Bulgarian Journal of Agricultural Science, 26 (No 6) 2020, 1183–1197

Origanum vulgare L. – a review on genetic diversity, cultivation, biological activities and perspectives for molecular breeding

Marina Alekseeva, Tzvetelina Zagorcheva, Ivan Atanassov and Krasimir Rusanov*

Agricultural Academy, Department of Agrobiotechnology, Molecular Genetics Group, AgroBioInstitute, 1164 Sofia, Bulgaria
**Corresponding author:* krusanov@abv.bg

Abstract

Alekseeva, M., Zagorcheva, Tz., Atanassov, I. & Rusanov, K. (2020). *Origanum vulgare* L. –a review on genetic diversity, cultivation, biological activities and perspectives for molecular breeding. *Bulg. J. Agric. Sci., 26 (6)*, 1183–1197

Origanum vulgare L. or simply called oregano is a flowering plant of the Lamiaceae family, which has been known since ancient times due to its diverse properties and applications as a culinary spice but also as a herb with medicinal properties. It is widespread throughout the Mediterranean, Western and Southwestern Eurasia and in the Irano-Turanian regions. Being a popular medicinal plant with well-known application in traditional medicine due to its antibacterial, antifungal and anti-inflammatory properties among others, *O. vulgare* has been a subject of extensive research related to analysis of genetic diversity, possibility for cultivation as well as analysis of the composition and biological activities of its essential oil and plant extracts. Here we present a short review on this species with an accent on the recent progress in genetic diversity studies, applying modern DNA marker systems, possibilities for cultivation, composition and evaluation of the biological activities of the obtained essential oil and different plant extracts as well as the perspectives for molecular breeding of improved cultivars with desired agronomic traits, based on the current and emerging knowledge.

Keywords: Origanum vulgare L.; taxonomy; genetic diversity; cultivation; essential oil; terpene synthase genes; molecular breeding

Introduction

The genus *Origanum* belongs to the Lamiaceae family of flowering plants and consists of more than 50 species including medicinal, fragrant, culinary and ornamental plants. Plants from this genus have been used in traditional medicine and as food additives since ancient times. The primary and secondary metabolites that this plants produce are central for their various beneficial properties and biological activities – antibacterial, antifungal, antiviral, insecticidal, antioxidant, anti-inflammatory, antitumor and more (Garcia-Beltran & Esteban, 2016).

Origanum vulgare L. is probably the most well-known species from the genus *Origanum*, which is applied in traditional medicine and as a culinary spice. It has two common names – oregano and wild marjoram. Sometimes called the "prince of herbs", the name *Origanum* was first used by the ancient Greek physician Hippocrates (460 – 370 B.C.). The name originates from the Greek words for mountain (*oros)* and joy *(ganos),* meaning "joy of the mountains". Oregano has been used since antique in folk medicine and for food flavouring and preservation. Fresh oregano has a spicy aroma reminiscent of clove and balsam, and the dried herb has a pungent flavour with notes of peppermint, pine and clove. Oregano is traditionally used in Italian, Greek and Mexican dishes. Flowering tops are used in beers and ales, and fresh and dried leaves can be added to soups, casseroles, sauces, stew, eggs, olives, teas, tomato-based dishes and strong-flavoured foods like chili and pizza (Meyers, 2005). In folk medicine *O. vulgare* is used for respiratory disorders (coughs, inflammation of the bronchial mucous membranes and as an expectorant), upset stomach, painful menstruation/as an emmenagogue, rheumatoid arthritis, to induce sweating, for urinary problems, muscle aches and as a diuretic. In Chinese medicine oregano is a remedy for colds, vomiting, fever, dysentery, and jaundice and childhood malnutrition. The essential oil is used commercially to scent soaps, lotions and perfumes (Meyers, 2005). Oregano essential oil is also used to kill lice and in homeopathy oregano is considered an aphrodisiac. Preparations include infusions, tea powders, gargles and baths (Fleming, 2000).

Distribution, Taxonomy and Botanical Description

O. vulgare is a perennial herb, which is widespread throughout the Mediterranean region, Western and Southwestern Eurasia and in the Irano-Turanian region (Lukas et al., 2015). According to the most widely accepted taxonomic reference for the genus, *Origanum vulgare* L. comprises six subspecies (Ietswaart, 1980), which are distinguished on the basis of differences in the indumentum, the size or colour of bracts and flowers and in the number of oil glands on the leaves (Lukas et al., 2015). Table 1 shows the six *O. vulgare* L. identified subspecies as well as their regions of distribution.

O. vulgare develops creeping roots, branched woody stems and opposite, petiolate and hairy leaves (Grieve, 1994). The plants from the genus *Origanum* have bracts or non-typical leaves, which surround the calyx and corolla. The leaves are spade-shaped, olive-green and are covered with fine hairs called trichomes. The leaves and flowering parts of the plant contain essential oil glands that produce volatile oils, which give the plant its fragrance (Meyers, 2005). *O. vulgare* has flowers that occur in spikes forming a panicle with multiple branched stems growing from a central stalk. Corollas are purple, pink or white, depending on the subspecies.

Genetic Diversity Based on DNA Markers

Usually, the identification of medicinal plants is based on the evaluation of phenotypic characteristics such as morphology, smell, taste, colour, texture and size. These characteristics, however, have certain limitations like insufficient variation among the samples, subjectivity of the analysis, plasticity of the character due to the influence of the environment and/or management practice as well as ability to be scored only at certain stages of the plant development. Recently the traditional methods of plant identification have been complemented by methods of phytochemistry and molecular biology. Molecular markers, based on DNA sequence variation, have become important for the identification and authentication of medicinal plants and for the estimation of genetic diversity (Nybom & Weising, 2007).

Studies on systematic, genetic diversity and identification of *Origanum* species have been mostly focused on the morphological characteristics and the composition of the essential oil. However, in the last decades, molecular markers such as AFLP, ISSR, SAMPL, SSR, RAPD and CAPS have been applied to detect DNA polymorphism between/within populations and to identify phylogenetic relationships (Jedrzejczyk, 2018).

Novak et al. (2008) developed the first simple sequence repeat (SSR) markers for the genus *Origanum.* Novak et al. (2008) elaborated 13 SSR markers from expressed sequence tags (ESTs) of essential oil glands of *O. vulgare.* The developed markers were also able to cross-amplify PCR products from *O. majorana*. The authors analyzed a total of 20 *O. vulgare* and 19 *O. majorana* plants. The number of alleles for a marker ranged between 2 and 4. The observed and expected heterozygosities ranged from 0.00 to 0.60, and from 0.14 to 0.67, respectively (Novak et al., 2008). In a later study ten microsatellite markers from the ones developed by Novak et al. were also used to investigate the genetic effects of recent colonization of *O. vulgare* in restored semi-natural grassland

Table 1. Subspecies of *O. vulgare* L. and regions of distribution

Subspecies	Region
O. v. ssp. *glandulosum* (Desfontaines) Ietsw.	Tunisia, Algeria
O. v. ssp. *gracile* (K.Koch) Ietsw.	From East Turkey to Afghanistan and South Siberia
O. v. ssp. *hirtum* (Link) Ietsw.	Balkan, Turkey
O. v. ssp. *virens* (Hoffmanns. & Link) Ietsw.	Azores, Madeira, Balearic Islands, Portugal, Spain, Morocco
O. v. ssp. *viridulum* (Martrin-Donos) Nyman	Widespread from Corsica to Nepal
O. v. ssp. *vulgare*	From Britain and Scandinavia to Taiwan; naturalized in North America and Venezuela (Skoula & Harborne, 2002)

patches (Helsen et al., 2013). The genetic diversity and differentiation of 14 recent populations were compared with that of 14 old, putative source populations. The study demonstrated that spontaneous plant colonization after habitat restoration can lead to new and viable populations in a relatively short time, overcoming pronounced founder effects, when several source populations are nearby. The rapid buildup of genetic diversity in restored populations, combined with low among population differentiation, could contribute positively to the overall viability of the *O. vulgare* metapopulation and also to mitigate the consequences of the genetic drift observed in the original source populations (Helsen et al., 2013).

Azizi et al. (2009) carried out a study using amplified fragment length polymorphism (AFLP) and selectively amplified microsatellite polymorphic loci (SAMPL) markers to investigate the genetic diversity and subspecies differentiation among 42 *O. vulgare* accessions – 39 from the Gatersleben Genebank (Germany) and 3 cultivated types. The results of the analysis confirmed the morphological classification of the subspecies. Both marker systems were suitable for taxonomic investigation and identification of subspecies of oregano, but SAMPL markers were slightly more efficient in differentiating accessions and subspecies than AFLPs (Azizi et al., 2009). Azizi et al. also examined 42 *O. vulgare* accessions (39 from the Gatersleben Genebank (Germany) and 3 cultivated types) to detect molecular, quantitative, morphological and chemotype polymorphisms and to discover possible correlations between them (Azizi et al., 2012). Again, AFLP and SAMPL markers were used for genotyping. A relatively high correlation between chemotypic patterns and genetic markers was identified, while a lower correlation was found between the morphological and genetic matrices. Cluster analysis, population inference and principal component analysis revealed a broad genetic and chemical variation among the accessions (Azizi et al., 2012).

Van Looy et al. (2009) investigated the within-population genetic variability and among-population genetic differentiation of 21 populations of *O. vulgare* along the river Meuse in Belgium, using dominant AFLP markers, in order to assess the effects of flood events on the genetic structure of riparian populations (Van Looy et al., 2009). The average observed within-population genetic diversity was high and suggested that river regulation and associated fragmentation of the populations had not strongly affected genetic diversity. The genetic differentiation between populations was high and could be explained by founder effects, rather than by genetic drift in isolated populations (Van Looy et al., 2009).

