Part 5

Pharmacology

8 The biological/pharmacological activity of the *Origanum* Genus

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INTRODUCTION

In the past, several classifications were made within the morphologically and chemically diverse *Origanum* (*Lamiaceae* family) genus. According to different taxonomists, this genus comprises a different number of sections, a wide range of species and subspecies or botanical varieties (Melegari *et al*., 1995; Kokkini, 1997). Respecting Ietswaart taxonomic revision (Tucker, 1986; Bernath, 1997) there exist as a whole 49 *Origanum* taxa within ten sections (*Amaracus* Bentham, *Anatolicon* Bentham, *Brevifilamentum* Ietswaart, *Longitubus* Ietswaart, *Chilocalyx* Ietswaart, *Majorana* Bentham, *Campanulaticalyx* Ietswaart, *Elongatispica* Ietswaart, *Origanum* Ietswaart, *Prolaticorolla* Ietswaart) the majority of which are distributed over the Mediterranean. Also, 17 hybrids between different species have been described, some of which are known only from artificial crosses (Kokkini, 1997). Very complex in their taxonomy, *Origanum* biotypes vary in respect of either the content of essential oil in the aerial parts of the plant or essential oil composition. Essential oil 'rich' taxa with an essential oil content of more than 2 per cent (most commercially known oregano plants), is mainly characterised either by the dominant occurrence of carvacrol and/or thymol (together with considerable amounts of γ-terpinene and *p*-cymene) or by linalool, terpinene-4-ol and sabinene hydrate as main components (Akgül and Bayrak, 1987; Tümen and Başer, 1993; Kokkini, 1997).

The *Origanum* species, which are rich in essential oils, have been used for thousands of years as spices and as local medicines in traditional medicine. The name hyssop (the Greek form of the Hebrew word 'ezov'), that is called '*za'atar*' in Arabic and *origanum* in Latin, was first mentioned in the Bible (Exodus 12: 22 description of the Passover ritual) (Fleisher and Fleisher, 1988). A comparative study of the traditional use of oregano-like herbs in the Mediterranean region, made by Fleisher and Fleisher (1988), established that the hyssop of the Bible is the carvacrol chemotype of the plant *Majorana syriaca* (L.) Feinbr. (syn.: *Origanum maru* L., *Origanum syriacum* L.). This plant, having a curative value in hypoglycaemic treatments (Yaniv *et al*., 1987), was an important part of the purification rites and was used as a medicine and as a condiment (Fleisher and Fleisher, 1988). A number of plants were found to have a similar flavour to that of hyssop, such as *Coridothymus capitatus* (L.) Reichenb. (Spanish oregano), *Satureja thymbra* L., *Thymbra spicata* L. and *Origanum vulgare* L. After the destruction of the Temple of Jerusalem at the beginning of the Christian era, the ritual use of hyssop ceased, while the tradition of using hyssop as a flavouring persisted, giving rise to two cultures of condiments, characterised by the high content of carvacrol in essential oils: '*za'atar*' in the Middle East and oregano in Europe.

Carvacrol-rich essential oils of *Origanum heracleoticum* L. (syn.: *O. hirtum* L., *O. creticum* Sieber × Bentham, *O. vulgare* L. subsp. *hirtum* (Link) Ietswaart), *O. maru* L. and *O. smyrnaeum* L. (syn.: *O. onites* L., *Majorana smyrnaea* (L.) Kostel, *M. onites* (L.) Bentham), that are native to Turkey and other East Mediterranean countries, have been used as a seasoning and in local materia medica in Turkey.

Aerial flowering parts of *O. vulgare* ssp. *viride* (Boiss.) Hayek (*O. viride* (Boiss.) Halacsy) are used in Iranian traditional medicine as diuretic, stomachic, antineuralgic, antitussive and expectorant. Afsharypuor *et al*. (1997) report on composition of essential oil of *O. vulgare* ssp. *viride* (Boiss.) Hayek, that grows wild in northern parts of Iran (with linalyl acetate, sabinene, β-caryophyllene as main components) and differs substantially from the composition of essential oil of the same species, growing wild in the Balkan area (Bulgaria, Albania, Turkey, Greece, Yugoslavia) (carvacrol chemotypes) or cultivated in Israel (thymol chemotype).

O. dubium Boiss., an endemic Mediterranean shrub, is widely spread on Cyprus (Paphos forest), in Greece and in Southern Turkey. In Cyprus it is locally known as 'rigani' and widely used in the preparation of local foods like 'souvla' and 'suovlakia'. In the traditional Cyprus medicine, an infusion of the leaves, flowering stems and flowers is used as a digestive and carminative, while carvacrol-rich essential oil is used externally as an antirheumatic (Arnold *et al*., 1993). In Greece, similar local names ('righani', 'aroani', 'rhoani') and traditional uses are found to be used for *Origanum onites* L. (= *O. smyrnaeum* L.). The essential oil of *Origanum majorana* L. var. *tenuifolium* Weston, which is endemic to Cyprus and locally named 'sampsishia', is traditionally used against common cold and fever or as a spasmolytic in gastro-intestinal disorders when applied internally or as an antirheumatic after external administration. *Cis*-sabinene hydrate (leaves: 33.3 per cent, flowers: 24.0 per cent, stems: 7.4 per cent), terpinene-4-ol (leaves: 21.6 per cent, flowers: 16.6 per cent, stems: 19.0 per cent), γ-terpinene (leaves: 8.3 per cent, flowers: 10.6 per cent, stems: 11.1 per cent) and α-terpineol (leaves: 7.3 per cent, flowers: 12.4 per cent, stems: 14.2 per cent) were found to be the leading compounds in 'sampsishia' essential oil, obtained after hydrodistillation of the above-ground plant parts. *Cis*-sabinene hydrate was also found to be the major component (21.5 per cent) of the essential oil of *Origanum rotundifolium* Boiss., aerial parts of which are used as a flavoured herbal tea in Turkey (Başer *et al*., 1995).

Aerial parts of *Origanum hypericifolium* O. Schwarz et P.H. Davis, an endemic Turkish species, collected before flowering, are used in Turkey (Burdur, Gölhisar) as a condiment, as meat flavouring and as herbal tea for treatment of common cold, stomach complaints and debility (Başer and Tümen, 1994). It was found, that aerial parts of this herb, when obtained in pre-flowering stage, contained relatively high amounts of essential oil (2.5 per cent), rich in carvacrol (64.3 per cent).

Aerial parts of *Origanum sipyleum* L. (syn.: *Majorana sipylea* (L.) Kostel, *Amaracus sipyleus* (L.) Rafin), a species of the eastern Mediterranean area, is widely used as a spice (central Anatolia) and against gastrointestinal disorders and cough (west Anatolia). The essential oil of this species was found to be rich in γ-terpinene and aromatic monoterpenes (Başer and Tümen, 1992). Dry leaves and flowering tips of *O. majorana* L. (syn. *Majorana hortensis* Moench.) and their tincture are used in the formulation of vermouths and bitters. The essential oil is used in the formulation of compounded oils for flavouring sauces, condiments, canned meats and other products (de Vincenzi and Mancini, 1997). In India this plant is traditionally used as an astringent, diuretic, antihysterial, antiasthmatic and antiparalytic drug (Yadava and Khare, 1995).

Based on ethnobotanical investigation in Lavras Novas (Brazil), where a rich folklore in the use of medicinal plants has been observed due to the remoteness of villages from modern medical facilities and the lack of a health care system, *O. majorana* L. is used for treatment of earache and influenza in children (Stehmann and Brandão, 1995).

