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Influence of harvesting time on composition of the essential oil of *Thymus capitatus* (L.) Hoffmanns. & Link. growing wild in northern Sicily and its activity on microorganisms affecting historical art crafts

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Abstract One of the main factors affecting historical art crafts material is the biodeterioration performed by bacteria and fungi, in archives, museums or private collections. Several microorganisms cause degradation to the natural organic material such as fibers, woods, and dyes as well as to stone objects. These alterations produce deterioration of physical, chemical, mechanical and esthetic properties. Consequently, in this publication, we report the high antibacterial and antifungal activities of wild thyme essential oil, oil that can be used as an alternative natural tool in the fight against microorganisms affecting historical art crafts.

Essential oil of the *Thymus capitatus* growing wild in northern Sicily has been extracted by hydrodistillation from aerial parts collected at different growth times. The constituents of the essential oil have been characterized by gas chromatography (GC) and GC–mass spectrometry.

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Fifty-five compounds have been identified. Essential oils were characterized by a high content of carvacrol (81.2–14.2%), γ -terpinene (34.4–2.6%) and *p*-cymene (22.8–5.0%) of the total oil content. Essential oil yield and composition vary throughout the vegetation time of the plant. The best time to harvest this species of thyme, for phenol content, is during or immediately before the full bloom. The related oils (**Tc2** and **Tc3**) showed a good antibacterial activity against *Bacillus subtilis* and *Staphylococcus epidermidis* and excellent antifungal properties against *Fusarium oxysporum* and *Aspergillus niger*.

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1. Introduction

The *Thymus* genus is one of the most taxonomically complex genera in the Lamiaceae family and includes 250–350 taxa (species and varieties) of wild growing evergreen species of herbaceous perennials and subshrubs, native to Southern Europe, North Africa and Asia (Könemann, 1999; Morales, 2002; Lawrence and Tucker, 2002); furthermore the presence of several chemotypes, according to the essential oil profiles associated with several species has been reported (Senatore, 1996). Thyme is a largely used medicinal plant. In ancient times it was used by the Egyptians as unguents for embalming and then by the Greeks and Romans for its therapeutic purposes (Barros et al., 2010). Thyme is used for its expectorant, spasmolytic and antiseptic properties and infusions are used for treating ulcers, dermatitis and rheumatic pains (Zaruelo and Crespo, 2002; Mulas, 2006). All the aforesaid activities can be ascribed to the considerable presence of polyphenol derivatives (Vila, 2002) in thyme extracts and/or infusions.

The essential oil of *Thymus* ssp. shows a broad spectrum of bioactivities. In fact, its application as food preservative (Bagamboula et al., 2004; Baydar et al., 2004; Rasooli et al., 2006; Omidbeygi et al., 2007; Solomakos et al., 2008; Nedorostova et al., 2009), as antioxidant (Lee et al., 2005; Bozin et al., 2006), and as additive to enhance organoleptic characteristics (Viuda-Martos et al., 2009) have been reported. The *in vitro* antimicrobial activity of the essential oils of several *Thymus* species has also been reported (De Feo et al., 2003; De Martino et al., 2009; Chedia et al., 2013; Kuecukbay et al., 2014). Thyme essential oils, from a chemical point of view, are characterized by a large amount of monoterpenes, which normally account for 90% of oil. Thymol and carvacrol occur more frequently, always accompanied by the couple *p*-cymene/ γ -terpinene, the four monoterpenes being biogenetically closely correlated (Poulose and Croteau, 1978a, 1978b). Also linalool, borneol, 1,8-cineole are often present, although in lesser amount (Stahl-Biskup, 2002, 2004).