The germplasm variability of Greek oregano (*O. vulgare* ssp. *hirtum*) was investigated using random amplification of polymorphic DNA (RAPD) markers (Katsiotis et al., 2009). Analysis of molecular variance revealed that genetic variability was distributed mainly within populations. Genetic distance between populations from different geographical locations was significant, supporting noteworthy genetic differentiation among *O. vulgare* ssp. *hirtum* populations (Katsiotis et al., 2009).

Amar & Wahab studied the genetic and chemical variability among four *Origanum* species including *O. vulgare, O. vulgare* ssp. *hirtum, O. syriacum var. syriacum* and *O. majorana,* grown in Egypt, using inter simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP) markers (Amar & Wahab, 2013). The results confirmed that *O. vulgare* and *O. vulgare* ssp. *hirtum* were highly correlated to each other. The SRAP marker system seemed to be more effective than ISSR for studies on intraspecific diversity and relationships among *Origanum* germplasm. SRAP had more sensitive, distinctive nature and higher discrimination capacity and could simultaneously detect several polymorphic markers per reaction (Amar & Wahab, 2013).

Ince et al. (2014) developed SSR and cleaved amplified polymorphic sequences (CAPS)-SSR markers from ESTs and mRNA sequences from *Origanum, Stenogyne, Thymus* and *Salvia* species. Microsatellite and CAPS-microsatellite markers were utilized to test whether they were useful in species identification and phylogenetic studies in 65 individual samples representing 8 *Origanum* species. The results indicated that they were very effective for species identification and to clarify taxonomic uncertainties within the genus and could be used to provide anchoring points for the integration of genetic maps among species (Ince et al., 2014).

ISSR markers were recently used in a study on genetic diversity between *Origanum* species (Jedrzejczyk, 2018). The species and subspecies included *O. heracleoticum* (from USA, this is the old name of *O. vulgare* ssp. *hirtum), O. majorana, O. syriacum, O. vulgare* ssp. *gracile, O. vulgare* ssp. *hirtum, O. vulgare* ssp. *vulgare.* The results of ISSR-PCR revealed polymorphism between species and subspecies of the genus *Origanum*, which ranged from 80 to 100%. The identification of all tested accessions was possible based on the banding profiles. Certain ISSR primers could be used to distinguish the *O. vulgare* subspecies. Specific bands enabled distinction between accessions of the same species but of different origin. The results suggested that ISSR primers can be successfully used for identification and estimation of genetic variability within *Oregano* genus (Jedrzejczyk, 2018).

Cultivation

Wild oregano is herbaceous perennial, native to the Mediterranean region, particularly in high locations. In these areas oregano is harvested mainly from wild populations at the flowering stage, once or twice a year. The interest towards cultivation of oreganoin Greece and Bulgaria is focused on the high essential oil subspecies of *O. vulgare* ssp. *hirtum* (syn. *O. heracleoticum*) also known as Greek oregano. Oregano harvests can be two or three annually. During the winter period the aerial parts are destroyed, but the roots maintain their vitality for the revegetation in spring. Oregano is tolerant to cold and dryness and grows in medium soils and in areas with high elevation and cool summer (Makri, 2002). The optimal climatic conditions for *O. vulgare* are temperatures 5-28°C with an annual precipitation of 0.4-2.7 mm and soil pH 4.5-8.7 (Marzi, 1997).

The demand and consumption of medicinal plants is increasing worldwide (Hoareau & DaSilva, 1999). The cultivation of herbs is a good alternative not only to avoid overexploitation of wild populations, but also because it gives the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants. Cultivation of herbs also creates opportunities for development of agriculture in mountainous regions where growing traditional crops is not a feasible option. Therefore, efforts have been made in domestication and cultivation of oregano, which can be grown as an annual plant in cold climates where it will not overwinter well. When grown as a perennial, roots should be divided every 3 years for best growth and flavour (Kintzios, 2012). Ploughing of the soil and fertilization with ammonium phosphate during November-December is sufficient for oregano cultivation. The necessary pest control is a simple weeding out (manually or by using herbicides) at least 4 times a year. The average yield is 2.5-3.5 t/ha and the essential oil yield ranges from <0.5% to > 2 % of dry weight depending on the subspecies (Bernath, 1997; Kintzios, 2012).

Oregano has a spreading root system and is usually propagated by seeds or cuttings. Direct sowing is difficult because oregano belongs to the species with the smallest fruits (approximately 60 µg per seed) (Thanos et al., 1995). The key to improving the direct sowing technique is selecting higher seed weight since seed quality; germinability and vigor depend on it. If seeds are used, they should be sown in a seed box in spring and planted outside when seedlings are 7.5 cm tall (Makri, 2002). Cuttings (transplants) of new shoots (about 30 cm long) are removed in late spring and placed in sand. The leaves should be firm enough to prevent wilting. Cuttings with well-developed roots are planted in the ground about 30 cm apart or in pots outside. Several studies have reported the establishment of tissue cultures and the regeneration of plantlets from oregano plants (Svoboda et al., 1995; Matsubara et al., 1996; Baricevic, 1997; Alves-Pereira & Fernandes-Ferreira, 1998; Fortunato et al., 2006). These studies enable the use of biotechnological methods for enhancing the production of planting material for propagation and breeding activities.

Gathering of leaves and stem tips should start when plants are at flowering stage, beginning 10 cm from the ground. In dry climates the best harvest time to collect the highest content of essential oil is when approximately 50% of plants in the field have started flowering. Harvesting usually finishes at full bloom. Plant material should be dried in the shade to avoid direct sunlight thus preserving the green colour and aroma (Makri, 2002; Kintzios, 2012).

Essential Oil Composition

Many of the *O. vulgare* biological activities as well as its characteristic aroma are due to the essential oil, synthesized in the trichomes, which are present in its areal parts. The highest level of essential oil content is observed during the flowering stage (Baranauskiene et al., 2013).Compared to leaves, the inflorescences showed to have 5-10 time more essential oil content measured as mg/g dry matter in *O. vulgare* ssp. *vulgare* (D'Antuono et al., 2000).

A number of factors have been known to affect the essential oil content including geographical location, developmental phase, solar radiation, harvesting time and growth conditions (Tibaldi et al., 2011; Baranauskiene et al., 2013; Morshedloo et al., 2017). In general, the subspecies from the southernmost distribution area (*O. vulgare* ssp. *glandulosum*, *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *gracile*) are rich in volatile compounds, whereas the subspecies from Central and Northern Europe (*O. vulgare* ssp. *virens*, *O. vulgare* ssp. *vulgare* and *O. vulgare* ssp. *viride*) have poor essential oil content (Lukas et al., 2015). The essential oil yield ranges from poor < 0.5% (*O. vulgare* ssp. *vulgare*, *O. vulgare* ssp. *gracile II*, *O. vulgare* ssp. *virens*, *O. vulgare* ssp. *viride II*) to intermediate 0.5 – 2.0% (*O. vulgare* ssp. *gracile I*, *O. vulgare* ssp. *viride I*) and high > 2% (*O. vulgare* ssp. *hirtum*, *O. vulgare* ssp. *glandulosum*) (Kintzios, 2012). Subspecies with higher essential oil content (2% or more) usually produce large amounts of monoterpenoid phenols resulting from the 'cymyl' pathway – carvacrol and/or thymol and chemically related compounds like γ-terpinen and *p*-cymene. The phenolic monoterpenes give the familiar oregano flavour and aroma. The subspecies with poor essential oil content usually produce low amount of 'cymyl' compounds and higher quantity of acyclic compounds (linalool, linalyl acetate, β-ocimene, myrcene) and/or bicyclic 'sabinyl'-compounds (sabinene, cis-/trans-sabinene hydrate and their acetates) and/or bornane type compounds (camphor, borneol, bornyl acetate). Also they often contain high amounts of sesquiterpenes (β-caryophyllene, germacrene D, bicyclogermacrene, α- and γ-muurolene, β-caryophyllene oxide) (Alves-Pereira & Fernandes-Ferreira, 1998; Lukas et al., 2015)

The high essential oil yield species *O. vulgare* ssp. *hirtum*, also known as the Greek oregano, produces less essential oil during the cool and wet vegetative period, more during the warm and dry flowering period and after flowering the leaves get older and drier and the essential oil content decreases (Skoula & Harborne, 2002). This subspecies includes two main chemotypes – thymol and carvacrol. There are also intermediate chemotypes, containing both thymol and carvacrol, and types with a high content of the two biosynthetic precursors – γ-terpinen and *p*-cymene (D'Antuono et al., 2000). The amounts of the two main constituents carvacrol and thymol show large variations in *O. vulgare* ssp. *hirtum* essential oil. For example, Kokkini et al. (2004) analyzed samples from six different locations in Greece and found that carvacrol amounts ranged from 1.7 to 69.6% and thymol ranged from 0.2 to 42.8%. The same was observed also for the other main constituent p-cymene, which varied 17.3 – 51.3%.

Biological Activity of *O. vulgare* Essential Oil and Plant Extracts

Antimicrobial activity

The demand for natural and safe food products is increasing worldwide. The possibility of using essential oils as antipathogenic agents is very important due to several reasons: the increase of antibiotic resistant strains, the rise in the number of people with lower immunity, the increased incidences of drug resistant biofilm associated infections, the demand for natural and clean-label food products (Leyva-Lopez et al., 2017).