Origanum vulgare L., commonly known as oregano or wild marjoram, is a well known flavouring for many international dishes and has antioxidant applications in human health (Dorofeev *et al*., 1989; Deighton *et al*., 1993). Antimicrobial action is reported for *O. vulgare* extracts, which contained phenolcarboxylic acids (cinamic, caffeic, *p*-hydroxybenzoic, syringic, protocatechuic, vanillic acid) as presumably active substances (Mirovich *et al*., 1989). Also, the fumigant toxicity of oregano essential oils for storeroom insects has been established (Shaaya *et al*., 1991; Baricevic *et al*., 2001). Traditionally, *Origani vulgaris herba* was used in respiratory tract disorders such as cough or bronchial catarrh (as expectorant and spasmolitic agents), in gastrointestinal disorders (as choleretic, digestive, eupeptic and spasmolitic agents) as oral antiseptic, in urinary tract disorders (as diuretic and antiseptic) and in dermatological affections (alleviation of itching, healing crusts, insect stings) (Blumenthal, 1998; Bruneton, 1999). Although, the monograph documentation of drug plant *O. vulgare* was submitted to the German Ministry of Health, the staff responsible for phytotherapeutic medicinal domain – Commission E – evaluated *O. vulgaris herba* negatively (Banz. Nr. 122 from 6th July 1988), because of lack of scientific proofs for the above mentioned indication areas (Blumenthal, 1998). Nevertheless, many of the studies confirmed the beneficial effects of oregano for human health. In view of the folk medicinal usage of New Zealand plants, the bioactivity of commercial essential oils of *O. vulgare* L. and of *O. majorana* L. was studied *in vitro* for their antibacterial, antifungal, antioxidative and spasmolytic activities. Oregano and marjoram were found to be effective antimicrobial agents and had a significant spasmolytic action on smooth muscle (Lis-Balchin *et al*., 1996). Extracts of *O. vulgare* exhibited a high capacity to compete with progesterone binding to intracellular receptors for progesterone, and showed considerable progestine activity *in vitro* (Zava *et al*., 1998).

Origanum vulgare, *O. majorana*, *O. dubium* and *O. dictamnus* contain in their leaves flavonoids (flavanone group – naringin, flavone group – apigenin and luteolin, flavonol group – quercetin) and flavonoid–glycosides (luteoline-7-glucoside, apigenin-7-glucoside, eriodictyol-7-glucoside) (Harvala and Skaltsa, 1986; Skaltsa and Harvala, 1987; Bohm, 1988; Soulèlès, 1990), some of which are known to possess spasmolitic activity. The antioxidative effect of plants belonging to the *Origanum* genus is probably the consequence of content of polar hydroxycinnamic derivatives and flavonoid glycosydes (Nakatani and Kikuzaki, 1987; Lamaison *et al*., 1993; Sawabe and Okamoto, 1994; Takácsová *et al*., 1995). These are found prevalently in essential oil 'poor' *origanum* taxa (less than 0.5 per cent of essential oil), such as *O. calcaratum* Jussieu or *O. vulgare* L. ssp. *vulgare*. Also non-polar phenolic compounds like thymol and carvacrol, which are major components of essential oil 'rich' *Origanum* taxa, possess remarkable antioxidant properties (Lagouri *et al*., 1993).

Interesting results were obtained in Poland, where Skwarek *et al.* (1994) discovered that *O. vulgare* extracts, when applied to $ECHO_9$ Hill virus, cultured in monkey kidney cells, induced the formation of a substance with interferon-like activity. The findings of relatively new investigations on bioactive compounds from spices show oregano's potential as a source of pesticidal and cancer preventive chemicals (Anonymus, 1997; Craig, 1999). Essential oils from *O. vulgare* ssp. *hirtum* (Greek oregano), *Origanum onites* (Turkish

oregano and *O. dictamnus* L. (syn. *Amaracus dictamnus* Benth., Cretan dittany), that are rich in phenolic compounds (carvacrol, thymol), possess antibacterial (Collier and Nitta, 1930; Kellner and Kober, 1954; Maruzzella and Sicurella, 1960; Aureli *et al*., 1992; Biondi *et al*., 1993; Vokou *et al*., 1993) and antifungal properties against pathogenic or nonpathogenic fungi (Maruzzella and Liguori, 1958; Arras and Picci, 1984; Guérin and Réveillère, 1985; Colin *et al*., 1989; Paster *et al*., 1993).

In the West Indies wild oregano (Lamiaceae) is the common name for a variety of botanical names such as *Coleus amboinicus* Launert., *C. aromaticus* Benth., *Plectranthus aromaticus* Roxb. and *P. amboinicus* Launert (French Origanum). It is cultivated for its sweat-inducing and insecticidal properties and is used also as a culinary flavour, against stings from scorpions and poisonous centipedes, for cleaning textiles, and as a shampoo (Chatterjee *et al*., 1958; Kuebel and Tucker, 1988; Prudent *et al*., 1995). In India, the plant and its preparations are used topically against ulcers and inflammation of the mouth and internally as a digestive. In Vietnam, *C. aromaticus* is employed in the preparation of cough mixtures (Prudent *et al*., 1995). According to Buznego *et al*. (1991) it possess antiepileptic properties. The essential oil of *C. aromaticus* is mainly composed of phenolic compounds, the major components depending on the geographical origin. *C. aromaticus* essential oil (Martinique origin), which contained carvacrol (60.96 per cent) and β-caryophyllene (13.26 per cent) as major components, showed both fungistatic (at MIC of 0.25 mg/ml towards human pathogens: *Aspergillus niger*, *Candida albicans* and towards plant pathogens: *Botrytis cinerea*, *Cylindrocarpon mali*, *Sclerotinia sclerotiorum*) and bacteriostatic activities *in vitro* (at MIC of 0.125 mg/ml towards *Mycobacterium smegmatis* and *Vibrio cholerae*, at MIC of 0.5 mg/ml towards *Staphylococcus aureus* and *Escherichia coli*) (Prudent *et al*., 1995). Stiles and co-workers (1995) observed similar results. They found, that *O. vulgare* essential oil was an effective anti-*C. albicans* agent (MIC against 3 different Candida strains <0.1 µg/ml), and that this activity might be due to the carvacrol content.

The world trends toward increasing usage of spices indicate an overall change in food habits and tastes. The appetiser effect of culinary herb mixtures with oregano was studied in animals and in human experiments. The authors generally agree that herb mixtures, when added to the diet in a moderate (about 0.3 per cent) quantity cause higher liveweight gains of experimental animal groups (pigs, calves) than the controls (Gunther, 1991; Stenzel *et al*., 1998), and act as appetisers in human diet (Yeomans, 1996). An improved feed conversion as well as protein and energy utilisation in fish that were fed an oregano supplemented (3 per cent) diet, was observed also by El-Maksoud *et al*. (1999). However, aversive postingestive feeding effects after initially increased preference for oregano flavoured feed as well as generalisation of aversion from familiar to novel feeds with a similar flavour were observed in animal (lambs) experiments (Launchbaugh and Provenza, 1994; Villalba and Provenza, 1996), causing lower preference for long-term feeding with oregano flavoured straw. Aversions to sauces flavoured with oregano have been reported also in human diet – in pregnant women (Hook, 1978).

ANTIFUNGAL ACTIVITY

When assessing the food-preservative and health promoting potential of spices, more attention in recent literature has been placed on the studies of inhibitory effects of oregano essential oil or its components to the fungal growth and/or sporulation than on

that of oregano crude drug or its extracts. The antifungal activity is strongly correlated with the type of essential oil (that depends on plant species and origin), its concentration and pH of the testing medium *in vitro* (Deans and Svoboda, 1990; Thompson, 1990; Biondi *et al*., 1993). Authors generally agree that there is a relationship between the chemical structure of the most abundant essential oil components and their antifungal and anti-aflatoxigenic potency. Phenols are believed to be the most potent antimicrobials followed by alcohols, ketones, ethers and hydrocarbons (Bullerman, 1977; Hitokoto *et al*., 1980; Hussein, 1990; Daw *et al*., 1994; Charai *et al*., 1996). These presumptions are in accordance with the findings of Biondi *et al*. (1993), who reported that carvacrol – rich *O. onites* L. essential oil showed more potent antifungal activity against *A. niger*, *Aspergillus terreus*, *C. albicans* and against *Fusarium* spp. than the oregano oil, composed prevalently of terpinene-4-ol and of γ-terpinene. A potent antifungal effect of *O. vulgare* essential oil (rich in carvacrol and thymol) at concentration of 1 µl/ml against the common spoilage fungus *A. niger* was observed also by Baratta *et al*. (1998a).