Thymus capitatus (L.) Hoffmanns. & Link. [syn. *Coridothymus capitatus* (L.) Reichenb. fil; *Thymbra capitata* (L.) Cav.; *Satureja capitata* L.] (Lamiaceae) is a Mediterranean endemic plant (<http://apps.kew.org/wcps/qsearch.do>) commonly used as a condiment for typical Mediterranean cuisine in Portugal, Spain, Italy, Cyprus, and Greece (Facciola, 1990). Leaves are eaten raw in salads or added as a flavoring to cooked foods (Hedrick, 1972) and are also used to prepare an aromatic tea (Huxley, 1992). The traditional use of this plant as food preservative has been experimentally confirmed on meat and fish (Mohammed

et al., 2010) and it could be explained by the its antimicrobial, antifungal and antioxidant activities (Blanco et al., 2010; Faleiro et al., 2005; Albano et al., 2012).

The essential oil from the plant, called “Spanish oregano oil”, is used for flavoring baked goods, condiments, beverages, and ice creams, (Facciola, 1990; Bown, 1995) and, in perfumery and soaps, as a mouth wash (Huxley, 1992; Bown, 1995), but should not be used in aromatherapy because it is highly irritant to the mucous membranes (Bown, 1995). *T. capitatus* essential oil has been shown to have antispasmodic (Al-Qura'n, 2009), anti-inflammatory (Albano and Miguel, 2011), parasiticide (Machado et al., 2010), nematocidal (Faria et al., 2013), antifungal, and antimicrobial properties (Cosentino et al., 1999; Arras and Usai, 2001; Bounatirou et al., 2007; Mohamed and Eddine, 2010; Palmeira-de-Oliveira et al., 2012; Palmeira-de-Oliveira et al., 2013; Saoud et al., 2013).

Several papers have been published on the chemical composition of the essential oil of *T. capitatus* (Cosentino et al., 1999; Arras and Usai, 2001; Miceli et al., 2006; El Ajjoury et al., 2008; Figueiredo et al., 2008; Blanco et al., 2010; Napoli et al., 2010; Tawaha and Hudaib, 2012; Yvon et al., 2012; Ballester-Costa et al., 2013; Chedia et al., 2013; Saoud et al., 2013; Hortigón-Vinagre et al., 2014; Bouayad Alam et al., 2014). It is very rich in the terpenes thymol, carvacrol, and *p*-cymene. Chemotypes thymol, thymol-carvacrol, and carvacrol have been described, the latter being the most abundant (Karousou et al., 2005; Miceli et al., 2006; Bounatirou et al., 2007).

Several heterotrophic microorganisms (bacteria and fungi) have the ability to interact with historical organic materials such as textile, leather, paper, paintings, wood, papyri, incunabula and books, all consisting essentially of natural fibers. Furthermore, the presence of residues of glues of vegetable and animal origin on the fibers and between the fibers, of dirt of various origins, dust and other contaminants, facilitates the processes of degradation caused by these microorganisms. Microbial growth causes loss of strength and elongation, oxidation state, discoloration, changes in appearance, degree of polymerization and breakdown of molecular structure. Species of genus *Bacillus*, frequent in archives, libraries and on museum cellulosic objects can cause deep deteriorations of the items but the most dangerous microorganisms both for cellulose fibers and fibers of animal origin (wool, silk) belong to some genera of fungi: *Chaetomium*, *Myrothecium*, *Memnoniella*, *Stachybotrys*, *Verticillium*, *Alternaria*, *Trichoderma*, *Fusarium*, *Penicillium* and *Aspergillus*. The degradation of cellulosic fibers can be ascribed to cellulases, a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobiose or oligosaccharides (Kamel et al., 2014) whereas wool is degraded by a

process of keratinolysis that includes firstly the denaturation of the substrate by fission of disulfide bridges, which are the source of the natural resistance of keratin, and then by hydrolytic degradation of protein via extracellular proteinases (Szostak-Kotowa, 2004).

Also stone monuments, in moderate and humid climates, can be colonized by fungal communities. The surfaces of stone monuments can be altered by fungal activity via hyphal penetration through the porous stone matrix and by the production of organic acids and pigments (Stupar et al., 2014).