Many scientists consider the use of oregano essential oil and different plant extracts as anti-pathogenic agents in the food industry. The antibacterial activity of essential oil and plant extracts from *O. vulgare* against common human pathogens and food spoilage bacteria have been tested in many researches. Some of the studies are focused on individual use of oregano essential oil (Govaris et al., 2011; Marques et al., 2015; Shange et al., 2019), while others are based on combination of essential oils from *O. vulgare* and other plant species, e.g. *Rosmarinus officinalis* (de Sousa et al., 2013; Barbosa et al., 2016; Diniz-Silva et al., 2019).Infusions of both essential oils and aqueous or ethanolic plant extracts can be used in antimicrobial formulations as they have activity against strains of Gram positive and negative bacteria. Oregano essential oil and extracts display activity against *Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus aureus, Enteroccocus faecalis, Bacillus cereus, Listeria monocytogenes, Salmonella enterica* and more (Licina et al., 2013; Teixeira et al., 2013; Mazzarrino et al., 2015; Sarikurkcu et al., 2015; Khan et al., 2018).

Different conditions such as low pH, temperature or oxygen levels enhance the antibacterial action of the essential oils (Garcia-Beltran & Esteban, 2016). As the pH decreases, the ability of the essential oils to dissolve in the bacteria's cell or its membrane increases due to the hydrophobic character of the oils (Burt, 2004). The diffusion rate through the bacterial cell membranes also depends on the extraction and purification method of the essential oils. Denaturalization, breakdown, volatilization and loss of functional properties of the active compounds should be avoided (Raybaudi-Massilia et al., 2009).The essential oil from *O. vulgare* has antimicrobial activity due to its high content of monoterpenoid phenolic compounds, especially thymol and carvacrol. The main compounds of the oregano essential oil, carvacrol and thymol, show a great variety of antibacterial mechanisms, responsible for the disruption of the cellular membrane such as inhibition of ATPase activity, release of intracellular ATP and other constituents resulting in cell death (Lambert et al., 2001; Burt, 2004; Raybaudi-Massilia et al., 2009). The monoterpenoid phenolic compounds from the essential oil can enter into the phospholipid bilayer and align between the fatty acid chains or they can bind to the proteins of the membrane. This results in changes of the membrane permeability and destabilization of the bacterial membrane (Lambert et al., 2001; Giannenas et al., 2018). The antibacterial activity of *O. vulgare* essential oil was also studied in different meat (Marques et al., 2015; Pesavento et al., 2015; Shange et al., 2019) and fish products (Cardoso et al., 2017). Some authors obtained good results from the use of combination of oregano essential oil and chitosan in the packaging of meat in modified atmosphere (Petrou et al., 2012; Paparella et al., 2016). The use of oregano essential oil in food packaging was shown to be safe – there was a lack of toxicity during a 90-day study on rats with doses 330-fold higher than those expected to be in contact with consumers in the worst scenario of exposure (Llana-Ruiz-Cabello et al., 2017).

Oregano essential oil is also used individually or in combination with other essential oils as antimicrobial agent in different types of cheeses (Govaris et al., 2011; de Souza et al., 2016; Gurdian et al., 2017; Bedoya-Serna et al., 2018; Diniz-Silva et al., 2019). The right concentration of essential oil should be evaluated carefully because it showed inhibitory effects on the growth of starter lactic acid culture (de

Souza et al., 2016). Also, there is a possibility of modifications of the organoleptic properties of the food. The use of edible coatings and films, which contain the essential oil, seems like a great solution to this problem (Cardoso et al., 2017; Gurdian et al., 2017).

O. vulgare essential oil and plant extracts have been used as antimicrobial agents in post-harvest fruit and vegetables – leafy vegetables (Barbosa et al., 2016), grapes (dos Santos et al., 2012; de Sousa et al., 2013), pomegranate (Thomidis & Filotheou, 2016) and apple (Rojas-Grau et al., 2007). In some studies the essential oil was in edible coating or film (Rojas-Grau et al., 2007; Du et al., 2009; dos Santos et al., 2012). Another great opportunity is the use of essential oil as preservatives in fruit juices (Raybaudi-Massilia et al., 2009; Dutra et al., 2019).

Oregano essential oil has also been shown to possess antifungal activity against yeasts from the genus *Candida,* which are the most common cause for fungal infections worldwide (Khosravi et al., 2011; Brondani et al., 2018).

The essential oil from oregano as well as different plant extracts have also been tested for potential antiviral activities. The essential oil showed inhibitory effect on the replication of the yellow fever virus *in vitro* (Meneses et al., 2009) and inactivated murine norovirus (Gilling et al., 2014). The essential oil also showed slight reduction on the infectivity of hepatitis A virus, while thymol alone showed no effect on the same virus but was effective against norovirus surrogates and murine norovirus (Sanchez & Aznar, 2015).Zhang et al. tested the antiviral effect of phenolic compounds from *Origanum vulgare* ethanolic extracts against respiratory syncytial virus, Coxsackie virus B3 and herpes simplex virus type 1, but most of them didn’t show inhibitory activities (Zhang et al., 2014).

Antioxidant activity

Oxidative stress due to the generation of free radicals and reactive oxygen species (ROS) causes damage to the cellular macromolecules. Oxidative damage has been related to various health problems such as ageing, arteriosclerosis, cancer, Alzheimer’s disease, Parkinson’s disease, diabetes and asthma (Wiseman & Halliwell, 1996; Aruoma, 1998; Nunomura et al., 2001). In living organisms cellular balance of free radicals is maintained by different antioxidant mechanisms (Nimse & Pal, 2015).

Phenolic compounds are a well-known class of secondary metabolites in plants, which possess strong antioxidant properties (Foti, 2007). The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity and/or singlet-oxygen-quenching capacity. They also play an important role in stabilizing lipid peroxidation and in the inhibition of enzyme oxidation (Shan et al., 2005).

Twelve from 21 phenolic compounds from oregano (e.g. rosmarinic acid, 2,5-dihydroxybenzoic acid) exhibited significant antioxidant activity comparable to that of ascorbic acid (Zhang et al., 2014). Both ethanolic and aqueous oregano extracts exhibited good antioxidant and free radical scavenging properties *in vitro* and strongly inhibited DNA damage (Majid et al., 2012), while methanolic extract showed antioxidant activity before and after gastrointestinal digestion *in vitro* and *in vivo* (Gayoso et al., 2018). Ding et al. (2010) isolated rosmarinic acid methyl ester from *O. vulgare* and demonstrated its antioxidant and depigmentation activity (Ding et al., 2010).

Some compounds from essential oils also possess significant antioxidant effects (Raut & Karuppayil, 2014).The monoterpenoid phenolic compounds from the essential oil not only act as preservatives but also can enhance some properties of the food - the antioxidant activity and stability of the food colour. Various studies suggest the use of oregano essential oil as natural antioxidant agent for the food industry (Sarikurkcu et al., 2015) with examples including cooked sausages (Fernandes et al., 2018), olive oil (Asensio et al., 2012), fish (Garcia-Beltran et al., 2018), cheese (Asensio et al., 2015) among others. Paraskevakis et al. (2015) tested the effect of oregano essential oil compounds on the quality and total antioxidant capacity of milk following dietary supplementation of dried *O. vulgare* ssp. *hirtum* in goats (Paraskevakis, 2015). The dietary intake of dried oregano plants positively affected some enzymatic and non-enzymatic antioxidant defenses of blood and milk and thus contributed to enhanced antioxidant capacity of the milk.

Cytotoxic and anti-proliferative activities

Compounds from oregano essential oil can be used as chemopreventive agents because they exert anti-proliferative and cytotoxic effects. The anti-proliferative properties have been demonstrated in several cancer cell models. The essential oil from oregano prevented the proliferation of human stomach cancer cell lines (AGS) – it altered the colony forming characteristics and the migration ability of cancer cells. The essential oil suppressed the transcription of the genes involved in fatty acid and cholesterol biosynthesis pathway and induced mitochondrial mediated apoptosis, resulting in the inhibition of cancer cell growth (Balusamy et al., 2018). Supercritical oregano extracts caused a reduction in cell viability in a dose-dependent manner in activated human THP-1 cells (Ocana-Fuentes et al., 2010). Acetone extract from *O. vulgare* exhibited dose-dependent cytotoxic effect on adenocarcinoma cervical cancer (HeLa) cell line

(Berrington & Lall, 2012). Oregano also showed anti-proliferative activity on hepatic cancer (HepG2) cell line (Marrelli et al., 2015) and on human colon adenocarcinoma (HT-29) (Begnini et al., 2014). After three months of a low uptake dose of oregano essential oil it has been established that it possesses anticancer activity in F1 DBA C57 Black hybrid mice – the engraftment of Lewis carcinoma decreased by 1.8 times, its size decreased by 1.5 times and the development of tumor was significantly suppressed (Misharina et al., 2013). Sankar et al. (2013) created silver nanoparticles, using the aqueous extract of *O. vulgare,* which showed dose dependent response against human lung cancer A549 cell line (Sankar et al., 2013).