However, there are many differences among genera of fungi with regard to sensitivity to the antifungal effects of oregano and of its essential oils. Also, the concentration of an essential oil and its origin may significantly influence their antifungal activity. When published results on the antimicrobial activity of oregano essential oils are compared, significant differences among results obtained by different research groups may be observed. These are due to different methods used in investigations, and also due to the incomplete specification (common name instead of botanical one) of the oregano species, especially, when no data are given about the chemical composition of the essential oil.

The ground *O. vulgare* (2 per cent) was found to possess a strong antifungal potential against several food-contaminating moulds, like *Trichoderma harzianum* Rifai, *Alternaria alternata* Keissler, *Fusarium oxysporum* Schlecht, *Mucor circinelloides f. griseo-cyanus* Schipper, *Cladosporium cladosporioides* de Vries, *Fusarium culmorum* Saac., *Aspergillus versicolor* Tiraboschi, but allowed selective growth of *Rhizopus stolonifer* Lind and *Penicillium citrinum* Thom in potato dextrose agar (Schmitz *et al*., 1993). A moderate antifungal activity of oregano crude drug, when applied in a concentration of 2 per cent (the upper level, that is most often used in food industry), against *Penicillium citrinum*, *P. roqueforti*, *P. patulum* and against three mycotoxigenic fungi (*Aspergillus flavus*, *A. parasiticus* and *A. ochraceus*) was reported also by Azzouz and Bullerman (1982). Deans and Svoboda (1990) observed a significant potency of *O. majorana* essential oil against filamentous fungi, in particular mycotoxigenic strains.

An interesting comparative study between the antimicrobial activity of crude drug (ground dried plant), of aqueous extracts and essential oils of *Origanum compactum* Bentham (section *Prolaticorolla* Ietswaart) and of *O. majorana* L. of Morocco origin was made, in order to ascertain their antimicrobial potential against yeasts (*Saccharomyces cerevisiae*, *Candida utilis*, *Candida tropicalis*, *Candida lipolytica*), moulds (*Penicillium parasiticus*, *Geotrichum candidum*, *A. niger*), bacteria (*Pseudomonas fluorescens*, *E. coli*, *S. aureus*, *Bacillus cereus*) and lactic acid bacteria (*Lactobacillus plantarum*, *L. mesenteroides*) (Charai *et al*., 1996). Sensitive microorganisms were generally more susceptible to *O. compactum* than to *O. majorana* regardless of the form of applied antimicrobial (crude drug, water extracts, essential oil). When considering the activity of the whole plant (at concentration of 1 per cent in solid media *in vitro*), yeasts proved to be the microorganisms most sensitive to the tested plant material, followed by lactic bacteria and other bacteria tested. Plants showed practically no

inhibition potential towards moulds. By contrast, when considering water extracts of both plant species, moulds were the most sensitive, bacteria showed only a scarce sensibility (especially towards *O. majorana* extracts) and yeasts were not affected. Moulds were completely inhibited (*O. compactum*) or partly inhibited (*O. majorana*) by water extracts at 40 per cent concentration. Inhibition of microbial growth in the case of water extracts was probably due to the presence of water-soluble polyphenols and tannins.

Essential oil of *O. compactum* was characterised by carvacrol (49.5 per cent), *p*-cymene (21.2 per cent) and γ-terpinene (14.2 per cent) as major components, while *O. majorana* was found to be a linalool chemotype (linalool 32.6 per cent, terpinene-4-ol 22.3 per cent and *p*-cymene 8.1 per cent). It was found that both essential oils have inhibitory potential against microbial growth, *O. compactum* being the more potent inhibitor, probably due to the presence of a highly active phenolic moiety (carvacrol) in its essential oil. The following concentrations of essential oils were found to completely inhibit growth of tested microorganisms: bacteria at 4 ppm (lactic bacteria in the case of *O. compactum* at 1 ppm), yeasts at 1.6 ppm (*O. compactum*) or at 5 ppm (*O. majorana*), moulds at 1 ppm (*O. compactum*) or at 5 ppm (*O. majorana*).

Phenolic compounds, characteristic major constituents of essential oils of *O. vulgare* L. (section *Origanum* Ietswaart) or of *O. dictamnus* L. (section *Amaracus* Bentham), are probably responsible for the high inhibitory activity of carvacrol/thymol chemotypes of oregano against fungal growth, conidial germination and production of *Penicillium digitatum* (at essential oil concentration of 250–400 ppm) (Daferera *et al*., 2000). Moreover, monoterpene components, which are present in essential oils in different proportions, seem to have more than an additive effect in fungal inhibition. Phenolic derivatives, present in essential oils, may also be involved in inhibition of yeast sporulation through depletion of cellular energy by reduction of respiration. It has been shown that oregano essential oil (at 100 ppm) significantly impaired the respiratory activity of *Saccharomyces cerevisiae* as evidenced by a reduction in CO_2 and ethanol production (Conner *et al*., 1984). The growth of variety of yeasts was significantly inhibited by oregano essential oils (at concentration of 200 ppm: *Brettanomyces anomalus*, *G. candidum, Kluyveromyces fragilis*, *Lodderomyces elongisporus*, *Metchnikowia pulcherrima*, *Pichia membranaefaciens*, *Saccharomyces cerevisiae*, *Torulopis glabrata*, at 100 ppm: *Candida lypolytica*, *Hansenula anomala*, *Kloeckera apiculata*, *Rhodotorula rubra*, *Debaryomyces hansenii*), that were rich in phenolic compounds (Conner and Beuchat, 1984a). When exposed to sublethal heat treatments (48–54 °C), which correspond to those during food processing, the increased sensitivity of yeasts to oregano essential oil (at levels 100–200 ppm) or to oregano oleoresins (at levels 250–500 ppm) was observed. This might be due to the synergistic or additive effect of oregano oil or oleoresin (in combination with heat) on inactivation of yeasts and due to the affected recovery of heat stressed yeast cells after treatment with oleoresin, resulting in low viable populations (Conner and Beuchat, 1984b; Conner and Beuchat, 1985). Karanika *et al*. (2001) studied the inhibitory effect of aqueous extract (at concentration of 5 g/l) of *O. dubium* on *Yarrowia lipolytica*. It was found that the increased lag time and suppressed specific growth rate of this yeast were not attributed to the direct effect of the extract on yeast cells, but to the *O. dubium*-induced chelation of metal ions, which are essential for microbial growth.

Biological assays showed strong fungitoxic activity of essential oil (at 1000 ppm) from Turkey native populations of *Origanum minutiflorum* Schwarz Davis against *Fusarium moniliforme*, *Rhizoctonia solani*, *S. sclerotiorum* and *Phytophthora capsici*. It was found that the antifungal effect was due to the presence of phenolic components in the

essential oil, like carvacrol and/or thymol, which are well known for their antifungal potency (Kurita *et al*., 1981; Farag *et al*., 1989; Curtis *et al*., 1996). Carvacrol or thymol, when applied in concentrations of more than 100 ppm led to a complete inhibition of growth of the above mentioned fungi *in vitro*. These phenolic compounds showed higher inhibition against *P. capsici* than the soil-applied systemic fungicide Previcur N (Muller-Riebau *et al*., 1995). Oregano essential oil, thymol and carvacrol (at 0.025 per cent or 0.05 per cent) were also found to be strong growth-inhibitory compounds against *Penicillium roqueforti, G. candidum* and *Mucor* spp. (Akgül and Kivanç, 1988; Akgül and Kivanç, 1989). Thompson (1990) established that carvacrol exhibited a fungicidal effect against *Aspergillus* spp., that was pH-dependent, and at pH 4 the fungicidal effect was more potent than at pH 6.

