 mm for *P. mirabilis*, 8 mm for *E. coli* and 7 mm for *P. aeruginosa, K. pneumonia, S. enteritidis* and *S. aureus*. The essential oil of *M. spicata* induced inhibition of the growth of all tested bacteria with inhibition zone diameters ranging from 8 to 12 mm (Table 3). The essential oil of *R. officinalis* revealed activity against *E. coli* and *S. aureus*, with inhibition zones of 8 mm and 7 mm, respectively. When compared with standard drugs, the essential oils of *F. vulgare* and *M. spicata* demonstrate comparable or higher antimicrobial activity against some pathogenic bacteria (Table 3). Analysis of variance revealed that, both, essential oils and microorganisms variations exhibited significant ($P<0.05$) effect on the antimicrobial activity. The growth of the fungi *A. niger* and *F. oxysporum* were totally inhibited by the *F. vulgare* essential oil (Table 3). The growth of the fungal strains *C. albicans, A. niger* and *F. oxysporum* were inhibited by the essential oil of *M. spicata*, who induced a growth inhibition zone of 31.5 mm for *C. albicans* and total inhibition for the remain strains. *C. albicans* strain revealed little sensibility to the essential oil of *R. officinalis* with inhibition zone of 8 mm.

As shown in Table 4, the minimum inhibitory concentration values of essential oils of *F. vulgare* and *M. spicata* were found more effective as compared to the *R. officinalis* essential oil ($P<0.05$). The essential oils of *F. vulgare* displayed significant antibacterial activity against five Gram-negative bacteria such as *S. enteritidis*, *K. pneumonia, P. mirabilis, E. coli* and *P. aeruginosa* and one Gram-positive (*S. aureus*) with their respective MIC values of 250, 500, 500, 750, 750 and 750 µg/mL. The essential oils of *M. spicata* showed strong activity against Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* ($P<0.05$) with MIC values of 250 µg/mL. On the other hand, the essential oil of *R. officinalis* showed lower activity against pathogenic bacteria, with MIC value of 1000 µg/mL against *E. coli*. The essential oil also exhibited potential antifungal effect against one pathogenic yeast and two phytopatogenic fungi (Table 4). The MIC values of *F. vulgare* essential oil were 250 µg/mL for *F. oxysporum* and 750µg/mL for *A. niger.* The MIC of *M. spicata* essential oil was 250 µg/mL for *C. albicans* and 500 µg/mL for *A. niger*. The MIC of essential oil of *R. officinalis* was 500µg/mL against to *C. albicans*.

There have been some reports on the essential oils activity of *F. vulgare* and *R. officinalis* inhibiting the growth of bacteria and fungi. According to Aprotosoaie *et al*. [25], the essential oil of *F.*

vulgare showed antimicrobial activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans* and *A. niger*. Fu *et al.* [23] reported that *R. officinalis* essential oil presented antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *A. niger* and *C. albicans*. Some antimicrobial activities of essential oil of *R. officinallis* are also reported by Gachkar *et al.* [24] and Bozin *et al.* [26].

The antimicrobial activity of essential oils have been associated to terpenes with aromatic rings and phenolic groups, alcohols, aldehydes and esters, which can be responsible for their activity despite being minor compounds. The action mechanism of the essential oils is not yet known but due to their hydrophobic character they can interact with membrane lipids destabilizing the membrane structure and turning it permeable [27].

The *in vitro* antimicrobial assays showed that all essential oils tested in the present work have antimicrobial activity. Essential oils of *F. vulgare* and *M. spicata* showed significant antibacterial activity either against Gram-positive and Gram-negative bacteria. Alls essential showed antimicrobial activity against *S. aureus* that is a major human pathogen, which can colonize many different tissues and organs, thereby causing a wide variety of diseases. The resulting complexity of staphylococcal pathogenesis poses an urgent challenge, especially in light of increasing resistance to antibiotics and the emergence of severe invasive infections [28]. *M. spicata* oil showed the highest activity against *S. aureus*, with MIC of 250 µg/mL. This essential oil was also the most active oil against some Gram-negative bacteria, namely *P. aeruginosa* and *E. coli*. However essential oil of *F. vulgare* showed also strong antibacterial activity (MICs ≤ 500 µg/mL) against some Enterobacteriaceae as *S. enteritidis, K. pneumonia* and *P. mirabilis*. These results are important because the Gram-negative bacteria showed frequently a greater resistance to some antibiotics and extracts of medicinal plants [29]. This fact could be due to the outer membrane of Gram-negative bacteria form a barrier and the periplasmic space have enzymes that are able to break down foreign molecules [30]. Essential oil of *M. spicata* and *R. officinalis* showed also strong antifungal activity against *C. albicans*, a harmless commensally yeast-like fungus in healthy humans, which can cause superficial as well as systemic infections under immune compromised situations. *C. albicans* can colonize or infect virtually all body sites because of its high adaptability to different host niches by the activation of appropriate sets of genes in response to complex environmental signals [31]. Essential oils of *F. vulgare* and *M. spicata* showed also significant antifungal activity against the food spoilage fungi *A. niger* and *F. oxysporum* and may have important applications as food additives.