Anti-inflammatory activity

Inflammation is a normal response of the body to tissue damage, infections and chemical or physical agents. If inflammation is not controlled, the pro-inflammatory mediators are overproduced, which can induce pathologic processes related to diseases – arthritis, atherosclerosis, cancer and others. There are studies, which confirm the ability of oregano essential oil to exert anti-inflammatory activity (Leyva-Lopez et al., 2017). Supercritical oregano extracts decreased the synthesis of pro-inflammatory cytokines and the production of anti-inflammatory cytokine (Ocana-Fuentes et al., 2010). Oregano essential oil also induced anti-proliferative effects and significantly inhibited several inflammatory and tissue remodeling biomarkers in a human skin disease cell model. It modulated global gene expression and altered signaling pathways, which are critical in inflammation, tissue-remodeling and cancer (Han & Parker, 2017).

Antiparasitic activity

Oregano essential oil and herbal extracts have been shown to exhibit different antiparasitic activities against parasites from the genus *Eimeria,* which cause the disease coccidiosis in animals like cattle and poultry. Oregano herbal extract had favorable effect on both the resistance and the production in rabbits (Nosal et al., 2014), while the essential oil exhibited anticoccidial effect in broiler chickens (Giannenas et al., 2003; Skoufos et al., 2011). Oregano essential oil also showed antimalarial activity (Hussain et al., 2011) and was effective against *Trypanosoma cruzi* (Santoro et al., 2007).Other studies evaluated the inhibition of human intestinal parasites *in vitro* by the essential oil and carvacrol (Machado et al., 2010; Gaur et al., 2018).

Other medical applications

Diabetes mellitus is characterized by chronic hyperglycemia with disturbance of glucose metabolism resulting from the defect in insulin secretion and/or insulin action. α-amylase and α-glucosidase are key enzymes in hydrolysis of starch and oligosaccharides. The inhibition of these enzymes is important for the management of the blood glucose level, but the inhibitors, which are currently in use, have adverse side effects. Sarikurkcu et al. (2015) tested the anti-diabetic activity of the essential oils from two *O. vulgare* subspecies (ssp. *vulgare* and ssp. *hirtum*) by analyzing the α-glucosidase and α-amylase inhibitory activity (Sarikurkcu et al., 2015). The essential oil from both *O. vulgare* subspecies exhibited similar inhibitory activity against α-amylase (0.13-1.14 mmol ACEs/g), however the essential oil from *O. vulgare* ssp. *vulgare* showed significantly higher inhibitory activity against α-glucosidase (6.04 mmol ACEs/g) compared to the essential oil from *O. vulgare* ssp. *hirtum* (0.88 mmol ACEs/g). In a study with C57BL/6 mice the methanolic extract from oregano protected them from development of diabetes (Vujicic et al., 2015). In streptozotocin diabetic rats the aqueous oregano extract exhibited anti-hyperglycemic activity without changing the insulin secretion (Lemhadri et al., 2004).

The oregano essential oil and some compounds like rosmarinic acid methyl ester and origanoside (novel phenolic glucoside isolated from *O. vulgare)* also showed anti-melanogenic activity against tyrosinase and other proteins and transcription factors that participate in the biosynthesis of melanin (Ding et al., 2010; Liang et al., 2010; Sarikurkcu et al., 2015). The essential oil from *O. vulgare* ssp. *hirtum* was more potent tyrosinase inhibitor (45.6 mg KAEs/g) than that from *O. vulgare* ssp. *vulgare* (8.3 mg KAEs/g) (Sarikurkcu et al., 2015). Therefore, oregano can be useful for dermatological disorders linked to excessive amounts of melanin.

Oregano as a natural pesticide

Excessive use of synthetic pesticides has negative effects on both human health and the environment. In recent years more and more efforts have been focused on the discovery of natural and eco-friendly products, including essential oils with herbicidal, insecticidal and fungicidal activity, to be used as substitutes of the synthetic pesticides (Mfarrej & Rara, 2019).

De Mastro et al. (2006) showed that chopped leaves and stems of oregano were able to reduce the number of weeds in pots and in the field (De Mastro et al., 2006). *In vitro* bioassay confirmed strong phytotoxic effect of oregano essential oil on *Arabidopsis thaliana* rosettes. The leaves were highly chlorotic and the plant growth was reduced (Araniti et al., 2018). Oregano was also tested for weed control in tomato and cotton crops, but the essential oil severely affected both crops and weed species (Argyropoulos et al., 2008). The essential oil of oregano had a significant larvicidal effect on mosquitos (Govindarajan et al., 2016) and diamondback moth(Nasr et al., 2017). The oil and its terpenoids also showed activity against the red flour beetle (*Tribolium castaneum*) (Kim et al., 2010; Licciardello et al., 2013). Licciardello et al. (2013) proposed the incorporation of essential oil in food packaging because of its repellent effect.

Genes Associated with the Synthesis of Mono- and Sesquiterpenes

Over sixty mono- and sesquiterpenes have been reported from the complex and highly variable essential oil from *O. vulgare* plants (Crocoll et al., 2010). The predominant compounds are the phenolic monoterpenoids thymol and carvacrol and their precursors γ-terpinene and *p*-cymene, the acyclic monoterpenoids lynalyl acetate and β-myrcene, and the sesquiterpenoids (E)-β-caryophyllene, β-bisabolene, germacrene-D, α-humulene, γ-muurolene and γ-cadinene (Morshedloo et al., 2017).

Studies on other Lamiaceae species such as mint (*Mentha* sp.) and sweet basil *(Ocimum basilicum)* have provided insights into essential oil biosynthesis. The essential oil is produced in glandular trichomes situated on the aerial parts of the plants. The glandular trichomes consist of a cluster of secretory cells covered by a subcuticular storage cavity, where the essential oil is accumulated (Turner et al., 1999; Crocoll, 2011). In the secretory cells terpenes are formed from the universal five-carbon precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), which are both synthesized by the methylerythritol pathway in the plastids and the mevalonate pathway in the cytosol (Sallaud et al., 2009). DMAPP and IPP are fused by prenyltransferases to form geranyl diphosphate (GPP, C_{10}), the usual precursor of the monoterpenes, and DMAPP and two units of IPP are fused to form farnesyl diphosphate (FPP, C_{15}), the precursor of most sesquiterpenes. Next, the linear carbon skeletons of GPP and FPP are converted to the basic terpene skeletons by terpene synthases, a class of enzymes responsible for the huge structural diversity of mono- and sesquiterpenes since these enzymes often form multiple products (Tholl, 2006; Crocoll, 2011). Several studies have shown that the structural diversity of plant mono- and sesquiterpene synthases originate from diverse reaction mechanisms (Degenhardt et al., 2009; Vattekkatte et al., 2018). The initial terpene synthase products are often further oxidized and/or conjugated by an important class of enzymes – cytochrome P450s monooxygenases (P450s). These enzymes further add to the complexity of the structural diversity of all mono- and sesquiterpenes (Jan et al., 2018).

While studying the mechanisms of terpene synthesis in *O. vulgare,* Crocoll et al. (2010) isolated a total of 6 active terpene synthases from *O. vulgare.*Three of them (*OvTPS1, OvTPS2 and OvTPS7*) were involved in the synthesis of monoterpenes including sabinene, γ-terpinene and*trans*-β-ocimene and three (*OvTPS3, OvTPS4 and OvTPS6*) in the synthesis of sesquiterpenes including (-)-germacrene D, bycyclogermacrene and E-β-caryophyllene (Crocoll et al., 2010). The isolated terpene synthases produced most of the terpene constituents of the *O. vulgare* essential oil. The enzyme responsible for γ-terpinene formation, *OvTPS2*, produced between 25.2 – 48.4% of the total terpene content of this species *in vitro* and therefore was suggested that it has a major role in the terpene synthesis *in vivo.* One of the compounds which was not directly formed by the characterized terpene synthases was thymol. It was predicted that γ-terpinene is most likely converted to thymol by the action of one or more cytochrome P450 oxidases via *p*-cymene. Crocoll et al. (2010) also suggested that the isolated terpene synthase genes from *O. vulgare* played a major role in controlling terpene composition in this species since the transcript levels of individual genes correlated closely with the amounts of the enzyme products found in the essential oil. The close correlation of γ-terpinene synthase expression and terpene composition indicated that transcript regulation of terpene synthase genes is the most important regulatory mechanism controlling terpene composition in *O. vulgare* (Crocoll et al., 2010). Further, Crocoll also demonstrated the involvement of cytochrome P450 monooxygenases in the conversion of γ-terpinene to thymol and carvacrol and isolated 11 cytochrome P450 gene sequences from oregano, thyme and marjoram that were assigned to 5 gene names, *CYP71D178* through *CYP71D182* (Crocoll, 2011). The transcript levels of most of these genes showed good correlation with the occurrence of thymol and carvacrol. Heterologous expression of 2 of them in yeast resulted in active proteins catalyzing the formation of *p*-cymene, thymol and carvacrol from γ-terpinene. The properties and sequence motifs of these P450s were similar to those of well-characterized monoterpene hydroxylases isolated from mint (Crocoll, 2011).

Recently, Jan et al. (2018) studied the tissue-specific expression patterns of 14 terpene synthase genes *(OvTPS)* and 5 cytochrome P450 monooxygenase *(CYP)* genes in 2 oregano species – *O. vulgare* and *O. majorana* (Jan et al., 2018). The maximum tissue-specific gene expression of both *OvTPS* and *CYP* sequences occurred in the flowers, then the leaves and finally, the stems, in both species. The roots exhibited minimum relative gene expression. Significant correlations between the relative expression of *OvTPS7, OvTPS5* and *CYP71D180* and the carvacrol content were also observed (Jan et al., 2018).