Adam *et al*. (1998) found a valuable therapeutic potency of essential oil of *O. vulgare* L. subsp. *hirtum* against experimentally induced dermatophytosis in rats (infection with *Trichophyton rubrum*). They report that carvacrol and thymol showed much higher antifungal activities against human pathogens (keratinophilic fungus *T. rubrum*, yeasts: *Malassezia furfur* and *Trichosporon beigelii*) than their biosynthetic precursors γ-terpinene and *p*-cymene.

Dose-dependent antiaflatoxinogenic effects of oregano (*O. vulgare* L.) essential oil and of carvacrol after exposure to toxigenic strains of *A. flavus* and *A. parasiticus* were observed in laboratory conditions (Llewellyn *et al*., 1981; Akgül *et al*., 1991; Özcan, 1998). Aflatoxins of *A. flavus* and *A. parasiticus* have been shown to be both toxic and carcinogenic in test animals and are also involved in the etiology of human liver cancer. Carvacrol, if used in sufficient amounts, can be an effective inhibitor of the growth and toxin production by *A. flavus* and *A. parasiticus*. At 500 ppm (28 °C) carvacrol completely inhibited growth and toxin production by both strains, aflatoxins (B_1, B_2, G_1) production being inhibited at lower concentrations than fungal growth. *A. flavus* was more sensitive to carvacrol than *A. parasiticus* (Akgül *et al*., 1991). Oregano (*O. vulgare* L.) essential oil, when applied in concentrations 1000 ppm or 750 ppm to a liquid culture medium, completely inhibited *A. ochraceus* growth and ochratoxin A production for up to 21 days and 14 days, respectively (Basilico and Basilico, 1999). When applied at 500 ppm, this essential oil was not effective. According to the results of Paster *et al*. (1990) higher concentrations of oregano essential oil are needed for total inhibition of spore germination (600 ppm) than for prevention of mycelial growth (400 ppm) of *A. flavus*, *A. ochraceus* or *A. niger*. The data obtained in the study of Hammer *et al*. (1998) showed inhibitory effect of *O. vulgare* essential oil (MIC = 0.12 per cent) against *C. albicans in vitro* (agar dilution method).

Oregano, its essential oil or isolated compounds, was studied also from the aspect of possible applications in plant protection, in post-harvest crop/fruit protection or in apiculture, where species specific fungi endanger the production systems.

The *in vitro* inhibitory effect of *O. vulgare* leaf extracts was observed in rice pathogen *Drechslera oryzae* (*Cochliobolus miyabeanus*) (Bisht and Khulbe, 1995). The strong antifungal effect of *O. vulgare* L. essential oil in relatively low concentrations (200 ppm) was observed against *Penicillium italicum* Wehm., *Botrytis cinerea* Pers., *Alternatia citri* Ell. et Pierce, that frequently infect fruits after harvesting and are difficult to control with synthetic phytopharmaceuticals due to the appearance of resistant strains (Arras and Picci, 1984). *Origanum compactum* Bentham essential oil (with carvacrol content of 37.7 per cent, Morocco origin) completely inhibited all three asexual reproduction steps (spore germination, mycelial elongation and sporulation) of common food

contaminating fungi at concentrations of 1 per cent (*A. niger*, *Penicillium italicum*) or 0.1 per cent (*Zygorrhynchus* spp.) (Tantaoui-Elaraki *et al*., 1993). The antifungal activity of essential oils, at all stages of asexual reproduction, was related to concentration, i.e. the higher the concentration the greater the inhibition. The fungistatic activity of essential oils of *O. syriacum* L. (syn.: *M. syriaca*) against mycelia of soil-borne pathogens (*Fusarium oxysporum*, *Macrophomina phaseolina*) and against foliar pathogens (*Botritis cinerea*, *Exserohilum turcicum*) was observed by Shimoni *et al*. (1993). Based on remarkable antifungal activity, these oils might serve as effective agents in control of *Fusarium oxysporum* and *Exserohilum turcicum* spread, although the authors are critical with regard to the toxicity of essential oils, which is comparably in the range of some synthetic fungicides.

Carvacrol rich essential oil of *O. vulgare* L. (Drôme origin) showed fungistatic as well as fungicide (minimal fungicidal concentration at 0.05 per cent) activity when assayed *in vitro* against bee pathogen *Ascosphaera apis*, and promises new possibilities for effective treatment of apiaries (in 0.1 per cent concentration) (Colin *et al*., 1989). Similar results were obtained by Calderone *et al*. (1994), who studied the sensibility of this chalkbrood causing pathogen towards Spanish *Origanum* (*Thymus capitatus*) oil, rich in thymol. Essential oil of *Thymus capitatus* and thymol completely inhibited *in vitro* growth of *Ascosphaera apis* for 72 h at concentrations of 100 ppm and 10 ppm, respectively. The relevance of carvacrol in essential oils of *O. syriacum* L. (*za'atar*) for their antifungal activity was stressed in the experiments of Daouk *et al*. (1995). They have considered *O. syriacum* as a potent mould inhibitor, which could be used as a food preservative in small amounts without changing much the odour or taste of the stored food. They found that essential oil of Lebanese '*za'atar*' completely inhibited growth of *Penicillium* spp., *A. niger* and *Fusarium oxysporum* at 100 ppm, and had a pronounced effect already when applied to the culture media in concentrations as low as 0.05 per cent (50 ppm). The fumigant toxicity of *O. vulgare* L. essential oils against fungi (*A. flavus*, *A. ochraceus* and *A. niger*) attached to stored wheat grain was studied in Israel. The minimal inhibitory concentrations (MIC) of oregano oil needed to inhibit the mycelial growth and sporulation of the fungi were 2.0 and 2.5 ppm, respectively (Paster *et al*., 1993; Paster *et al*., 1995). Better inhibitory effect was achieved when the grain contained a relatively high moisture content (15–20 per cent). The results of this study indicate the possibility of using the oregano-based fumigants as an alternative to chemicals for preserving grains destined for human or animal consumption. Contrary to this, oregano essential oils could not be used in seed preservation, because the treatments considerably affected wheat germination. No phytotoxic effect on germination and corn growth was detected when maize grain was treated with *O. vulgare* essential oils against *A. flavus* contamination, but the effective essential oil concentration (>10 per cent) was too high for practical applications (Montes-Belmont and Carvajal, 1998). Similar results were obtained in Greece, where essential oils of *Origanum* spp. were studied for their antifungal effects against *Botrytis cinerea* (Thanassoulopoulos and Yanna, 1997). Although, oregano essential oils (at 500 ppm) were fungicidal towards *Botrytis cinerea* culture *in vitro*, the small reduction of Botryris-rot in kiwifruit was meaningless for the control of storage rot. Hence, the essential oils destroyed the qualitative characteristics of the fruits (flavour, flesh colour, taste) and were unmarketable.

High antifungal activities of isolated components of essential oils, like carvacrol and thymol against food-storage fungi (Thompson, 1989) and of carvone against *Penicillium hirsutum*, responsible for post-harvest *Penicillium* rot on tulip bulbs, were reported (Smid *et al*., 1995).

Moulds, most frequently spread on bread (*Aspergillus glaucus* spp., *Eurotium repens* de Bary, *Cladosporium herbarum* Link ex Gray, *Penicillium expansum* Link ex Gray), can be effectively suppressed by oregano or its essential oils and, according to the authors (Kunz, 1994; Kunz *et al*., 1995), offer new possibilities for the use of oregano in bakery and bakery products. On the other hand, only a weak fungistatic activity of oregano oleoresin was observed by Nielsen and Rios (2000), who studied antifungal effects towards bread spoilage fungi *Penicillium commune, P. roqueforti, A. flavus* and *Endomyces fibuliger*.

