Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. & Reut and *Thymus vulgaris* L. essential oils

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ABSTRACT

Aromatic plant species of genus *Thymus* are important medicinal plants, highly recommended due to a range of therapeutic properties of their essential oil (thyme oil): antirheumatic, antiseptic, antispasmodic, antimicrobial, cardiac, carminative, diuretic and expectorant. Oil is also beneficial in boosting the immune system, and helps to fight colds, flu, infectious diseases and chills. It is proved to be a urinary antiseptic, being very helpful for cystitis and urethritis. Scientific validation of traditional uses, and phytochemical and bioactivity evaluation of essential oils from *Thymus serpyllum*, *Thymus algeriensis* and *Thymus vulgaris* was performed.

GC/MS analysis revealed thymol as major component of *T. algeriensis*, *T. vulgaris and T. serpyllum*, with a contribution of 56.02%, 48.92% and 38.50%, respectively. The three essential oils (EOs) exhibited a significant antimicrobial activity against all the tested strains, *T. serpyllum* oil being the most potent (MIC 2.5-5 µg/mL, MBC 5-10 µg/mL; MIC 1-2 µg/mL, MFC 2-4 µg/mL). In addition, *T. serpyllum* oil revealed the highest antioxidant activity in all the assays and was also the most effective one against all the tested cell lines, presenting GI_{50} values of 7.02-52.69 µg/mL. Moreover, the EOs did not show any toxicity, at the tested concentrations (<400 µg/mL), for porcine liver primary cell culture. In addition to their traditional use in food and cosmetics, the great potential of the tested *Thymus* essential oils for application in oral disease and anticancer treatments, encourage further investigation.

Keywords: T. serpyllum, *T. algeriensis*, *T. vulgaris*, essential oils, chemical composition, cytotoxic, antioxidant, antimicrobial activity.

1. Introduction

The genus *Thymus* L. belongs to the family Lamiaceae, and consists of about 215 to 350 species, according to different literature data (Cronquist, 1988; Zaide and Crow, 2005). They are usually herbaceous perennials, small shrubs occurring within the Mediterranean region, which is a center of the entire genus, and are also characteristic for Asia, Southern Europe and North Africa (Maksimovic et al., 2008). Throughout the history, the aerial parts and the volatile constituents of *Thymus* species are highly recommended; they are commonly used as herbal teas, condiments and spices, so as for various medicinal purposes (Stahl-Biskup and Saez, 2002). Many ethnomedicinal properties are attributed to infusions, decoctions and essential oils of the aerial parts of *Thymus* species, which are used due to their tonic, carminative, digestive, antispasmodic, antimicrobial, antioxidant, antiviral, anti-inflammatory and expectorant activity, so as for the treatment of colds (Nickavar et al., 2005; Pirbalouti, 2009). Thyme oil is among the world's top 10 essential oils also used as a preservative for food (Stahl-Biskup and Saez, 2002). The aromatic and medicinal properties of the *Thymus* species have made it one of the most popular herbs. The genus *Thymus* has numerous species and varieties and their essential oils have been studied earlier (Guillen and Manzanos, 1998). However, there are considerable research interests to continue with studying of many other biological properties of *Thymus* essential oils (Stahl-Biskup and Saez, 2002; Shin and Kim, 2005).

Thymus vulgaris L. is a perennial herb indigenous in central and southern Europe, Africa and Asia. It is rich in essential oils and antioxidative phenolic substances (WHO, 1999). It is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders, anthelmintic, carminative, sedative, diaphoretic (Rustaiyan et al., 2000). It has been reported that its essential oil possesses numerous biological activities including antiworm, antiseptic, antispasmodic, antimicrobial

(Marino et al., 1999) and antioxidant (Miura et al., 2002; Soliman and Badeaa, 2002; Pina-Vaz et al., 2004). *T. vulgaris* is well-known species of the genus *Thymus* and extensively studied for chemical and biological activity (Simandi et al., 2001; Soković et al., 2008, 2009)

T. algeriensis is the most widespread North African species. It is endemic to Libya, Tunisia, Algeria and Marocco (Houmani et al, 2012). *T. algeriensis* is largely used, fresh or dried, only as a culinary herb. Its chemical compositions have been studied previously (Giordiani et al., 2008; Hazzit et al., 2009; Giweli et al., 2013), tough results of its biological activity are scarce. This species is also used in traditional medicine in the form of a fresh or dry spicy herb, in respiratory disorders, against illnesses of the digestive tube and anti-abortion (Giweli et al., 2013).