Breeding and Perspectives for Marker Assisted Selection

Breeding *O. vulgare* is in general related to improvement in both yield-related parameters like growth habit, leaf/stem ratio, stress (salt, cold) tolerance, and resistance to diseases as well as quality-related parameters including better aromatic characteristics, colour, essential oil content and composition, antioxidant and antimicrobial properties.

O. vulgare is characterized by high levels of genetic, chemical and morphological heterogeneity observed inside and among its six different subspecies. The subspecies of *O. vulgare* show high variability in the essential oil content and the main compounds in the oil and also for morphological characters as leaf and flower colour, trichome density, yield, leaf/stem ratio and others (Franz & Novak, 2002). High chemical variation has been observed among and also within individual wild populations (Kokkini et al., 1991; Kintzios, 2012). The observed high heterogeneity for both morphological and chemical characters in wild oregano populations has a negative impact on the quality and uniformity of the obtained plant material from natural populations. However, the observed heterogeneity is also a prerequisite for fruitful breeding programs. Breeding of *O. vulgare* in general starts from selection of best performing plants from wild populations. Recently, Sarou et al. (2017) reported the development of a conventional breeding program in Greece based on selection of elite self-plants of the high essential oil content *O. vulgare* ssp. *hirtum* from a natural population in Samothraki Island (Sarrou et al., 2017).Further, interspecific hybridization is common in the genus Origanum and therefore breeding of *O. vulgare* may also include cross pollination between different Origanum species in search for a heterosis effect. Several *O. vulgare* cultivars like *O. vulgare* ssp. *vulgare* 'Aureum' (golden foliage, mild taste) and *O. vulgare* 'Jim Best' (gold and green marbled leaves) have been developed as ornamentals. However, when developing *O. vulgare* cultivars targeting medicinal and culinary properties based on high essential oil content and quality (e.g. high carvacrol composition), the commonly applied selection methods should be coupled with analytical methods like GC-MS. Recently developed techniques for analysis of flower extracts rather than distillation of essential oil by Clevenger apparatus is a promising tool for faster and more straight-forward evaluation of the essential oil composition of individual plants from natural populations (Zagorcheva et al., 2016). Such methods allow during-the-season evaluation of a high number of individual plants followed by collection of seeds or vegetative material from the ones with desired characteristics later in the season.

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS) (Collard & Mackill, 2008). Many of the traits of interest to breeders are not easily assessed; therefore, selection based on linked DNA markers is much more efficient. Selection based on markers can be carried out at an early age (plantlets), thus it can significantly reduce the number of individuals assessed by the breeder. MAS has great potential for efficient gene pyramiding (combining several important genes in one cultivar). The prerequisites for MAS are the DNA markers and linkage analysis, which identifies the markers that are linked to the genes controlling the trait(s) of interest (Ben-Ari & Lavi, 2012). MAS rely on DNA markers, which are closely linked to agricultural traits of interest and therefore allow tremendously improved speed and efficiency over the traditional breeding approaches. The discovery of DNA markers linked to agricultural traits of interest including essential oil content and composition typically involves the development of genetic maps, identification of QTLs and DNA markers located in the close vicinity of the QTLs.

To date, however, there have been relatively few reports of molecular marker-based approaches to medicinal plant improvement, and not even the most skeletal genetic maps are available for any of the important species including oregano (Canter et al., 2005). Although diverse genetic marker systems have been applied for evaluation of the genetic diversity in *O. vulgare* and other *Origanum* species, so far,no genetic maps have been developed and QTL analysis has not been carried out.

A different approach for oregano improvement may be based on the growing knowledge regarding genes involved in key steps of terpenoid synthesis for different *Origanum* species, which expression has already been proven to be closely related to the content of essential oil and its composition (Crocoll et al., 2010; Crocoll, 2011). Recently, Habibi et al. (2016) reported successful development of genetic transformation and regeneration system from *O. vulgare* hairy roots. Although the modification of *O. vulgare* secondary metabolites by manipulating the expression of key genes in terpene biosynthesis through genetic transformation or gene editing is already a feasible option, the genetically modified plants are still not well accepted in many of the European countries, which may hamper the commercial application of such plants. However, although not as straight forward as are the genetic transformation or gene editing, analysis of the allele variants of such genes may be used for development of gene-specific markers and direct testing of the influence of particular alleles and allele combinations on the content and composition of secondary metabolites in the essential oil. For example, Lukas et al. (2010) analyzed part of the sequence of the γ-terpinene synthase gene (intron 2, exon 3 and intron 3) in 4 *Origanum* species from the section Majorana and *O. vulgare* (section Origanum). The study revealed 6 different variants of the gene that were distinguishable according to sequence characteristics in intron 3. The formation of "cymyl" compounds (γ-terpinene, *p*-cymene, carvacrol and other related compounds) in *O. vulgare* was found to be associated with the presence of intron pattern 1 and/or 3 and/or 4. In plants lacking these gene variants "cymyl"- compounds were completely absent or present in trace amounts only (Lukas et al., 2010).

There is a high degree of similarity in the DNA sequences of functional genes between different plant species, therefore, DNA probes from one species can often be used to identify homologous sequences in another closely related species (Canter et al., 2005). The constantly growing number of sequenced plant genomes may be used as a source of valuable genetic sequence information. The genome sequences assembled at different levels of several medicinal plant species from the Lamiaceae family including *Salvia splendens*, *Pogostemon cablin*, *Perilla citriodora*, *Mentha longifolia*, *Ocimum tenuiflorum* and *Scutellaria baicalensis* are already available in the NCBI genome database and can be used as a source of information and design of degenerated primers targeting particular genes of interest in *O. vulgare*. Following cloning of such genes and their functional analysis, the set of already known gene sequences from *O. vulgare* may be enlarged and used for development of gene-specific markers and future breeding programs based on MAS.

Conclusions

O. vulgare is a medicinal and culinary plant species with high degree of morphological, quantitative, genetic and chemotype variation both among and within its six subspecies. The essential oil distilled from its areal parts as well as the different plant extracts obtained from *O. vulgare* have shown a number of biological activities and find diverse applications in the food, cosmetics and pharmaceutical industries. Currently the vast amounts of utilized plant material from *O. vulgare* are obtained directly from the environment, which causes direct negative effect on the wild populations. Further the large variation observed within the subspecies and its wild populations is a major concern for the uniformity of the obtained material in terms of its further processing and standardization of the final products.

Therefore, the transition from gathering of plants from wild populations towards cultivation of *O. vulgare* as an agricultural crop and especially its high essential oil-bearing subspecies like *O. vulgare* ssp. *hirtum* is a necessary process, which is expected to progress in the next few years. The cultivation of *O. vulgare* will go in parallel with the selection of wild accessions and breeding for superior cultivars. Although modern breeding in *O. vulgare* is currently hampered by the lack of genetic maps and mapped QTLs, substantial knowledge including cloning of key enzymes involved in the synthesis of terpenoids in *O. vulgare* has already been obtained. In addition, the genome sequences of several species from the Lamiaceae family are already available in online databases allowing transfer of knowledge between species based on syntheny and sequence homology. Until QTL studies and DNA markers linked to particular traits become available in *O. vulgare*, breeding programs in the near future should focus on the development of allele specific DNA markers for candidate genes, which expression is associated with desired traits and test for the influence of allele variants on the trait of interest. Additionally, breeding in *O. vulgare* could involve inter- and intra-specific hybridization, which is common in the genus *Origanum* and could be a valuable source of heterosis effects.

Acknowledgements

This work was supported by Operational Program "Science and Education for Smart Growth" 2014-2020, co-financed by European Union through the European Structural and Investment Funds, Grant BG05M2OP001-1.002-0012 "Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products"

References

Alves-Pereira, I. M. S. & Fernandes-Ferreira, M. (1998). Essential oils and hydrocarbons from leaves and calli of *Origanum vulgare* ssp. *virens*. *Phytochemistry, 48(5)*, 795-799.

Amar, M. & Wahab, M. A. E. (2013). Compartive genetic study among *Origanum* L. plants grown in Egypt. *Journal of Biodiversity and Environmental Sciences, 3(12)*, 208-222.

Araniti, F., Landi, M., Lupini, A., Sunseri, F., Guidi, L. & Abenavoli, M. R. (2018). *Origanum vulgare* essential oils inhibit glutamate and aspartate metabolism altering the photorespiratory pathway in *Arabidopsis thaliana* seedlings. *J. Plant Physiol., 231*, 297-309.

Argyropoulos, E. I., Eleftherohorinos, I. & Vokou, D. (2008). *In vitro* evaluation of essential oils from Mediterranean aromatic plants of the Lamiaceae for weed control in tomato and cotton crops. *Allelopathy Journal, 22(1)*, 69-78.

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil*

Chemists Society, 75(2), 199-212.

Asensio, C. M., Grosso, N. R. & Juliani, H. R. (2015). Quality preservation of organic cottage cheese using oregano essential oils. *Lwt-Food Science and Technology, 60(2)*, 664-671.

Asensio, C. M., Nepote, V. & Grosso, N. R. (2012). Sensory attribute preservation in extra virgin olive oil with addition of oregano essential oil as natural antioxidant. *Journal of Food Science, 77(9),* 294-301.

Azizi, A., Hadian, J., Gholami, M., Friedt, W. & Honermeier, B. (2012). Correlations between genetic, morphological, and chemical diversities in a germplasm collection of the medicinal plant *Origanum vulgare* L. *Chemistry & Biodiversity, 9(12)*, 2784-2801.