ANTIBACTERIAL ACTIVITY

Based on a broad spectra of antibacterial activity, oregano seems to be one of the most inhibitory spices tested. However, when considering the reported data, questions on the accuracy of the interpreted information often arise. When assessing the antimicrobial potency of herbs, spices and medicinal plants, that are used traditionally in gastronomy for preservative reasons or in prevention of human–plant diseases, one has to consider many factors which influence the efficacy of an extract or essential oil. These include the plant species used and its origin, concentration and composition of an extract/essential oil, the mode of dispersal of extract/oil to the medium, the concentration of tested organism in the growing medium, susceptibility of bacterial strains, the method used – *in vitro* or *in vivo* – pH, temperature . . .). Moreover, Skandamis *et al*. (2000) have stressed the importance of the fluidity of the culture medium for accurate estimation of the potency of essential oils against microorganisms. Different culture media (liquid culture or gel matrix) were found to influence the rate of consumption of glucose, thus influencing the growth of bacteria as well as their susceptibility toward extracts/essential oils.

Altogether, 52 essential oils (including *O. vulgare* and *O. majorana* essential oils) and extracts of different plant genera have been investigated (Hammer *et al*., 1999) for their activity against *Acinetobacter baumanii*, *Aeromonas veronii* biogroup *sobria*, *C. albicans*, *Streptococcus faecalis*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serotype *typhimurium*, *Serratia marcescens* and *S. aureus*. It was found that *O. vulgare* (Australian origin) yielded one of the most potent antibacterial agents, which considerably inhibited the growth of all tested microorganisms. The lowest minimum inhibitory concentration of *O. vulgare* essential oil was 0.12 per cent (v/v) and 0.25 per cent (v/v) of *O. majorana*. Among the tested bacteria, the most resistant was *Pseudomonas aeruginosa*, that was inhibited by *O. vulgare* essential oil at 2 per cent (v/v), but not by *O. majorana* oil.

Screening of Italian Medicinal Plants for their antibacterial activity using the *in vitro* paper disk diffusion method (paper disks Whatman No. 1) revealed the strong activity of *O. vulgare* L. dimethylsulphoxide (DMSO) extracts against Gram-positive (*Bacillus subtilis*, *S. aureus*, *Streptococcus haemolyticus*) and Gram-negative bacteria (*E. coli* 7075, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi* H) (Izzo *et al*., 1995). The minimal inhibitory concentration (MIC) of applied extracts towards all tested bacteria was less than 4 μg/disk, with the exception of *Pseudomonas aeruginosa*, which was not susceptible. The essential oils of *Origanum onites* L. of Sicilian origin and of commercial oregano (which was found to be a mixture of two species, i.e. *O. vulgare* L. and *O. majorana* L.) exhibited bactericidal or bacteriostatic activity against a variety of Gram-positive

(*S. aureus*, *Streptococcus faecalis*, *Micrococcus luteus* and *Bacillus subtilis*) and Gram-negative bacteria (*Proteus vulgaris*, *E. coli*, *Hafnia alvei*) (Biondi *et al*., 1993). GC analysis revealed the carvacrol (61.68 per cent) as the main component of *O. onites* essential oil, while the commercial oregano sample consisted of terpinene-4-ol (24.87 per cent), γ-terpinene (15.91 per cent) and thymol (11.61 per cent) as leading compounds. When comparing the two species, *O. onites* essential oil was found to be more effective, inducing bactericidal effects against all G(−) tested organisms and against *S. aureus* and bacteriostatic effects against *Micrococcus luteus* and *Bacillus subtilis* at dilutions (in absolute ethanol) of 1:10 (final concentration of essential oil 1 µl/disk). The bactericidal effect of essential oil, distilled from commercial oregano sample showed bactericidal effects only against *Streptococcus faecalis* (dilution 1:2, final conc. of EO 5 µl/disk), *E. coli* and *Proteus vulgaris* (dilution 1:5, final conc. of EO 2 µl/disk), whilst bacteriostatic effects (dilution 1:10) were observed against other tested bacteria. *Pseudomonas aeruginosa* was not susceptible to any of the tested oils or concentrations. Similar results were obtained by Paster *et al*. (1990), who found that *Pseudomonas aeruginosa* was not affected by oregano (*O. vulgare* L.) essential oil at concentrations of up to 500 µg/ml. Under aerobic conditions the *O. vulgare* oil was very effective against *Campylobacter jejuni* (microaerophile) and *Clostridium sporogenes* (anaerobe) at 250 µg/ml (Paster *et al*., 1990). Also, good bacteriostatic (at concentration of 225 mg/l) and bactericidal (at concentration of 900 mg/l) effects *in vitro* against *Erwinia amylovora* were observed with *O. vulgare* essential oil (Scortichini and Rossi, 1989; Scortichini and Rossi, 1993).

Origanum vulgare essential oil (agar dilution method: 10 µl oil/Petri dish), characterised by high thymol (32.4 per cent) and carvacrol (16.7 per cent) content, showed a strong inhibitory (inhibition zone >20 mm) effect against a broad spectrum of tested bacteria, that were both G(+) or G(−) (*Alcaligenes faecalis*, *Bacillus subtilis*, *Beneckea natriegens*, *Brevibacterium linens*, *Brocothrix thermosphacta*, *Citrobacter freundii*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Erwinia carotovora*, *Klebsiella pneumoniae*, *L. plantarum*, *Leuconostoc cremoris*, *Moraxella* spp., *Proteus vulgaris*, *Salmonella pullorum*, *Serratia marcescens*, *S. aureus*, *Streptococcus faecalis*, *Yersinia enterolitica*) (Baratta *et al*., 1998a). Good inhibitory activity (inhibition zone >10 mm <20 mm) was observed also against *E. coli*, *Flavobacterium suaveolens*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, whilst two bacteria (*Acinetobacter calcoaceticus*, *Aeromonas hydrophila*) were not susceptible to oregano essential oil. In affected organisms, the origin of the bacterial strain or the fact of being G(+) or G(−) did not influence their susceptibility towards the oil. This is in agreement with the observations of Deans (Deans and Ritchie, 1987; Deans *et al*., 1992), who found that volatile oils of *O. vulgare* ssp. *hirtum* were equally effective against both G(+) and G(−) microorganisms.

Using the same bacterial strains and test conditions, the essential oils of *O. majorana* – consisting prevalently of terpinen-4-ol (20.8 per cent), γ-terpinene (14.1 per cent) and α-terpinene (14.1 per cent) – showed similar, but less potent antibacterial activity than *O. vulgare*. However, *O. majorana* exhibited a strong inhibitory effect against *Acinetobacter calcoaceticus* and *Aeromonas hydrophila* (Baratta *et al*., 1998b), against *Beneckea natriegens*, *Erwinia carotovora* and *Moraxella* spp. (Deans and Svoboda, 1990) but was not active against *Brevibacterium linens* and *Leuconostoc cremoris*.

Essential oils of *O. vulgare* of Turkish origin exhibited still more potent antibacterial activity as observed by Kivanç and Akgül (1986), who have studied the bactericidal effects of essential oils by both the agar diffusion and the serial dilution methods. Essential oils of *O. vulgare* showed pronounced bacteriostatic (dilution levels of

1:20–1:160) and bactericidal effects (dilution level of 1:20) against seven bacteria (*Aerobacter aerogenes*, *Bacillus subtilis*, *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus albus*, *Staphylococcus aureus*).

The growth of a wide range of bacteria (*Clostridium sporogenes*, *Enterobacter*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella pullorum*, *S. aureus*, *Streptococcus faecalis*, *Yersinia enterolitica*) was strongly inhibited (zone inhibition $>$31.5 mm $<$71.2 mm), when grown in an agar medium supplemented with essential oil of *Origanum officinalis* (dilution 1:10), a special breed from Israel, that was selected for elevated oil yields (Deans *et al*., 1992).