The growing interest on natural products that can be used as an alternative to synthetic chemicals in order to prevent and reduce the dangerous effects of microorganisms on historical artifacts (Mansour, 2013; Rakotonirainy and Lavèdrine, 2005; Stupar et al., 2014; Casiglia et al., 2014a, 2014b; Casiglia et al., 2015) prompted us, in the frame of our previous reports on Sicilian *Thymus* ssp. (De Feo et al., 2003; De Martino et al., 2009), to investigate on the chemical composition and anti-microbial properties against several microorganism, including *Bacillus subtilis*, *Fusarium oxysporum* and *Aspergillus niger*, species infesting historical material (Kamel et al., 2014; Gupta, 2013), of *T. capitatus*, collected on the northern coast of Sicily at three different vegetative stages. Considering that in addition to microorganisms using biochemical processes of degradation, may be found on art craft materials microorganisms whose growth can be connected with the presence of substances (adhesives, dyes, finiture, etc.) added during the production process were also found, and we have extended the study to other species potentially present and pathogenic for example *Staphylococcus epidermidis*, *Escherichia coli* and *Proteus vulgaris*.

2. Materials and methods

2.1. Plant material

Aerial parts of *Thymus capitatus* (L.) Hofm. & Lk. were collected at Salinelle Beach, Lascari, Palermo (38°01'48"N, 13°18'40"E, 6 m s/l), 50 km east of Palermo, Sicily (Italy), on the 14th of May 2014 (Tc1), on the 19th of June 2014 (Tc2) and on the 16th of July 2014 (Tc3). Typical specimens (PAL 14/97 MB), identified by Mr. Emanuele Schimmenti, have been deposited in the Department STEBICEF, University of Palermo, Palermo, Italy.

2.2. Essential oil isolation

The air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h using *n*-hexane as a solvent, according to the standard procedure previously described (Ben Jemia et al., 2013). The oils were dried over anhydrous sodium sulfate and then stored in sealed vials, at -20 °C, ready for the GC and GC-MS analyses. The samples yielded 0.92% (Tc1), 0.65% (Tc2) and 0.88% (Tc3), respectively, of yellow oils (w/w), with a very characteristic smell.

2.3. Qualitative and quantitative analyses of essential oil

2.3.1. Gas chromatography-mass spectrometry

The essential oil samples were analyzed to determine the chemical components at the Department of Pharmacy of the

University of Naples "Federico II" by GC and GC-MS. Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness), a split-splitless injector heated at 250 °C and a flame ionization detector (FID) at 280 °C. Column temperature was initially kept at 40 °C for 5 min, then gradually increased to 250 °C at 2 °C/min, held for 15 min and finally raised to 270 °C at 10 °C/min. The injection volume was 1.0 μL (split ratio 1:20). A fused silica HP Innowax polyethyleneglycol capillary column (50 m × 0.20 mm, 0.25 μm film thickness) was also used for analysis. In both cases helium was the carrier gas (1 mL/min). GC-MS analysis was performed on an Agilent 6850 Series II apparatus, fitted with a fused silica HP 5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; and source temperature 250 °C. Mass spectra were scanned in the range 35–450 amu, scan time 5 scans/s. Gas chromatographic conditions were the same as those for GC; transfer line temperature, 295 °C.

2.3.2. Identification of components

Most of the constituents were identified by GC by comparison of their retention indices (R_i) with either those in the literature (Davies, 1990; Jennings and Shibamoto, 1980) or with those of authentic compounds available in our laboratories or purchased from the Sigma-Aldrich Co. Retention indices were determined in relation to a homologous series of *n*-alkanes (C₈–C₃₀) under the same conditions. Further identification of oil components was achieved by comparing their mass spectra on both columns, either with those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature (Jennings and Shibamoto, 1980; Adams, 2007; E.P.I.M.S., 1983) and our personal library. Component relative concentrations were calculated based on GC peak areas without using correction factors. These results are shown in Table 1.