T. serpyllum, known as wild thyme, is native to Mediterranean Europe and North Africa, mainly at the higher altitudes. It is acknowledged for its use in home remedies. The plant is aromatic, antiseptic, diaphoretic, analgesic, carminative, expectorant and diuretic; also it acts as an emmanagogue, carminative, and stimulant, also being used in mouth washes, gargles, cough and colds (Farooqi et al., 2005). Its essential oil contains various compounds that are very powerful, proven disinfectants enhancing the immune system and fighting infections. The oil relieves rheumatism, and is also used in hear loss-treatments (Aziz and Rehman, 2008).

To the best of our knowledge, as far as the literature is concerned, this study represents the first report on cytotoxic activity of the three thyme oils on the following tumor cell lines: lung, breast, cervical, colon and gastric cancer. In addition, toxicity of the oils to non-tumor cells was also evaluated. The oils were submitted to bioactivity evaluation by measuring *in vitro* antioxidant potential. Even though, the antibacterial and antifungal activity exhibited by *Thymus* species has already been demonstrated (Cruz et al., 1989; Karaman et al., 2001; Rota et al., 2004; Couladis 2004; Soković et al., 2008, 2009) unfortunately, there

are only few quantitative data (minimal inhibitory concentration or minimal bactericidal/fungicidal concentration) related to the antimicrobial activity of the oils against the human oral microorganisms.

Therefore, our primary objective was to characterize the essential oils of *T. serpyllum*, *T. algeriensis* and *T. vulgaris*, and to evaluate their antimicrobial, antioxidant, antitumor and cytotoxic attributes, in an attempt to contribute to their use, as alternatives, in microbial control and cancer therapy in humans.

2. Material and methods

2.1. Essential oil

Wild thyme oil (*Thymus serpylum* L.) is commercial sample from Greece local pharmacy. The samples from wild growing *Thymus algeriensis* plants were collected during the flowering stage in May 2010 from Zentan (Libya), which is located on the top of Western mountain (Aljabel Algarbi) at altitude about 700 m a.s.l. The plants were identified by Dr A. Felaly, Faculty of Science, Al-Gabel Al-Garbi University Libya. The samples were dried in shadow at room temperature for 10 days. Voucher specimens where deposited in Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" (BEOU), (voucher No. 16614). *Thymus vulgaris* L. plants were collected during the summer (July) in 2006 at the experimental field of the Institute for Medicinal Plant Research “Josif Pančić” in Pančevo (Serbia). The species was identified bz Prof. Petar Marin, at the Institute of Botany, Faculty of Biology of the University of Belgrade, where a voucher specimen is deposited (voucher No 17432).

2.2 Isolation of the essential oil

Air-dried aerial parts of *Thymus vulgaris* and *T. algeriensis* deprived from wooden parts (100 g) were submitted to hydrodistillation, using Clevenger-type apparatus for 3 h, according to

the standard procedure. The obtained essential oils were dried over Na_2SO_4 and stored in a sealed dark vials, then kept at 4ºC prior to further analysis.

2.3. Essential oil analysis

The EO sample was diluted in ethanol (1 μL) and injected in a split-mode (1:30). Gas chromatography was performed on GC Agilent Technologies 7890A apparatus, equipped with the split-splitless injector attached to HP-5 column (30 m × 0.32 mm, film thickness 0.25 μm) and fitted to flame-ionisation detector (FID). Operating conditions were as follows: carrier gas was H_2 (1 mL/min/210°C); temperatures were set as follows: injector at 250°C and detector at 280°C, while the column temperature was linearly programmed 40–260°C at 4°C/min. The percentage composition was computed from the peak areas, without correction factors.

The GC-MS was performed on HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). Carrier gas was He (1 mL/min). Other chromatographic conditions were as those for GC-FID. Transfer line was heated at 260°C. Mass spectra were recorded in EI mode (70 eV), in a range of m/z 40–450.

The identification of individual constituents was accomplished by comparison of their spectra with those from available MS libraries (NIST/Wiley) and by comparison of their experimentally determined retention indices (calibrated AMDIS), with data from the literature (Adams, 2009).