Origanum majorana L. essential oil (Vojvodina origin), at concentration of 0.15 per cent showed moderate inhibitory activity against four bacteria (*E. coli*, *Proteus vulgaris*, *Salmonella enteritidis*, *Pseudomonas fluorescens*), that are frequently present as undesirable flora in the meat-processing industry (Sirnik and Gorišek, 1983). Deans and Ritchie (1987) reported, that *Origanum majorana* L. essential oil had a broad spectrum of antibacterial activities (at dilution level of 1:10) against bacteria of animal or human origin (*E. coli*, *Salmonella pullorum*, *Streptococcus faecalis*, *S. aureus*, *Clostridium sporogenes*) against soil bacteria (*Bacillus subtilis*, *Serratia marcescens*), plant pathogen (*Erwinia carotovora*) and aquatic bacteria (*Beneckea natriegens*). At dilution level of 1:5 of *O. majorana* essential oil, a remarkable effect was detected against *Yersinia enterocolica* and *Pseudomonas aeruginosa* (agar dilution method).

In a way comparable to antifungal activity, the antibacterial effects of oregano essential oils, as experienced in *O. vulgare* ssp. *hirtum*, *O. dictamnus* and commercial Greek *Origanum* oil, were mainly due to the presence of phenolic constituents of essential oils (carvacrol and/or thymol), whilst their biosynthetic precursors γ-terpinene and *p*-cymene were inactive (Pellecuer *et al*., 1980; Gergis *et al*., 1990; Panizzi *et al*., 1993; Sivropoulou *et al*., 1996; Adam *et al*., 1998). Hence, synergistic antibacterial activities of carvacrol and thymol were reported (Didry *et al*., 1993; Sivropoulou *et al*., 1996). According to Sivropoulou and co-workers (1996) *P. aeruginosa* exhibited resistance to all three tested essential oils as well as towards the compounds tested (carvacrol, thymol, γ-terpinene, *p*-cymene), although later findings of Dorman and Deans (2000) confirmed good inhibitory effects of *O. vulgare* essential oils and of carvacrol against this G(−) bacteria.

The essential oils of *O. vulgare* ssp. *hirtum* and *O. dictamnus* were extremely bactericidal (in *S. aureus*) at 1:4000 dilution, and even at dilutions as high as 1:50000 caused considerable decrease in bacterial growth rates. Essential oils of *O. vulgare*, rich in carvacrol (49.1 per cent) and of *Thymus vulgaris* L., rich in thymol (67.3 per cent), showed approximately the same range of antibacterial efficiency (MIC: 1:2000–1:3000) against *E. coli*, *S. aureus*, *Bacillus megaterium*, *Salmonella hadar* (Remmal *et al*., 1993). Dorman and Deans (2000) have studied the effects of essential oils of *O. vulgare* ssp. *hirtum* and of *Thymus vulgaris* toward a range of G(+) and G(−) bacteria. When comparing the relative efficacy of the two species, they found that thyme was generally more effective against the majority of G(+) bacteria (with the exception of *Acinetobacter calcoacetica* and *Yersinia enterolitica*) and against all G(−) bacteria (especially toward *Alcaligenes faecalis*, *Flavobacterium suaveolens*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella pullorum* and *Serratia marcescens*), but not toward *Pseudomonas aeruginosa*. This was more sensitive to essential oil of *O. vulgare*. The same results have been obtained by isolated phenolic compounds, showing a more pronounced effect of thymol against G(+) bacteria (with the exception of *Clostridium sporogenes*, that was well inhibited by *O. vulgare* and carvacrol) or G(−) bacteria (with the exception of *Pseudomonas aeruginosa*). This might indicate that the relative

position of the hydroxyl group in the phenolic structure might contribute to the antibacterial potency of essential oil components (Dorman and Deans, 2000).

While most of the reported studies on the antimicrobial activity of oregano involved pathogenic bacteria, only a limited number of authors studied the inhibitory effects of oregano on lactic bacteria. Authors generally agree that, like all susceptible bacteria, lactic bacteria are also inhibited by *Origanum* essential oil in a concentration-dependent manner. High sensitivity of *Vibrio parahaemolyticus* was observed in media containing oregano (Beuchat, 1976). Zaika and Kissinger (1981) have found that oregano extracts were bactericidal toward lactic acid bacteria (at 8 g/l against *L. plantarum*, 4 g/l against *Pediococcus cerevisiae*), but these organisms became resistant toward the toxic effects of oregano, when sublethal concentrations (3 g/l in *L. plantarum*, 2 g/l in *P. cerevisiae*) were applied to the starter culture of bacteria. Instead of inhibition, low concentrations of oregano in culture medium stimulated the growth and production of acid production in resistant bacteria. Moreover, a phenomenon of cross-resistance of bacteria against different herb species was observed (Zaika *et al*., 1983). This means that bacteria which had acquired a resistance to one herb species (oregano) were also resistant to other herbs (sage, rosemary, thyme). So far, the mechanism by which the starter cultures acquire their resistance is not known. Kivanç and co-workers (1991) studied the effects of *Origanum onites* leaves and essential oil on growth and acid production of *L. plantarum* and *L. mesenteroides*. They found that oregano leaves (0.5 per cent, 1.0 per cent or 2.0 per cent) had no significant influence on the growth of *L. plantarum*, but after 2 days of fermentation *in vitro* they stimulated its acid production. By contrast, the growth and acid production of *L. plantarum* were strongly inhibited by *O. onites* essential oil (150, 300 or 600 ppm). When considering *Leuconostoc mesenteroides*, both the leaves and the essential oil of *O. onites* at all tested concentrations inhibited the growth and acid production.

The stimulative effects of extracts of *O. majorana* on the growth of non-lactic acid bacteria have been reported by Adlova *et al*. (1998). They observed that the phenolic fraction of water extracts of *O. majorana*, present in media at low concentration (0.0001 per cent), exhibited a stimulating effect *on E. coli* and *Streptococcus pyogenes*, but had no influence on the growth of *Corynebacterium xerosis*. Vokou and Liotiri (1999) established that essential oil of *O. vulgare* L. ssp. *hirtum*, when added to soil samples (0.1 ml per 150 g of soil) of Mediterranean ecosystems, could be used by soil bacteria as a carbon and energy source, and that it stimulated soil microbial activity. Dose-dependent bactericidal (at $\geq$1 mmol/l) or bacteriostatic (MIC = 0.75 mmol/l) effects of isolated carvacrol on the foodborne pathogen *Bacillus cereus* were detected *in vitro* (Ultee *et al*., 1998). The pH of the medium and the growth temperatures (8 °C or 30 °C) considerably influenced the bactericidal activity. Sensitivities recorded at pH 5.5 and 8.0 were two- and six-fold higher than those at pH 7.0, where the lowest sensitivity of *B. cereus* was detected. The study of the mechanism of action showed that the inhibitory effect of carvacrol was due to the interaction with membranes of *B. cereus*, changing their permeability for cations (K^+, H^+). The dissipation of the ion gradient influenced the membrane transport and led to impairment of cell essential processes and finally to cell death (Ultee *et al*., 1999). However, a considerable decrease in sensitivity of *B. cereus* against carvacrol was observed after growth in the presence of sublethal concentrations of carvacrol (0.4 mmol/l). Concomitantly, a lower membrane fluidity of adapted cells was detected, indicating the changes in the fatty acid composition and rearrangement of the phospholipid bilayer in bacterial cell membranes during adaptation process (Ultee *et al*., 2000). Pol and Smid (1999) report on the synergistic effects of carvacrol and nisin (bactericidal peptide, used

as a biopreservative in certain foods) against *Bacillus cereus* and *Listeria monocytogenes in vitro*, which resulted in a much higher sensitivity of both pathogens (at 20 °C) to combined exposure (*B. cereus*: $MIC_{combination}$ = 0.63 mmol/l carvacrol and 1.25 μg/ml nisin, *L. monocytogenes*: $MIC_{combination}$ = 1.25 mmol/l carvacrol and 0.63 μg/ml nisin) than to individual compounds (*B. cereus*: $MIC_{carvacrol}$ = 1.25 mmol/l or MIC_{nisin} = 10 μg/ml, *L. monocytogenes*: $MIC_{carvacrol}$ = 2.50 mmol/l or MIC_{nisin} = 10 μg/ml). This means that lower concentrations of both carvacrol and nisin are needed for effective decrease in the number of colony-forming units of food-borne pathogens.