2.4. Antimicrobial assay

2.4.1. Microbial strains

The antimicrobial and antifungal activities of essential oil were tested against a panel of eight bacteria species, selected as representative of the class of Gram positive and Gram negative, *B. subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *P. vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853) one yeast, *Candida albicans* (ATCC 10231); two molds, *F. oxysporum* (ATCC 695) and *A. niger* (ATCC 16401). The strains were grown on Tryptone Soya Agar (Oxoid, Milan, Italy) for the bacteria, Sabouraud Dextrose Agar (SDA) with chloramphenicol for yeasts and SDA for molds. For the antimicrobial tests, Tryptone Soya broth (Oxoid, Milan, Italy) for bacteria and Sabouraud dextrose broth (SDB) for yeasts and fungal strains were used.

2.4.2. Antimicrobial screening

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), which includes minimum

Table 1 Essential oil composition from *Thymus capitatus* Hoff. et Link. (Lamiaceae) growing wild in northern Sicily.

K_i^a	K_i^b	Ident. ^c	Compounds	14/5	19/6	16/7
Monoterpene hydrocarbons				65.8	13.9	13.5
930	1014	1,2	α -Thujene	2.4	1.0	1.5
938	1032	1,2,3	α -Pinene	2.6	0.5	0.7
953	1076	1,2,3	Camphene	0.2	0.1	0.1
973	1132	1,2	Sabinene	0.3		
978	1118	1,2,3	β -Pinene	0.5	0.1	0.2
993	1174	1,2,3	Myrcene	2.1	1.1	1.4
1005	1150	1,2,3	α -Phellandrene	0.3	0.1	0.3
1009	1157	1,2	δ^3 -Carene	t	0.1	0.1
1012	1189	1,2,3	α -Terpinene	0.2	0.8	1.0
1025	1278	1,2,3	<i>p</i> -Cymene	22.8	5.8	5.0
1029	1218	1,2	β -Phellandrene		0.3	0.4
1049	1262	1,2	(<i>E</i>)- β -Ocimene			0.1
1057	1256	1,2,3	γ -Terpinene	34.4	3.8	2.6
1086	1265	1,2,3	Terpinolene		0.2	0.1
Oxygenated monoterpenes				0.2	0.6	1.7
1097	1553	1,2,3	Linalool			0.4
1063	1555	1,2	(<i>Z</i>)-Sabinene hydrate	t	0.1	t
1098	1475	1,2	(<i>E</i>)-Sabinene hydrate	t		
1167	1718	1,2,3	Borneol		0.1	0.1
1176	1611	1,2,3	Terpineol-4	0.1	0.3	0.5
1189	1706	1,2,3	α -Terpineol			0.1
1195	1624	1,2	<i>cis</i> -Dihydrocarvone	0.1		
1238	1694	1,2	Neral		0.1	0.2
1243	1752	1,2	Carvone			0.1
1268	1741	1,2	Geranial			0.3
Sesquiterpene hydrocarbons				2.5	3.3	2.1
1363	1492	1,2	Cyclosativene	0.2		
1407	1538	1,2	α -Gurjunene	t		
1415	1612	1,2,3	(<i>E</i>)- β -Caryophyllene	0.6	2.3	1.7
1437	1628	1,2	Aromadendrene		0.1	
1455	1689	1,2	α -Humulene		0.1	0.1
1463	1667	1,2	<i>allo</i> -Aromadendrene		t	
1487	1679	1,2	α -Amorphene	t		
1489	1743	1,2	Eremophilene	0.1		
1508	1746	1,2	(<i>Z</i>)- α -Bisabolene	0.7	0.3	0.1
1511	1743	1,2	β -Bisabolene	0.5	0.4	0.2
1513	1709	1,2	Ledene		t	
1526	1773	1,2	δ -Cadinene	0.2	0.1	
1494	1687	1,2	Viridiflorene	0.2		
Oxygenated sesquiterpenes				3.2	1.5	0.5
1578	2150	1,2,3	Spathulenol		0.2	t
1580	2008	1,2,3	Caryophyllene oxide	2.2	1.3	0.5
1612	2018	1,2	Humulene oxide II	0.3		
1615	2324	1,2	Caryophylla-4(12),8(13)-dien-5 α -ol; Caryophylladienol II	0.3		
1640	2158	1,2	t-Cadinol	t		
1642	2209	1,2	τ -Muurolool	0.1		
1716	2478	1,2	14-Hydroxy- α -humulene	0.3		
Diterpenes				1.1	0.1	
1896	2147	1,2	Rimuene			t
1908	2176	1,2	Isopimara-9-(11),15-diene	0.7		
2054	2524	1,2	Abietatriene	0.4	0.1	
Phenolic compounds and derivatives				19.8	76.7	81.5
1245	1975	1,2,3	Carvacrol methyl ether	1.9	0.5	0.2
1297	2239	1,2,3	Carvacrol	14.2	76.1	81.2
1298	1956	1,2	Carvacrol ethyl ether	0.7		
1353	2186	1,2,3	Eugenol	0.8	0.1	0.1
1367	1885	1,2,3	Carvacryl acetate	1.5		
1472	1985	1,2	Carvacryl isobutyrate	0.7	t	