2.4. Microorganisms

The following six clinical oral isolates were tested: *Streptococcus mutans* (IBR S001), *Streptococcus sanguis* (two strains, IBR S002 & IBR S003), *Streptococcus pyogenes* (two

strains, IBR S004 & IBR S005), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (IBR P001), and *Lactobacilus sp.* (IBR L002). In antifungal assay, fifty eight clinical isolates of *Candida* spp., and two ATCC strains were used (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750). The reference strains were obtained from the Laboratory of Mycology at the Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The bacteria species were maintained in Mueller Hinton Agar and Triptic Soy Agar (MHA, TSA, Merck, Germany). Strains of *Candida* spp. were maintained on Sabourand Dextrose Agar (SDA, Merck, Germany). All clinical oral isolates were obtained by rubbing a sterile cotton swab over oral mucosa from patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia.

The colonies obtained were analyzed for morphological, cultural and physiological characteristics. Proper identification of oral bacteria (Cecchini et al. 2012) and fungi (Nikolic et al. 2012) colonies were performed.

2.5. Antimicrobial activity

Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method in 96 well microtitre plates described by CSLI (2006) with modifications. Briefly, fresh overnight cultures of bacteria and yeasts were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/per well. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spores suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The inocula were stored at 4°C for further use. Essential oils were added in TSB (Merck, Germany) medium for bacteria, SDB medium for *C. albicans*, and MB medium for fungi. The

microplates were incubated for 24 h at 37°C for bacteria and yeasts, while 72 h at 28°C for fungi. The MIC/MBC values for bacteria and yeasts were detected following the addition of 40 μL of p-iodonitrotetrazolium violet (INT) 0.2 mg/mL (Sigma I8377) and incubation at 37°C for 30 min (Tsukatani, 2012). For the fungi, the lowest concentrations without visible growth for 72 h at 28°C were defined as MIC, while MFC was determined by serial subcultivation of 10 μL into microtiter plates containing 100 μL of broth per well and further incubation for 72 h at 28°C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. Positive controls of antibiotics (Ampicillin and Streptomycin), mycotic (Fluconazole) and commercial antimicrobial preparation (Hexoral and Chlorhexidine 0.05%) were used in both experiments.

2.6 Cytotoxicity in human tumor cell lines and non-tumor primary culture

Five human tumor cell lines were used: MCF7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HCT15 (colon carcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) and 2 mM glutamine (MCF-7, NCI-H460 and HCT-15) or in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin (HeLa and HepG2 cells), at 37 ºC, in a humidified air incubator containing 5% CO_2. Each cell line was plated at an appropriate density (7.5×10^3 cells/well for MCF-7, NCI-H460 and HCT15 or 1.0×10^4 cells/well for HeLa and HepG2) in 96-well plates. Sulforhodamine B assay was performed according to a procedure previously described by the Vichai & Kirtikara (2006).

For hepatotoxicity evaluation, a cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughter house, according to a procedure established by Guimarães et al. (2013), designed as PLP2. Cultivation of the cells has been carried on with

direct monitoring by the phase contrast microscope, every two to three days. Before confluence was reached, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells/well, and cultivated in DMEM medium with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. Ellipticine was used as positive control (0.24-65.2 μg/mL). Three independent experiments were performed in triplicate, and the results were expressed as mean values ± standard deviation (SD).

2.7. Antioxidant activity

2.7.1.DPPH radical-scavenging activity

DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, USA), and calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH} - A_S) / A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution (Reis et al., 2012).

2.7.2. Reducing power

The sample solutions (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% (w/v), 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% (w/v), 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells plate, the same with deionized water (0.8 mL) and ferric chloride (0.1% (w/v), 0.16 mL), and the absorbance was measured at 690 nm in the Microplate Reader, as mentioned above (Reis et al., 2012).

2.7.3. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two milliliters of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 °C under vacuum, and linoleic acid (40 mg), Tween 80 emulsifier (400 mg) and distilled water (100 mL) were added to the flask, with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing sample solutions (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene leaching inhibition was measured by the following formula: β-carotene absorbance after 2 h/initial absorbance) × 100 (Reis et al., 2012).

2.7.4.Thiobarbituric acid reactive substances (TBARS) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtered animals, dissected, and homogenized with Polytron in an ice cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate, which was centrifuged at 3000 × *g* for 10 min. An aliquot (100 μL) of the supernatant was incubated with the sample solutions (200 μL) in the presence of $FeSO_4$ (10 mM; 100 μL) and ascorbic acid (0.1 mM; 100 μL), at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% (w/v), 500 μL), followed by thiobarbituric acid (TBA, 2% (w/v), 380 μL), and then the mixture was heated at 80 °C for 20 min. After centrifugation at 3000 × *g* for 10 min, in order to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where the A and B represent the absorbance of the control and the sample solution, respectively (Reis et al., 2012).