Oregano essential oils have been considered as an alternative natural additive in gastronomy and in the food processing industries. It was found that *O. vulgare* essential oil was effective in inactivation of *E. coli* in concentrations as low as 0.7 per cent, acting synergistically with the pH and storage temperatures, thus contributing to the intrinsic safety of home-made eggplant salad (Skandamis and Nychas, 2000). Also, Dorman and Deans (2000) believe that volatile oils (but not spices as integral ingredients) may have the greatest potential use as food preservatives. Due to their high antimicrobial potency they could be added to foodstuffs in small quantities and would cause no loss of organoleptic properties of the food. However, when assessing the potential use of essential oils in food and the food industries, one has to consider the whole system (food, essential oil, processing, storage temperatures, marketable/sensory characteristics of the processed food) to be able accurately to judge the usable value of plant essential oils/extracts. A considerable reduction in antimicrobial activity, when plant essential oils or extracts were evaluated in food systems/actual foods, was found by several authors (Shelef *et al*., 1984; Ismaiel and Pierson, 1990b; Hao *et al*., 1998). Similar observations were reported by Aureli *et al*. (1992), who found that the high antilisteric activities (4 strains of *L. monocytogenes*, one strain of *Listeria innocua*) of *Origanum* or *Thymus* essential oil (dilution 1:5 and 1:50 in absolute ethanol) *in vitro* were significantly reduced when essential oils were tested in a meat matrix (minced pork meat). Accordingly, the leaf extract of *O. majorana* exhibited no remarkable antilisteric effect, when assayed in cooked chicken breasts (Hao *et al*., 1998). By contrast, a significant inhibitory activity of 0.8 per cent (v/w) oregano essential oil against *L. monocytogenes* on naturally contaminated beef meat fillets was observed under various packaging conditions at 5 °C (Tsigarida *et al*., 2000). Based on the findings on the sporostatic and growth inhibiting activities of *Origanum* essential oil (at 150 and 200 ppm) against food contaminant *Clostridium botulinum in vitro* (TYG: thiotone yeast extract glucose medium) (Ismaiel and Pierson, 1990a), the same authors examined the potential antibotulinal effect of *Origanum* oil in minced pork. They observed considerable diminishing of antibotulinal activity of *Origanum* oil in the meat system. *Origanum* essential oil was effective only when used at 400 ppm and in combination with 50–100 ppm of sodium nitrite, depending on the spore inocula. The absence of inhibition by oregano oil in the meat system, in contrast to TYG medium, could be due to the high solubility of the oil components in the lipid fraction of the meat. Such concentrations (≥400 ppm) of *Origanum* essential oil are questionable with regard to the possible effects on marketable characteristics of cured meat (flavour, colour, taste, and structure), although these were not assessed in the study of Ismaiel and Pierson (1990b). In view of practical implications in the preservation of food products, where the antimicrobial efficacy and sensory attributes of food have to be considered, the approach of combining different food-preservative compounds, proposed by Pol and Smid (1999), seems the most appropriate.

ANTIOXIDANT ACTIVITY

A large number of reports on the antioxidant effects of *Origanum* species have been published. A survey of the potential use of *Origanum* or oregano based preparations, that would replace synthetic substances such as BHT, as protectors of highly unsaturated lipids in foodstuffs has been made by numerous research groups. However, limited industrial applications are often ascribed to the characteristic oregano aroma and flavour, that influence the sensorial characteristics of processed food, so deodorization steps would be required (Nguyen *et al*., 1991; Moure *et al*., 2001). Dietary supplies of antioxidants from *Origanum* species were also considered as effective scavengers of the free radicals that are generated by metabolic pathways in the body, and in sufficient amounts prevent cellular damages and human diseases.

In these studies different methods, test/model systems (lard, bulk oils, emulsions, meat products, human cells as oxidation substrates), different plant preparations (whole spices, essential oils, hydrophilic or hydrophobic extracts, isolated phenolic compounds, etc.) have been used in the quantification of antioxidative potential (Madsen and Bertelsen, 1995; Madsen *et al*., 1997; Pearson *et al*., 1997; Moure *et al*., 2001). Comparison between the different reports, which is often difficult due to a high variability in experimental design, mostly refer to the different potency of antioxidant activity of tested *Origanum* species or their compounds. This is why several authors (Laughton *et al*., 1989; Frankel *et al*., 1994; Pearson *et al*., 1997) claim that a variety of testing systems is required when assessing the antioxidant potential of a substance, since a substance exhibiting high antioxidant activity in one system may have a prooxidant effect in another system. In the current literature, relatively little information is available on mechanisms of the antioxidative action, although phenolic compounds are most frequently cited as active ingredients, responsible for the antioxidant effect (Madsen *et al*., 1997; Moure *et al*., 2001). Comparison of the antioxidant activity of model phenolic compounds has shown that polymeric phenolic compounds are generally more potent antioxidants than simple monomeric phenolics (Moure *et al*., 2001). Yamaguchi *et al*. (1999) observed that the degree of polymerisation of flavanols correlates with the superoxide-scavenging capacity. The antioxidant activity relies also on the polarity of tested compounds, depending on the type and polarity of the extracting solvent. It was found that hydrophilic antioxidants are generally more effective in bulk oil, whereas lipophilic antioxidants exhibit more potent effects in emulsions ('polar paradox' phenomenon) (Moure *et al*., 2001).

Investigations on the antioxidative activity of herbs and spices date about 40 years ago, when Chipault with co-workers (Chipault *et al*., 1952; Chipault *et al*., 1955; Chipault *et al*., 1956) screened the effects of 32 different spices in various model systems/substrates, measuring their persistence with antioxidant index (AI). This was defined as a ratio between substrate, containing herb ingredient, and substrate without herb addition. In order to investigate the stabilising capacity of *O. vulgare* in different substrates, these were exposed to autooxidation at substrate-specific temperature regimes: lard (at 99 °C), egg yolk (at 63 °C), oil in water emulsion (at 40 °C), minced pork (at −5 °C) and mayonnaise (at 20 °C). In all tested substrates dry oregano (at concentration of 0.1 per cent in o/w emulsion, at 0.25 per cent in minced pork and at 0.2 per cent in all other substrates) displayed high antioxidant activity, the AI being between 2.7 (egg yolk) and 8.5 (mayonnaise). The length of the induction period in autooxidation of lard, which was used as an indicator of antioxidative potency, showed a higher antioxidant potency of dry oregano

(*O. vulgare*) when compared with that of marjoram (*O. majorana*), although Saito *et al*. (1976) had found marjoram to be a more potent antioxidant herb than oregano at the same concentration tested (Gerhardt and Schröter, 1983).

Methanol extracts of *O. vulgare* and of *O. majorana* exhibited strong hydroxyl radical-scavenging activity, inhibiting the oxidation of 2-deoxyribose by more than 50 per cent at the 1 μg/ml concentration, however, the scavenging effect was much less evident when the antioxidant potential was measured by benzoic acid hydroxylation method (Chung *et al*., 1997). By contrast with the high antioxidant activity of *O. vulgare* methanol extracts, as observed in the lard test system (Herrmann *et al*., 1981; Banias *et al*., 1992), oregano and marjoram extracts showed only scarce antioxidant effect in the β-carotene model system (Dapkevicius *et al*., 1998).

Essential oil of *O. vulgare* showed the ability to form stable free radicals upon reaction with potassium superoxide (Deighton *et al*., 1993). The essential oil monophenols, carvacrol and thymol, were identified as molecules which react with the superoxide anion (O_2^-), probably through hydrogen atom donation, and form stable paramagnetic species (free radicals) as found by EPR spectroscopy.