Table 1 (continued)

K_i^a	K_i^b	Ident. ^c	Compounds	14/5	19/6	16/7
Others					0.1	0.5
980	1452	1,2	1-Octen-3-ol		0.1	0.4
992	1394	1,2	3-Octanol			0.1
Total				92.6	96.2	99.8

^a K_i : linear retention index: on HP 5MS column.

^b K_i : linear retention index: on HP Innowax column.

^c K_i : linear retention index: (1) retention index identical to the literature, (2) MS: comparison of mass spectra with MS libraries, (3) Co-GC: co-injection with authentic compound; t, trace, less than 0.05%.

bactericidal (MBC) and minimum fungicidal concentrations (MFC), using the broth dilution method (Barry, 2007). In order to facilitate the dispersion of the oil in the aqueous nutrient medium, it was diluted with Tween 20 at a concentration of 10%. The mixture was thoroughly agitated over 2 min to disperse the oil. Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 100 to 0.8 µg/mL. The sample, previously sterilized with Millipore filter of 0.20 µm, was stirred and inoculated with 50 µL of suspension of the tested microorganisms, containing $2.0 \cdot 10^6$ CFU/mL for bacteria and $2.0 \cdot 10^5$ CFU/mL spore for fungal strains, and incubated for 24 h at 37 °C for bacteria, 48 h at 30 °C for yeasts and 10 days at room temperature for molds. The MIC value was determined as the lowest concentration of the sample at which the tested microorganisms did not demonstrate any visible growth after incubation. Control containing only Tween 20 instead of the essential oil was not toxic to the microorganisms. As positive control cultures containing only sterile physiologic solution Tris buffer were used. Chloramphenicol, Amphotericin B and ketoconazole were used as standard antimicrobial agents. The MMC is defined as the lowest concentration of the essential oil at which inoculated microorganisms were completely killed. MMC was determined by subculture of the test tubes containing samples concentrations equal or higher than the MIC in 5 ml of sterile nutrient broth. After incubation the tubes were observed. When the germs did not grow, the sample denoted a microbicidal action. Oil samples were tested in triplicate.

3. Results and discussion

3.1. Chemical composition of the essential oils

T. capitatus aerial parts wild growing in Salinelle, Sicily, were collected in three different vegetative stages: before blooming on 14th of May 2014 (Tc1), at the beginning of blooming on the 19th of June 2014 (Tc2) and at full blooming stage on the 16th of July 2014 (Tc3). Hydrodistillation of *T. capitatus* aerial parts (Tc1, Tc2 and Tc3) gave pale yellow oils. Overall, 55 compounds were identified, representing 92.6%, 96.2% and 99.8%, respectively, of the total components. The components are listed in Table 1 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into 7 classes (Table 1).