Azizi, A., Wagner, C., Honermeier, B. & Friedt, W. (2009). Intraspecific diversity and relationship between subspecies of *Origanum vulgare* revealed by comparative AFLP and SAMPL marker analysis. *Plant Systematics and Evolution, 281(1-4),* 151-160.

Balusamy, S. R., Perumalsamy, H., Huq, M. A. & Balasubramanian, B. (2018). Anti-proliferative activity of *Origanum vulgare* inhibited lipogenesis and induced mitochondrial mediated apoptosis in human stomach cancer cell lines. *Biomed Pharmacother, 108*, 1835-1844.

Baranauskiene, R., Venskutonis, P. R., Dambrauskiene, E. & Viskelis, P. (2013). Harvesting time influences the yield and oil composition of *Origanum vulgare* L. ssp *vulgare* and ssp *hirtum*. *Industrial Crops and Products, 49*, 43-51.

Barbosa, I. D., Medeiros, J. A. D., de Oliveira, K. A. R., Gomes-Neto, N. J., Tavares, J. F., Magnani, M. & de Souza, E. L. (2016). Efficacy of the combined application of oregano and rosemary essential oils for the control of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enteritidis* in leafy vegetables. *Food Control, 59*, 468-477.

Baricevic, D. (1997). Experiences with oregano *(Origanum spp.)* in Slovenia. In: *Oregano. Promoting the conservation and use of underutilized and neglected crops. 14. Proceedings of the IPGRI International/Workshop on Oregano*, Italy, 110-120.

Bedoya-Serna, C. M., Dacanal, G. C., Fernandes, A. M. & Pinho, S. C. (2018). Antifungal activity of nanoemulsions encapsulating oregano (*Origanum vulgare*) essential oil: *in vitro* study and application in Minas Padrao cheese. *Braz J Microbiol, 49*(4), 929-935.

Begnini, K. R., Nedel, F., Lund, R. G., Carvalho, P. H., Rodrigues, M. R., Beira, F. T. & Del-Pino, F. A. (2014). Composition and antiproliferative effect of essential oil of *Origanum vulgare* against tumor cell lines. *J. Med. Food, 17(10),* 1129-1133.

Ben-Ari, G. & Lavi, U. (2012). Marker-assisted selection in plant breeding. *Plant Biotechnology and Agriculture: Prospects for the 21st Century*, 163-184.

Bernath, J. (1997). Some scientific and practical aspects of production and utilisation of oregano in Central Europe. In: *Oregano. Promoting the conservation and use of underutilized and neglected crops. 14. Proceedings of the IPGRI International Workshop on Oregano*, Italy, 75-92.

Berrington, D. & Lall, N. (2012). Anticancer activity of certain herbs and spices on the Cervical Epithelial Carcinoma (HeLa) cell line. *Evid Based Complement Alternat Med, 2012*, 564927.

Brondani, L. P., Neto, T. A. d. S., Freitag, R. A. & Lund, R. G. (2018). Evaluation of anti-enzyme properties of *Origanum vulgare* essential oil against oral *Candida albicans*. *Journal de Mycologie Médicale, 28(1)*, 94-100.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology, 94(3),* 223-253.

Canter, P. H., Thomas, H. & Ernst, E. (2005). Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. *Trends Biotechnol, 23(4)*, 180-185.

Cardoso, L. G., Santos, J. C. P., Camilloto, G. P., Miranda, A. L., Druzian, J. I. & Guimardes, A. G. (2017). Development of active films poly (butylene adipate co-terephthalate) - PBAT incorporated with oregano essential oil and application in fish fillet preservation. *Industrial Crops and Products, 108*, 388-397.

Collard, B. C. Y. & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B-Biological Sciences, 363(1491),* 557-572.

Crocoll, C. (2011). Biosynthesis of the phenolic monoterpenes, thymol and carvacrol, by terpene synthases and cytochrome P450s in oregano and thyme. Dissertation, Max-Planck-Institut für chemische Ökologie, Jena, Germany.

Crocoll, C., Asbach, J., Novak, J., Gershenzon, J. & Degenhardt, J. (2010). Terpene synthases of oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of terpene biosynthesis. *Plant Mol. Biol., 73(6),* 587-603.

D'Antuono, L. F., Galletti, G. C. & Bocchini, P. (2000). Variability of essential oil content and composition of *Origanum vulgare* L. populations from a North Mediterranean Area (Liguria Region, Northern Italy). *Annals of Botany, 86(3)*, 471-478.

De Mastro, G., Fracchiolla, M., Verdini, L. & Montemurro, P. (2006). Oregano and its potential use as bioherbicide. *Acta Horticulturae, 723*, 335-345.

de Souza, G. T., de Carvalho, R. J., de Sousa, J. P., Tavares, J. F., Schaffner, D., de Souza, E. L. & Magnani, M. (2016). Effects of the essential oil from *Origanum vulgare* L. on survival of pathogenic bacteria and starter lactic acid bacteria in Semihard Cheese broth and slurry. *J Food Prot, 79(2),* 246-252.

de Sousa, L. L., de Andrade, S. C., Athayde, A. J., de Oliveira, C. E., de Sales, C. V., Madruga, M. S. & de Souza, E. L. (2013). Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic Aspergilli and autochthonous mycoflora in *Vitis labrusca* L. (table grapes). *Int J Food Microbiol, 165(3),* 312-318.

Degenhardt, J., Kollner, T. G. & Gershenzon, J. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry, 70(15-16),* 1621-1637.

Ding, H. Y., Chou, T. H. & Liang, C. H. (2010). Antioxidant and antimelanogenic properties of rosmarinic acid methyl ester from *Origanum vulgare*. *Food Chemistry, 123(2),* 254-262.

Diniz-Silva, H. T., de Sousa, J. B., Guedes, J. S., Queiroga, R. C. R. E., Madruga, M. S., Tavares, J. F., de Souza, E. L. & Magnani, M. (2019). A synergistic mixture of *Origanum vul-*

gare L. and *Rosmarinus officinalis* L. essential oils to preserve overall quality and control *Escherichia coli* O157:H7 in fresh cheese during storage. *LWT*.

dos Santos, N. S., Aguiar, A. J. A., de Oliveira, C. E., de Sales, C. V., de Melo, E. S. S., da Silva, R. S., Stamford, T. C. & de Souza, E. L. (2012). Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). *Food Microbiol, 32(2),* 345-353.

Du, W. X., Olsen, C. W., Avena-Bustillos, R. J., McHugh, T. H., Levin, C. E., Mandrell, R. & Friedman, M. (2009). Antibacterial effects of allspice, garlic, and oregano essential oils in tomato films determined by Overlay and Vapor-Phase methods. *Journal of Food Science, 74(7),* 390-397.

Dutra, T. V., Castro, J. C., Menezes, J. L., Ramos, T. R., do Prado, I. N., Machinski, M., Mikcha, J. M. G. & de Abreu, B. A. (2019). Bioactivity of oregano (*Origanum vulgare*) essential oil against *Alicyclobacillus* spp. *Industrial Crops and Products, 129*, 345-349.

Fernandes, R. P. P., Trindade, M. A., Lorenzo, J. M. & de Melo, M. P. (2018). Assessment of the stability of sheep sausages with the addition of different concentrations of *Origanum vulgare* extract during storage. *Meat Science, 137*, 244-257.

Fleming, T. (2000). PDR for herbal medicines (2nd ed.). Medical Economics, Montvale (US).

Fortunato, I. M., Avato, P. & Ruta, C. (2006). Glandular hairs and essential oils in micropropagated plants of *Origanum vulgare* L. In: *Proceedings of the 1st International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation*, Italy, 295-298.

Foti, M. C. (2007). Antioxidant properties of phenols. *Journal of Pharmacy and Pharmacology, 59(12)*, 1673-1685.

Franz, C. & Novak, J. (2002). Breeding of oregano. In: *Oregano: The genera Origanum and Lippia*, S. E. Kintzios. Taylor and Francis CRC Press, USA, *25,*163-174.

Garcia-Beltran, J. M., Espinosa, C., Guardiola, F. A. & Esteban, M. A. (2018). *In vitro* effects of *Origanum vulgare* leaf extracts on gilthead seabream (*Sparus aurata* L.) leucocytes, cytotoxic, bactericidal and antioxidant activities. *Fish & Shellfish Immunology, 79*, 1-10.

Garcia-Beltran, J. M. & Esteban, M. A. (2016). Properties and applications of plants of *Origanum* sp. genus. *SM Journal of Biology, 2(1)*, 1006.

Gaur, S., Kuhlenschmidt, T. B., Kuhlenschmidt, M. S. & Andrade, J. E. (2018). Effect of oregano essential oil and carvacrol on *Cryptosporidium parvum* infectivity in HCT-8 cells. *Parasitology International, 67(2),* 170-175.

Gayoso, L., Roxo, M., Cavero, R. Y., Calvo, M. I., Ansorena, D., Astiasarán, I. & Wink, M. (2018). Bioaccessibility and biological activity of *Melissa officinalis*, *Lavandula latifolia* and *Origanum vulgare* extracts: Influence of an in vitro gastrointestinal digestion. *Journal of Functional Foods, 44*, 146-154.

Giannenas, I., Bonos, E., Christaki, E. & Florou-Paneri, P. (2018). Oregano: A feed additive with functional properties. *Therapeutic Foods, 8*, 179-208.

Giannenas, I., Florou-Paneri, P., Papazahariadou, M., Christaki, E., Botsoglou, N. A. & Spais, A. B. (2003). Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. *Arch Tierernahr, 57(2),* 99-106.