3. Results and Discussion

3.1. Chemical composition

The results obtained by chemical analysis by GC-MS of *T. serpyllum*, *T. algeriensis* and *Thymus vulgaris* essential oils are presented in Table 1.

In total, 48 compounds were identified. Results showed that oxygenated monoterpenes are the major portion of all EOs samples, with highest content observed in *T. algeriensis* (74.61%), and similar content in *T. speryllum* and *T. vulgaris* (54.49% and 58.11%, respectively). Twenty nine compounds were identified in *T. serpyllum* oil, which accounts for 99.98% of the total oil. The major constituent of the oil was thymol (56.02%), followed by carvacrol (14.00%) and *p*-cymene (6.27%). GC-MS analysis of *T. algreriensis* oil showed 45 compounds representing 99.64% of the total oil. Thymol was the main constituent (38.50%) followed by *p*-cymene, terpinene and bornyl acetate and borneol (8.91%, 7.19%, 7.03% and 6.07%, respectively). In the oil of *T. vulgaris*, 26 constituents represented 99.06% of the total oil, with thymol also being the major constituent (49.10%) along with *p*-cymene (20.01%).

According to presented results it is obvious that the oils from all the three Thymus species belong to "thymol chemotype".

Many studies on the chemical composition of the oils from the plants belonging to the genus *Thymus* were conducted, including *T. serppyllum*, *T. algeriensis* and *T. vulgaris* (Stahl-Biskup, 1991; Houmania et al., 2002; Dob et al, 2006; Kizil and Uyart, 2006, Saad et al., 2010).

Our results on chemical profiling of *T. serpyllum* essential oil are in agreement with several other studies (Raal et al., 2004; Verma et al., 2009; Verma et al., 2011;), except for results of Sfaei-Ghomi et al. (2009), where α-pinene and carvacrol were reported to be the major oil components. Besides thymol (30%), carvacrol (20%) was reported to be the second

main component of the wild thyme oils (Thompson et al., 2003), while results of Rasooli and Mirmostafa (2002), showed thymol being the third major component (>18%) in the wild thyme oil, after the content of γ-terpinene (>22%) and *p*-cymene (>20%).

Regarding the essential oil composition of *T. algeriensis*, it is already known from the literature that it shows really great chemical polymorphism, even in samples collected from the same locality (Hazzit et al., 2009), which seems to be common characteristics for the oils from *Thymus* species (Ozguven and Tansi, 1998; Naghdi et al., 2004), and is most frequently attributed to the origin, environmental conditions and developmental stage and/or the harvesting time (season) of the sourcing plant material (Marković, 2011). Although *T. algeriensis* is one of the rarest *Thymus* species, various authors already testified the occurrence of different oil chemotypes, such as thymol (Hazzit et al., 2009) lilalool (Houmani et al, 2002; Dob et al., 2006), carvacrol, and geranyl acetate (Raal et al., 2004) and terpynyl acetate (Hazzit et al., 2009), the first two being the most common ones. Present study on the chemical profile of *T. algeriensis* oil reveals that it belongs to thymol chemotype, as it is quite common for Maroccan samples of *T. algeriensis* (Benjulali et al., 1987; Houmani et al, 2002).

On the other hand, chemical profile of our *T. vulgaris* essential oil sample is in agreement with several other reports (Hudaib et al., 2002; Ghasemi et al., 2013), also reporting the thymol as a major constituent of this species oil.

3.2. Antimicrobial activity

The results from the antimicrobial activity tested by microdilution method are summarized in Table 2.

The three EOs exhibited a significant antimicrobial activity against all the tested strains. Inhibition values range from MIC 2.5-160 μg/mL and MBC 5-320 μg/mL for bacteria, and