The oil of Tc1 is particularly rich in monoterpene hydrocarbons (65.8%, 11 compounds) with γ -terpinene (34.4%) and

p-cymene (22.8%) as the main components of the oil. Phenolic compounds and derivatives, the second class of the oil, represent the 19.8% (6 compounds), being carvacrol (14.2%) and its derivatives (4.8%) the most abundant components. It is noteworthy the very poor amount (0.2%) of oxygenated monoterpenes and sesquiterpenes (5.7%).

The profiles of Tc2 and Tc3 oils are quite similar both in composition and quantity of the metabolites, but quite different from Tc1. In fact, in these cases, the main class was phenolic compounds and derivatives, accounting for the 76.7% and 81.5%, respectively. The two oils were extremely rich of carvacrol (76.1% and 81.2%, respectively), and, practically, devoid of its derivatives. Monoterpene hydrocarbons are present in lesser amount with respect to Tc1 (13.9% and 13.5% for Tc2 and Tc3, respectively) with γ -terpinene (3.8–2.6%) and *p*-cymene (5.8–5.0%) as the main components of the class.

Our results on Tc2 and Tc3, when compared with the data reported in the literature for the oil of *T. capitatus* collected in the southern part of Sicily (Napoli et al., 2010), indicate a similar profile. In fact, the range of carvacrol (70–83%), γ -terpinene (t-10%) and *p*-cymene (4-8%) is similar to those of Tc2 and Tc3. The only exceptions are the population of Alcamo containing minor quantity of carvacrol (48.9%) and larger amount of γ -terpinene (7.1%) and *p*-cymene (19.1%) and the population of Galati Mamertino which is practically devoid of carvacrol (2.7%) but quite rich of thymol (16.1%), γ -terpinene (10.4%) and *p*-cymene (40.4%). It is noteworthy that our three oils are completely devoid of thymol according to the studies that demonstrate that the composition of the essential oil depends on plant type, geographical location and collection time (Adzet et al., 1977; Circella et al., 1995; Hornok, 1988; Senatore, 1996; Vidic et al., 2010).

As previously stated, chemotypes thymol, thymol-carvacrol, and carvacrol have been described for *T. capitatus* (Karousou et al., 2005; Miceli et al., 2006; Bounatirou et al., 2007) and our population clearly belongs to the last one.

The analysis of our oils showed that before the early flowering stage (Tc1), the monoterpene hydrocarbon content peaked at 65.8%. Successively, at the beginning (Tc2) and during the flowering (Tc3), this value decreases (13.9% and 13.5%, respectively), but the amount of carvacrol increases drastically (76.7% and 81.5%, respectively). The large amount of monoterpene hydrocarbons in Tc1 can be justified since the monoterpenes γ -terpinene and *p*-cymene are considered the biogenetic precursors (via enzymatic hydroxylation) of the phenolic terpene carvacrol (Poulose and Croteau, 1978a, 1978b; Vernet et al., 1986).

Table 2 MIC ($\mu\text{g/mL}$) and MMC^a ($\mu\text{g/mL}$) of essential oils from *Thymus capitatus* growing wild in northern Sicily.

Strain	Tc1	Tc2	Tc3	Ch	Am	Ke
<i>Bacillus subtilis</i> ATCC 6633	50(100)	12.5(25)	25	12.5	NT	NT
<i>Staphylococcus aureus</i> ATCC 25923	50(100)	25(50)	25(50)	25	NT	NT
<i>Staphylococcus epidermidis</i> ATCC 12228	25(50)	12.5(25)	12.5	3.12	NT	NT
<i>Streptococcus faecalis</i> ATCC 29212	50(100)	50	25(50)	25	NT	NT
<i>Escherichia coli</i> ATCC 25922	50(100)	25(50)	25	12.5	NT	NT
<i>Klebsiella pneumoniae</i> ATCC 10031	100	50(100)	50(100)	50	NT	NT
<i>Proteus vulgaris</i> ATCC 13315	100	100	100	25	NT	NT
<i>Pseudomonas aeruginosa</i> ATCC 27853	100	100(> 100)	100(> 100)	100	NT	NT
<i>Candida albicans</i> ATCC 10231	50(100)	50	25(50)	NT	1,56	NT
<i>Fusarium oxysporum</i> ATCC 695	12.5(25)	6.25(12.5)	6.25	NT	NT	3.12
<i>Aspergillus niger</i> ATCC 16401	12.5	6.25(12.5)	6.25	NT	NT	3.12