Gilling, D. H., Kitajima, M., Torrey, J. R. & Bright, K. R. (2014). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *Journal of Applied Microbiology, 116(5),* 1149-1163.

Govaris, A., Botsoglou, E., Sergelidis, D. & Chatzopoulou, P. S. (2011). Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. *Lwt-Food Science and Technology, 44(4),* 1240-1244.

Govindarajan, M., Rajeswary, M., Hoti, S. L. & Benelli, G. (2016). Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). *Research in Veterinary Science, 104*, 77-82.

Grieve, M. (1994). A modern herbal. Tiger, London.

Gurdian, C., Chouljenko, A., Solval, K. M., Boeneke, C., King, J. M. & Sathivel, S. (2017). Application of edible films containing oregano (*Origanum vulgare*) essential oil on Queso Blanco Cheese prepared with flaxseed (*Linum usitatissimum*) oil. *Journal of Food Science, 82(6),* 1395-1401.

Habibi, P., de Sa, M. F., da Silva, A. L., Makhzoum, A., da Luz Costa, J., Borghetti, I. A. & Soccol, C. R. (2016). Efficient genetic transformation and regeneration system from hairy root of Origanum vulgare. *Physiol Mol Biol Plants, 22(2),* 271-277.

Han, X. & Parker, T. L. (2017). Anti-inflammatory, tissue remodeling, immunomodulatory, and anticancer activities of oregano (*Origanum vulgare*) essential oil in a human skin disease model. *Biochim Open, 4*, 73-77.

Helsen, K., Jacquemyn, H., Hermy, M., Vandepitte, K. & Honnay, O. (2013). Rapid buildup of genetic diversity in founder populations of the Gynodioecious plant species *Origanum vulgare* after semi-natural grassland restoration. *PLoS One, 8(6).*

Hoareau, L. & DaSilva, E. J. (1999). Medicinal plants: a re-emerging health aid. *Electronic Journal of Biotechnology, 2*, 56-70.

Hussain, A. I., Anwar, F., Rasheed, S., Nigam, P. S., Janneh, O. & Sarker, S. D. (2011). Composition, antioxidant and chemotherapeutic properties of the essential oils from two *Origanum* species growing in Pakistan. *Revista Brasileira de Farmacognosia, 21*, 943-952.

Ietswaart, J. H. (1980). A taxonomic revision of the genus *Origanum* (Labiatae). Leiden University Press, The Hague (NL).

Ince, A. G., Karaca, M. & Elmasulu, S. Y. (2014). New microsatellite and CAPS-microsatellite markers for clarifying taxonomic and phylogenetic relationships within *Origanum* L. *Molecular Breeding, 34(2),* 643-654.

Jan, S., Mir, J. I., Shafi, W., Faktoo, S. Z., Singh, D. B., Wijaya, L., Alyemeni, M. N. & Ahmad, P. (2018). Divergence in tissue-specific expression patterns of genes associated with the terpeniod biosynthesis in two oregano species *Origanum vulgare* L., and *Origanum majorana*. *Industrial Crops and Products, 123*, 546-555.

Jedrzejczyk, I. (2018). Study on genetic diversity between *Origanum* L. species based on genome size and ISSR markers. *Indus-*

trial Crops and Products, 126, 201-207.

Katsiotis, A., Nikoloudakis, N., Linos, A., Drossou, A. & Constantinidis, T. (2009). Phylogenetic relationships in *Origanum* spp. based on rDNA sequences and intra-genetic variation of Greek *O. vulgare* subsp *hirtum* revealed by RAPD. *Scientia Horticulturae, 121(1),* 103-108.

Khan, M., Khan, S. T., Khan, N. A., Mahmood, A., Al-Kedhairy, A. A. & Alkhathlan, H. Z. (2018). The composition of the essential oil and aqueous distillate of *Origanum vulgare* L. growing in Saudi Arabia and evaluation of their antibacterial activity. *Arabian Journal of Chemistry, 11(8),* 1189-1200.

Khosravi, A. R., Shokri, H., Kermani, S., Dakhili, M., Madani, M. & Parsa, S. (2011). Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis. *Journal de Mycologie Médicale, 21(2),* 93-99.

Kim, S. I., Yoon, J. S., Jung, J. W., Hong, K. B., Ahn, Y. J. & Kwon, H. W. (2010). Toxicity and repellency of origanum essential oil and its components against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults. *Journal of Asia-Pacific Entomology, 13(4),* 369-373.

Kintzios, S. E. (2012). Oregano. In: *Handbook of herbs and spices. 2nd edition*, K. V. Peter. Woodhead Publishing Limited, UK, *2*, 417-436.

Kokkini, S., Vokou, D. & Karousou, R. (1991). Morphological and chemical variation of *Origanum vulgare* L. in Greece. *Botanica. Chronica, 10*, 337-346.

Kokkini, S., Karousou, R., Hanlidou, E. & Lanaras, T. (2004). Essential oil composition of Greek (*Origanum vulgare* ssp. *hirtum*) and Turkish (*O. onites*) Oregano: a Tool for Their Distinction. *Journal of Essential Oil Research*, *16(4),* 334-338.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J. & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology, 91(3)*, 453-462.

Lemhadri, A., Zeggwagh, N. A., Maghrani, M., Jouad, H. & Eddouks, M. (2004). Anti-hyperglycaemic activity of the aqueous extract of *Origanum vulgare* growing wild in Tafilalet region. *J Ethnopharmacol, 92(2-3),* 251-256.

Leyva-Lopez, N., Gutierrez-Grijalva, E. P., Vazquez-Olivo, G. & Heredia, J. B. (2017). Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules, 22(6),* 989.

Liang, C.-H., Chou, T.-H. & Ding, H.-Y. (2010). Inhibition of melanogensis by a novel origanoside from Origanum vulgare. *Journal of Dermatological Science, 57(3),* 170-177.

Licciardello, F., Muratore, G., Suma, P., Russo, A. & Nerin, C. (2013). Effectiveness of a novel insect-repellent food packaging incorporating essential oils against the red flour beetle (*Tribolium castaneum*). *Innovative Food Science & Emerging Technologies, 19*, 173-180.

Licina, B. Z., Stefanovic, O. D., Vasic, S. M., Radojevic, I. D., Dekic, M. S. & Comic, L. R. (2013). Biological activities of the extracts from wild growing *Origanum vulgare* L. *Food Control, 33(2),* 498-504.

Llana-Ruiz-Cabello, M., Maisanaba, S., Puerto, M., Pichardo, S., Jos, A., Moyano, R. & Camean, A. M. (2017). A subchronic 90-day oral toxicity study of *Origanum vulgare* essential oil in rats. *Food and Chemical Toxicology, 101*, 36-47.

Lukas, B., Samuel, R. & Novak, J. (2010). Oregano or marjoram? The enzyme gamma-terpinene synthase affects chemotype formation in the genus *Origanum*. *Israel Journal of Plant Sciences, 58(3-4),* 211-220.

Lukas, B., Schmiderer, C. & Novak, J. (2015). Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). *Phytochemistry, 119*, 32-40.

Machado, M., Dinis, A. M., Salgueiro, L., Cavaleiro, C., Custodio, J. B. A. & Sousa, M. d. C. (2010). Anti-*Giardia* activity of phenolic-rich essential oils: effects of *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *sylvestris*, and *Lippia graveolens* on trophozoites growth, viability, adherence, and ultrastructure. *Parasitology Research, 106(5)*, 1205-1215.

Majid, R., Ahmad, D. & Zargar, M. (2012). Antioxidant screening of ethanolic and aqueous extracts of *Organum vulgare* L. in-vitro. *African Journal of Biochemistry Research, 6(13)*, 165-171.

Makri, O. (2002). Cultivation of oregano. In: *Oregano: The genera Origanum and Lippia*, S. E. Kintzios. Taylor and Francis CRC Press, USA, *25,*153-162.

Marques, J. D., Volcao, L. M., Funck, G. D., Kroning, I. S., da Silva, W. P., Fiorentini, A. M. & Ribeiro, G. A. (2015). Antimicrobial activity of essential oils of *Origanum vulgare* L. and *Origanum majorana* L. against *Staphylococcus aureus* isolated from poultry meat. *Industrial Crops and Products, 77*, 444-450.

Marrelli, M., Cristaldi, B., Menichini, F. & Conforti, F. (2015). Inhibitory effects of wild dietary plants on lipid peroxidation and on the proliferation of human cancer cells. *Food Chem Toxicol, 86*, 16-24.

Marzi, V. (1997). Agricultural practices for oregano. In: *Oregano. Promoting the conservation and use of underutilized and neglected crops. 14. Proceedings of the IPGRI International Workshop on Oregano*, Italy, 61-68.

Matsubara, S., Ino, M., Murakami, K., Kamada, M. & Ishihara, I. (1996). Callus formation and plant regeneration of herbs in Perilla family (Labiatae). *Scientific Reports of the Faculty of Agriculture - Okayama University (Japan)*, 23-30.

Mazzarrino, G., Paparella, A., Chaves-Lopez, C., Faberi, A., Sergi, M., Sigismondi, C., Compagnone, D. & Serio, A. (2015). *Salmonella enterica* and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils. *Food Control, 50*, 794-803.

Meneses, R., Ocazionez, R. E., Martinez, J. R. & Stashenko, E. E. (2009). Inhibitory effect of essential oils obtained from plants grown in Colombia on Yellow fever virus replication *in vitro*. *Annals of Clinical Microbiology and Antimicrobials, 8*, 8.