MIC 1-40 μg/mL and MFC 5-80 μg/mL for fungi. *T. serpyllum* EO showed the strongest activity in both cases (MIC 2.5-5 μg/mL, MBC 5-10 μg/mL; MIC 1-2 μg/mL, MFC 2-4 μg/mL), while *T. vulgaris* exhibited the lowest antimicrobial potential (MIC 80-160 μg/mL, MBC 160-320 μg/mL; MIC 20-40 μg/mL, MFC 40-80 μg/mL). *T. algeriensis* inhibited the growth of selected microorganisms in medium range of MIC 20-80 μg/mL, MBC 40-160 μg/mL (for bacteria) and MIC 5-10 μg/mL, MFC 10-20 μg/mL (for fungi). Fungi appear to be more sensitive compared to bacteria, which could be explained by their different cell organization. Comparing the results of essential oils with that of standard drug, hexoral, it was concluded that oils are more potent anti-oral-pathogen activity. Essential oil of *T. serpyllum* expressed higher antibacterial activity than both antibiotics tested. Oil of *T. algeriensis* showed equal antibacterial potential as streptomycin but higher than ampicillin on the following bacteria: *S. sanguis, L. acidophilus, S. pyogenes* and *S. aureus*. *T. vulgaris* oil also exhibited higher activity than ampicillin on *S. pyogenes* and *S. aureus*. All the oils tested expressed much better antifungal potential than chlorhexidine 0.05%, and only 11 isolates of *C. albicans*, among the 55 tested, possessed the same susceptibility on oils and fluconazole. *T. serpyllum* and *T. algeriensis* oils were more active than fluconazole against *C. krusei* and two isolates of *C. glabrata.*

Overall, the essential oils of *T. serpyllum*, *T. algeriensis* and *T. vulgaris* showed significant antibacterial activity, especially against *S. mutans*, a recognized cariogenic species. The oils also efficiently inhibited the growth of *Candida* spp., which is crucial since *C. tropicalis*, *C. krusei* and *C. glabrata* proved to be involved in the disease course and together with *C. albicans* represent more than 80% of human cavity clinical isolates (Akpan and Morgan, 2002). Hence, the present results support traditional use of thyme herb against various infections; therefore, the bioactive properties could be easily attributed to its essential

oil. Furthermore, the obtained results also imply that thyme oils could be also useful against oral pathogen infections.

The correlation between antimicrobial activity of the EOs and their chemical composition suggests that the activity of the oils could be attributed to the presence of the major constituent, thymol, in all the studied EOs. As a sole component, thymol was already presented as a good antimicrobial agent in several studies (Penalver et al., 2005; Sokovic et al., 2008, 2009). On the other hand, although the lowest thymol content among the three thyme EOs, the oil of *T. serpyllum* exhibited the strongest activity, implying that, although the thymol is the major oil constituent, obviously it is not the only one responsible for achieved good antimicrobial activity; the involvement of less abundant constituents should also be considered.

In numerous studies, active natural compounds have been compared with antibacterial compounds currently employed in dentistry, such as chlorhexidine and triclosan, in order to determine their relative effectiveness (Hwang et al., 2004). Taking into account some pre-set criteria from the relevant literature, agents with MIC values of isolated phytochemicals below 20 mg/mL may be considered useful for development products for application against oral infections. Otherwise, plant derivatives with MIC values above 100 mg/mL are unlikely to be useful chemotherapeutic agents, because such high concentrations are almost impossible to achieve *in vivo* and often problems of toxicity occurs (Cecchini et al., 2012).

3.3. Antioxidant activity

Numerous and diverse techniques are available to evaluate the antioxidant properties of compounds or complex mixtures such as essential oils; however, a single procedure cannot identify all possible mechanisms characterizing an antioxidant. Therefore, in the present study, four different assays were conducted in order to evaluate *in vitro* antioxidant properties

of the EOs' samples: scavenging activity on DPPH radicals, reducing power, inhibition of lipid peroxidation in a β-carotene–linoleate system, and TBARS assay.

In the DPPH assay, the radical scavenging capacity of the tested EOs increased in a concentration dependent manner. The values for 50% scavenging activity (EC_{50}) are presented in Table 3. *T. serpyllum* essential oil showed the highest radical scavenging activity (EC_{50}: 0.96 μg/mL), followed by *T. algeriensis* (EC_{50}: 1.64 μg/mL) and *T. vulgaris* (EC_{50}: 4.80 μg/mL) oils. For the measurements of the EOs reductive abilities, the transformation of Fe^{3+} - Fe^{2+} in the presence of oils was investigated. The highest reducing power was detected for *T. serpyllum*, being similar to that of *T. algeriensis* (0.66 μg/mL and 0.68 μg/mL, respectively), both more than twice higher than that of *T. vulgaris* oil (1.54 μg/mL). Table 3 also shows the results of β-carotene bleaching inhibition based on the loss of the yellow color of β-carotene due to its reaction with radicals, which are formed by linoleic acid oxidation in an emulsion. Again, *T. serpyllum* (0.11 μg/mL) essential oil was slightly better than *T. vulgaris* (0.18 μg/mL). *T algeriensis* (1.56 μg/mL) showed the lowest capacity of inhibition in this test. The antioxidant activity of the essential oils was also assessed by TBARS inhibition assay and the results are presented in Table 3. In this assay, the greater effectiveness was shown by *T. serpyllum* (0.004 μg/mL), while slightly lower activity was detected for *T. vulgaris* (0.005 μg/mL), and the lowest for *T. algeriensis* (0.31 μg/mL).