^a MBC are reported in brackets when different from MIC; NT: not tested; **Tc1**: collected in May; **Tc2**: collected in June **Tc3** collected in July; **Ch**: Chloramphenicol; **Am**: Amphotericin B; **Ke**: Ketoconazole.

3.2. Antimicrobial activity

Table 2 shows the inhibition of the oils obtained from *T. capitatus* aerial parts collected in three different vegetative stages. The oils affected the tested microorganisms with different degrees. The samples **Tc2** and **Tc3** have comparable level of potency while sample **Tc1** appears less effective. Among the bacteria *B. subtilis* and *S. epidermidis* appear to be most sensitive to the biocidal effect of the samples. This finding totally agrees with the observations derived from studies with essential oils from other thyme species. Nedorostova et al. (2009) reported, in particular, the antimicrobial activity in vapor phase of essential oils of *Thymus vulgaris*, *T. pulegioides* and *T. serpyllum*; in addition, Saoud et al. (2013) reported high antifungal activity for the thyme essential oils isolated at the post-flowering development phase.

The three oils show a similar significant activity against *C. albicans* and a strong effect on the other fungal strains, particularly samples **Tc2** and **Tc3**. Comparatively the oils were more active against Gram positive strains as evidenced by the lower MIC values. The significant activity of the samples is expected by considering their main constituent such as carvacrol that possesses considerable antibacterial and antifungal activities as reported in previous works (Dorman and Dean, 2000; Valero and Giner, 2006; Bouayad Alam et al., 2014; Kordali et al., 2008).

In the last few years, an increasing interest on the antimicrobial action of the different types of essential oils against microorganisms has been reported and in many cases these activities are due to the presence of active constituents such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols. The antimicrobial action of the essential oil components is due to both the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of

their functional groups. Some essential oils containing phenolic structures, such as eugenol, carvacrol and thymol, are highly active against a broad spectrum of microorganisms and several researches have shown the importance of the phenolic group, in fact, low activity was observed with components containing only an aromatic ring with alkyl substituents as in *p*-cymene. Among the non aromatic oxygenated monoterpenes 1,8-cineole and the ketones pulegone, fenchone, α -thujone and camphor were reported to have antimicrobial activities. Also sabinene, terpinenes, α -pinene, β -pinene and limonene, belonging to monoterpenes hydrocarbons, have shown good activity especially against Gram positive bacteria. Essential oils with a high quantity of sesquiterpenes have been also reported to have antibacterial and antifungal properties. The main active components have been identified as 8-cadinene, (*Z*)- β -farnesene, γ -muurolene, caryophyllene oxide, (*E*)-caryophyllene, α -eudesmol, β -eudesmol, spathulenol, hexahydrofarnesyl acetone and α -selinene (Koroch et al., 2007; Negri et al., 2014).

4. Conclusion

The analysis of the essential oils of *T. capitatus* (L.) Hofm. & Lk. collected in the northern coast of Sicily clearly indicated that this population belongs to the carvacrol chemotypes and also has a strong influence of harvesting time on composition of the essential oil.

The high antimicrobial activity of the oils detected against *B. subtilis*, *F. oxysporum* and *A. niger*, microorganisms infesting quite frequently archives, libraries and historical art craft objects, comparable with that of chloramphenicol (**Tc2** MIC = 12.5) and ketoconazole (**Tc2** and **Tc3** MIC = 6.25), respectively, makes this plant interesting for possible applications in the protection and disinfestation of museum objects.

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