Conclusion

As discussed above, essential oils studied showed good antioxidant and a broad activity against bacteria and pathogenic fungi. *Foeniculum vulgare, Mentha spicata* and *Rosmarinus officinalis* essential oils showed antioxidant activity either by the DPPH and β-carotene/linoleic acid methods. However, these three essential oils showed larger potential on the scavenging activity than on the inhibition of the linoleic acid oxidation. *F. vulgare* and *M. spicata* essential oils showed a valuable antimicrobial spectrum, against Gram positive and Gram negative bacteria and some yeast and molds, while the *R. officinalis* showed a poor activity against the tested microorganisms, but further studies will be required on other microbial strains to evaluate if this essential oil has therapeutic potential. The antioxidant, antimicrobial and aromatic properties of the essential oils studied support the use of these plants on the Portuguese traditional food by its contribution to control the foodstuff spoilage and by its aromatic properties. Assays *in vitro* and *in vivo* to estimate the toxicity of these essential oils will be performed, considering the possibility on their incorporation into pharmaceutical formulations or their use as additives in the food industry.

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Table 3- Antimicrobial activity of essential oils and comparison to commercial antibiotics and the fungicide nystatin

Microorganism		*E. coli* (ATCC 25922)	*k. pneumonia* (ATCC 13883)	*S. enteritidis* (ATCC 13076)	*P. mirabilis* (ATCC 7002)	*P. aeruginosa* (ATCC 8603)	*S. aureus* ATCC 29213)	*C. albicans*	*A. niger*	*F. oxysporum*
		Inhibition zone (mm)								
Esential oil (5 µL)	*F. vulgare*	8 ± 1^{a}	7 ± 1^{a}	7 ± 1^{a}	9 ± 1^{a}	7 ± 1^{a}	7 ± 1^{a}	nd	ti ^{a}	ti ^{a}
	M. spicata	12 ± 2^{b}	8 ± 1^{a}	7 ± 1^{a}	8 ± 2^{a}	$8\pm1^{a,b}$	9 ± 1^{a}	32 ± 3^{a}	ti ^{a}	ti ^{a}
	R.officinalis	8 ± 1^{a}	nd	nd	nd	nd	7 ± 1^{a}	8 ± 1^{b}	nd	nd
Antibiotics	SXT (25µg)	nd	14 ± 1^{b}	18 ± 1^{b}	17 ± 2^{b}	nd	17 ± 1^{b}	-	-	-
	CT (30µg)	14 ± 1^{b}	9 ± 1^{a}	14 ± 2^{c}	$16\pm1^{b,c}$	nd.	nd	-	-	-
	GM (10µg)	ne	13 ± 1^{b}	12 ± 2^{c}	13 ± 1^{c}	nd.	10 ± 1^{a}	-	-	-
	AK (30µg)	9 ± 1^{a}	ne	ne	ne	14 ± 1^{c}	ne	-	-	-
Fungicide	Nystatin (33µg)	-	-	-	-	-	-	21 ± 1^{c}	10 ± 3^{b}	10 ± 3^{b}

nd- not detected; ne- not evaluated; ti- total inhibition; SXT- Trimetroprim+Sulfamethoxazole;
AMC- Amoxicilin +Clavulamic acid; P- Penicillin G; CAZ- Ceftazidime; TE- Tetracycline;
GM- Gentamicin; PB- Polimicine B; AK- Amikacin.
Values are means ± standard deviation of three separate experiments.
Different letters in superscript along each column indicate significant differences ($P < 0.05$).

Table 4 - Minimum inhibition concentration (MICs) of essential oils

Essential oil	Minimum inhibitory concentration (µg/mL)								
	E. coli	*k. pneumonia*	*S. enteritidis*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. niger*	*F. oxysporum*
F. vulgare	750^{a}	500^{b}	250^{c}	500^{b}	750^{a}	750^{a}	nd	750^{c}	750^{c}
M. spicata	250^{c}	500^{b}	250^{c}	750^{a}	500^{b}	250^{c}	250^{a}	500^{b}	500^{b}
R. officinalis	1000^{d}	nd	nd	nd	nd	1000^{d}	500^{b}	nd	nd

nd– not detected for [essential oil] at 1000 µg/mL.
Values are means ± standard deviation of three separate experiments.
Different letters in superscript indicate significant differences ($P< 0.05$).