Meyers, M. (2005). Oregano and marjoram: An Herb Society of America Guide to the Genus *Origanum*. *The Herb Society of America.* https://www.herbsociety.org/

Mfarrej, M. F. B. & Rara, F. M. (2019). Competitive, sustainable natural pesticides. *Acta Ecologica Sinica, 39(2),* 145-151.

Misharina, T. A., Burlakova, E. B., Fatkullina, L. D., Alinkina, E. S., Vorob'eva, A. K., Medvedeva, I. B., Erokhin, V. N., Semenov, V. A., Nagler, L. G. & Kozachenko, A. I. (2013). Effect of oregano essential oil on the engraftment and development of Lewis carcinoma in F1 DBA C57 black hybrid mice. *Prikl Biokhim Mikrobiol, 49(4),* 423-428.

Morshedloo, M. R., Craker, L. E., Salami, A., Nazeri, V., Sang, H. & Maggi, F. (2017). Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono- and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. *Plant Physiol Biochem, 111*, 119-128.

Nasr, M., Sendi, J. J., Moharramipour, S. & Zibaee, A. (2017). Evaluation of *Origanum vulgare* L. essential oil as a source of toxicant and an inhibitor of physiological parameters in diamondback moth, *Plutella xylustella* L. (Lepidoptera: Pyralidae). *Journal of the Saudi Society of Agricultural Sciences, 16(2),* 184-190.

Nimse, S. B. & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances, 5(35),* 27986-28006.

Nosal, P., Kowalska, D., Bielanski, P., Kowal, J. & Kornas, S. (2014). Herbal formulations as feed additives in the course of rabbit subclinical coccidiosis. *Ann. Parasitol., 60(1),* 65-69.

Novak, J., Lukas, B., Bolzer, K., Grausgruber-Groger, S. & Degenhardt, J. (2008). Identification and characterization of simple sequence repeat markers from a glandular *Origanum vulgare* expressed sequence tag. *Molecular Ecology Resources, 8(3),* 599-601.

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E. K., Jones, P. K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C. S., Petersen, R. B. & Smith, M. A. (2001). Oxidative damage is the earliest event in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 60(8),* 759-767.

Nybom, H. & Weising, K. (2007). DNA profiling of plants. In: *Medicinal plants biotechnology, from basic to industrial applications*, O. Kayser & W. J. Quax. Wiley-VCH, Weinheim, 73-95.

Ocana-Fuentes, A., Arranz-Gutierrez, E., Senorans, F. J. & Reglero, G. (2010). Supercritical fluid extraction of oregano (*Origanum vulgare*) essentials oils: anti-inflammatory properties based on cytokine response on THP-1 macrophages. *Food Chem. Toxicol., 48(6),* 1568-1575.

Paparella, A., Mazzarrino, G., Chaves-Lopez, C., Rossi, C., Sacchetti, G., Guerrieri, O. & Serio, A. (2016). Chitosan boosts the antimicrobial activity of *Origanum vulgare* essential oil in modified atmosphere packaged pork. *Food Microbiol., 59*, 23-31.

Paraskevakis, N. (2015). Effects of dietary dried Greek oregano (*Origanum vulgare* ssp *hirtum*) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. *Animal Feed Science and Technology, 209*, 90-97.

Pesavento, G., Calonico, C., Bilia, A. R., Barnabei, M., Calesini, F., Addona, R., Mencarelli, L., Carmagnini, L., Di Martino, M. C. & Lo Nostro, A. (2015). Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control, 54*, 188-199.

Petrou, S., Tsiraki, M., Giatrakou, V. & Savvaidis, I. N. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Food Microbiol., 156(3),* 264-271.

Raut, J. S. & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops and Products, 62*, 250-264.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Soliva-Fortuny, R. & Martin-Belloso, O. (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Comprehensive Reviews in Food Science and Food Safety, 8(3),* 157-180.

Rojas-Grau, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J., McHugh, T. H. & Martin-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology, 45(2),* 254-264.

Sallaud, C., Rontein, D., Onillon, S., Jabes, F., Duffe, P., Giacalone, C., Thoraval, S., Escoffier, C., Herbette, G., Leonhardt, N., Causse, M. & Tissier, A. (2009). A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl pyrophosphate in the wild tomato *Solanum habrochaites*. *Plant Cell, 21(1),* 301-317.

Sanchez, G. & Aznar, R. (2015). Evaluation of natural compounds of plant origin for inactivation of enteric viruses. *Food and Environmental Virology, 7(2)*, 183-187.

Sankar, R., Karthik, A., Prabu, A., Karthik, S., Shivashangari, K. S. & Ravikumar, V. (2013). *Origanum vulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. *Colloids Surf B Biointerfaces, 108*, 80-84.

Santoro, G. F., Cardoso, M. d. G., Guimaraes, L. G., Salgado, A. P., Menna-Barreto, R. F. & Soares, M. J. (2007). Effect of oregano (*Origanum vulgare L.*) and thyme (*Thymus vulgaris L.*) essential oils on *Trypanosoma cruzi* (Protozoa: Kinetoplastida) growth and ultrastructure. *Parasitology Research, 100(4),* 783-790.

Sarikurkcu, C., Zengin, G., Oskay, M., Uysal, S., Ceylan, R. & Aktumsek, A. (2015). Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. *vulgare* and subsp. *hirtum*) essential oils. *Industrial Crops and Products, 70*, 178-184.

Sarrou, E., Tsivelika, N., Chatzopoulou, P., Tsakalidis, G., Menexes, G. & Mavromatis, A. (2017). Conventional breeding of Greek oregano (*Origanum vulgare* ssp. hirtum) and development of improved cultivars for yield potential and essential oil quality. *Euphytica, 213(5).*

Shan, B., Cai, Y. Z., Sun, M. & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry, 53(20),* 7749-7759.

Shange, N., Makasi, T., Gouws, P. & Hoffman, L. C. (2019). Preservation of previously frozen black wildebeest meat (*Connochaetes gnou*) using oregano (*Oreganum vulgare*) essential oil. *Meat Sci, 148*, 88-95.

Skoufos, I., Tsinas, A., Giannenas, I., Voidarou, C., Tzora, A. & Skoufos, J. (2011). Effects of an oregano based dietary supplement on performance of broiler chickens experimentally infected with *Eimeria Acervulina* and *Eimeria Maxima*. *Poultry*

Science, 48(3), 194-200.

Skoula, M. & Harborne, J. B. (2002). The taxonomy and chemistry of origanum. In: *Oregano: The genera Origanum and Lippia*, S. E. Kintzios. Taylor and Francis CRC Press, USA, *25,* 67–108.

Svoboda, K. P., Finch, R. P., Cariou, E. & Deans, S. G. (1995). Production of volatile oils in tissue culture of *Origanum vulgar*e and *Tanacetum vulgare.* In: *Proceedings of the International Symposium on Medicinal and Aromatic Plants*, Japan, 147-152.

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N. R., Nogueira, J. M. F., Saraiva, J. A. & Nunes, M. L. (2013). Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. *Journal of the Science of Food and Agriculture, 93(11),* 2707-2714.

Thanos, C. A., Kadis, C. C. & Skarou, F. (1995). Ecophysiology of germination in the aromatic plants thyme, savory and oregano (Labiatae). *Seed Science Research, 5(3),* 161-170.

Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr Opin Plant Biol, 9(3),* 297-304.

Thomidis, T. & Filotheou, A. (2016). Evaluation of five essential oils as bio-fungicides on the control of *Pilidiella granati* rot in pomegranate. *Crop Protection, 89*, 66-71.

Tibaldi, G., Fontana, E. & Nicola, S. (2011). Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. ssp hirtum (Link) Ietswaart. *Industrial Crops and Products, 34(3),* 1516-1522.

Turner, G., Gershenzon, J., Nielson, E. E., Froehlich, J. E. & Croteau, R. (1999). Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiol, 120(3)*, 879-886.

Van Looy, K., Jacquemyn, H., Breyne, P. & Honnay, O. (2009). Effects of flood events on the genetic structure of riparian populations of the grassland plant *Origanum vulgare*. *Biological Conservation, 142*, 870-878.

Vattekkatte, A., Garms, S., Brandt, W. & Boland, W. (2018). Enhanced structural diversity in terpenoid biosynthesis: enzymes, substrates and cofactors. *Org Biomol Chem, 16(3)*, 348-362.

Vujicic, M., Nikolic, I., Kontogianni, V. G., Saksida, T., Charisiadis, P., Orescanin-Dusic, Z., Blagojevic, D., Stosic-Grujicic, S., Tzakos, A. G. & Stojanovic, I. (2015). Methanolic extract of *Origanum vulgare* ameliorates type 1 diabetes through antioxidant, anti-inflammatory and anti-apoptotic activity. *Br J Nutr, 113(5),* 770-782.

Wiseman, H. & Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. *Biochemical Journal, 313*, 17-29.

Zagorcheva, T., Rusanov, K., Stanev, S. & Atanassov, I. (2016). A simple procedure for comparative GC-MS analysis of lavender (*Lavandula angustifolia* Mill.) flower volatile composition. *Journal of Pharmacy and Biological Sciences, 11(4),* 9-14.

Zhang, X. L., Guo, Y. S., Wang, C. H., Li, G. Q., Xu, J. J., Chung, H. Y., Ye, W. C., Li, Y. L. & Wang, G. C. (2014). Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food Chemistry, 152*, 300-306.

Received: November, 28, 2019; *Accepted:* August, 13, 2020; *Published:* December, 31, 2020