Strong antioxidants profile of thyme oils, especially, *T. serpyllum* is reported by several studies (Kulisic et al., 2005; Stanisavljevic et al., 2011). The difference between *T. algeriensis* and *T. vulgaris* in two different tests could be explained by different mechanisms involved in corresponding assays; therefore, each plant had different compounds with specific capacities to participate in those mechanisms. Antioxidant activity exhibited by the oils tested is an evidence of traditional uses of these plants. Antioxidants are used as food additives to help protect against food deterioration; in 2007, the worldwide market for industrial

antioxidants had a total volume of around 0.88 million tons. This created revenue of approximately 3.7 billion US-dollars (2.4 billion Euros) (Market research). The observed antioxidant potential should be addressed to the phenolic oil constituents (Hazzit et al., 2009) and reported chemoprotective effects against oxidative stress-mediated disorders, mainly due to its free radical scavenging and metal chelating properties.

3.4. Cytotoxic activity for human tumor cell lines and non-tumor liver primary culture

The effects of the oils on the growth of four human tumor cells lines (NCI-H460, MCF7, HCT15, HeLa and HepG2), represented as the concentrations that caused 50% of cell growth inhibition (GI_{50}), are summarized in Table 5.

T. serpyllum was the most potent in all the tested cell lines, presenting GI_{50} values that ranged from 7.02 - 52.69 µg/mL. Less activity was found for *T. algeriensis*, which showed similar activity against all the cell lines in the range of 62.12 - 64.79 µg/mL. The lowest antitumor activity was shown by *T. vulgaris* oil, with GI_{50} values of 76.02 -180.40 µg/mL. The three EOs did not show any effect in the tested concentrations (up to 400 µg/mL) against non-tumor liver primary culture PLP2. The HCT15 cell line was the most susceptible to the oils. Among the tumor cell lines employed, MCF-7 was the most resistant. Ellipticine was used as positive control for antitumor activity evaluation assays, but should not be considered as a standard, and comparison with EOs results should be avoided, because it is an individual compound and not a mixture.

Until now, various authors have reported antitumor activities of essential oils as well as their components. For instance, thyme oil appears to be the most effective against PC3, A549 and MCF-7 cell lines. According to Ait et al. (2010), the thyme oil containing carvacrol as the major oil constituent has an important *in vitro* cytotoxic activity against tumor cells.

Our data demonstrated that thyme essential oils inhibited the viability of several tumor cell lines in a concentration-dependent manner. In some cases, this activity was attributed to specific components of the oil. There is evidence that thymol, a constituent of the essential oil, could be involved in the stimulation of active proliferation of pulp fibroblasts (Tsukamoto et al., 1989). Whether thymol, alone or in combination with other components of the oil, is responsible for the observed cytotoxicity against tumor cells still remains to be revealed, being an important limitation of the present study.

At non-toxic concentrations, thyme extract was also identified as a natural antimutagen with the possibility of enhancement of error-free DNA repair (Vukovic et al., 1993). GI_{50} values below 100 mg/mL for mixtures are considered as relevant cut off points for activity. On this basis, and according to published guidelines, we can conclude that all the essential oils are promising in developing novel cytotoxic agents.

4. Conclusion

Thymol is identified as the main oil component in the three *Thymus* essential oils.. The results of antimicrobial activity of the essential oils supported the use of the tested plant species in the treatment of minor wounds and disorders of the oral cavity, and as an antibacterial agent in oral hygiene. Furthermore, strong antioxidant and antitumor activity supports the traditional use for the treatment of dyspepsia and other gastrointestinal disturbances bronchitis and pertussis; and laryngitis and tonsillitis. In all the assays, *T. serpyllum* oil showed the strongest biological activity. In addition to *Thymus* oils use in food and cosmetics, they have a great potential for applications in anti-cancer treatments and deserves further exploration.

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