Origanum vulgare essential oil (Italian origin) demonstrated protective antioxidant properties in an egg yolk assay. At high concentrations (1000 ppm and 750 ppm), the antioxidative potency of oregano oil was higher than those of butylated hydroxytoluene (BHT) or of α-tocopherol. However, in a rat liver assay the antioxidative effect of oregano essential oil was much lower, at 750 ppm exhibiting the same range of antioxidant activity as α-tocopherol at 250 ppm (Baratta *et al*., 1998a). Similar results were observed by Lagouri *et al*. (1993), who studied the antioxidative potency of *O. vulgare* L. ssp. *hirtum* Ietswaart and *Origanum onites* L. essential oils (at 1000 ppm) by measuring the autooxidation rate (peroxide value) of lard stored at 35 °C. The antioxidant activities of essential oils were attributed to their high phenol moiety (carvacrol and thymol) and were comparable to BHT (at 200 ppm). Still more potent activity was observed with *O. majorana* essential oil, the antioxidant activity being much higher than that of α-tocopherol and comparable to that of BHT at all concentration levels (100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm) (Baratta *et al*., 1998b).

A survey of the antioxidant activity of methanol extracts of *Origanum* species of Greek origin showed that *O. vulgare*, *O. dictamnus* and *O. majorana* at concentration of 0.02 per cent significantly prolonged the induction period of lard autooxidation at 75 °C and slightly decreased the rate of peroxide formation. However, their relative antioxidant efficiencies (oregano > dittany > marjoram) in comparison to those of BHT or rosemary extracts were much lower (Economou *et al*., 1991).

Turkish oregano (*O. vulgare* L.) and Chilean oregano (*Origanum onites* L.) showed high antioxidative effects, measured by the oxygen depletion method as well as by EPR spectroscopy. The study of oregano water extracts, using both methods, allowed Madsen and co-workers (1996) to find that the antioxidant activity of oregano was due to at least two different antioxidative mechanisms. The activity might be due both to the non-phenolic group of compounds and to the phenolic group. The group of non-phenolic compounds act as scavengers of free radicals and are effective in early stages of oxidation. The group of phenolic compounds were effective in interrupting the chain processes responsible for oxygen consumption by a mechanism similar to that for tocopherols. Among the phenolic compounds that have been isolated from oregano, there were isolated at least five different groups of substances which were highly antioxidative active (rosmarinic acid, water soluble phenolic glycosides, flavonoids, carvacrol and

thymol) (Madsen and Bertelsen, 1995). The ESR spin-trapping assay showed that the free radical scavenging capacity of the *O. dictamnus* water and methanol extracts correlated with the content of phenolics. When compared to acetone or ethanol extracts (poor in phenolics), the aqueous and methanol extracts (rich in phenolic compounds) were also the most effective in reducing oxygen consumption and thus had high chain-breaking properties, as evidenced by the oxygen depletion assay (Møller *et al*., 1999). The ability of aqueous extracts of *O. dictamnus* to inhibit development of secondary lipid oxidation products was also confirmed in model food systems (turkey thigh meat homogenate), where dittany dose-dependently (from 0.0018 mg dittany/g meat upwards) inhibited development of thiobarbituric reactive substances.

Vekiari *et al*. (1993a) have studied the extracts of *O. vulgare* of different polarity, to find the active compounds responsible for the antioxidative effect of oregano. The main antioxidant factor of the non-polar hexane extract was isolated by repeated fractionations, and consisted mainly of terpene derivatives. Among polar compounds that were extracted from *O. vulgare* leaves, the most effective in stabilising lard against oxidation, with potency equal to BHT, were flavonoids (flavanone eriodictyol, the dihydroflavonols dihydrokaempferol and dihydroquercetin and flavone apigenine) (Vekiari *et al*., 1993b). These compounds also showed marked antioxidant activity when tested on vegetable oils (corn, soybean and olive) under storage or frying conditions.

Nakatani (1997) reports that both the polar and non-polar fractions of oregano leaves significantly retarded oxidation of linoleic acid, measured by the ferric thiocianate (FTC) and thiobarbituric acid (TBA) methods. From a water-soluble fraction of methanol extracts, phenolic compounds with high antioxidant activities have been purified, the most potent being derivative of rosmarinic acid (2-caffeoyloxy-3-[2-(4-hydroxybenzyl)-4,5-dihydroxy] phenylpropionic acid and a new glycoside of protocatechuic acid ester, identified as 4-(3,4-dihydroxybenzoyloxymethyl)phenyl-β-D-glucopyranoside (Nakatani and Kikuzaki, 1987; Kikuzaki and Nakatani, 1989; Nakatani, 1992). These were more effective against linoleic acid oxidation than the natural α-tocopherol, and are comparable to the synthetic antioxidants, BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene). Similar polyphenolic compounds were found in *O. majorana* leaves, but these also contain compounds such as 6-*O*-4-hydoxybenzoyl arbutin and 2-hydroxy-3-(3,4-dihydroxyphenyl)propionic acid, which possess moderate antioxidant activity (Nakatani, 1997). These findings are in agreement with Herrmann (1994), who observed that antioxidant active plant phenols often possess a 3,4-dihydroxybenzoyl- or 3-methoxy-4-hydroxybenzoyl group in their structure. Lamaison and co-workers (Lamaison *et al*., 1990; Lamaison *et al*., 1991; Lamaison *et al*., 1993), who extensively studied the antioxidant activity of members of Lamiaceae, reported that the content of rosmarinic acid and of total hydroxycinnamic derivatives in hydroalcoholic extracts of *Origanum* taxa (*O. onites*, *O. tytthantum*, *O. vulgare* ssp. *hirtum*) was only partly correlated with their antioxidant effect, estimated by measuring the free radical scavenger effect on DPPH (1,1-diphenyl-2-picrylhydrazyl). They stressed the importance of flavonoid content for oregano antioxidant activity.

The antioxidant activity of isolated carvacrol and thymol in liposomal systems was confirmed by Aeschbach *et al*. (1994), and in biological systems (human aortic endothelial cells, HAEC) by Pearson *et al*. (1997). The potency of antioxidant activity of thymol ($ID_{50} = 4.02$ μM), measured as per cent of inhibition of HAEC-mediated human LDL oxidation, was significantly higher than that of carvacrol ($ID_{50} = 5.53$ μM), but it was

found that both monophenols (thymol or carvacrol) had much lower antioxidant activities than rosmarinic acid (ID_{50} = 0.74 μM) (Pearson *et al*., 1997).

It was also found that combinations of spices or compounds with high antioxidant activities exhibited synergistic antioxidant effects, which would practically result in a better protection of foods from oxidation (Madsen *et al*., 1996). However, no practically important synergistic effects were observed, when *O. vulgare* methanol extracts – which were highly effective in stabilising lard (at concentration 0.02 per cent) stored at 75 °C – were mixed in lard with less potent antioxidants (methanol extracts of thyme, marjoram, spearmint, basil) (Economou *et al*., 1991).

In the study of Banias *et al*. (1992), combinations of methanol extracts of oregano, dittany or marjoram with primary antioxidants were used in the lard autooxidation process. The results showed that significant positive synergism in antioxidant activity existed in combinations of oregano or marjoram (0.1 per cent) with BHT (0.005 per cent) and in a combination of dittany (0.1 per cent) with ascorbyl palmitate (0.01 per cent). By contrast, high negative synergism was observed in combinations of oregano (0.1 per cent) or marjoram (0.1 per cent) with propyl gallate (PG) (0.01 per cent) and in a combination of oregano (0.1 per cent) or marjoram (0.1 per cent) with α-tocopherol. Milos *et al*. (2000) have studied the antioxidant activity of volatile aglycons, that are bound glycosidically in dry *O. vulgare* plants, in comparison to that of essential oil. Although, the total content of volatile aglycons in plant material (0.002 per cent) was significantly lower than the content of essential oil (2.9 per cent), a mixture of volatile aglycons (thymoquinone, benzyl alcohol, eugenol, thymol, carvacrol) showed similar antioxidant activity to that of essential oil. They inhibited hydroperoxide formation in lard stored at 60 °C even after 80 days and were significantly more effective than α-tocopherol. Thymoquinone, which was found to be a potent inhibitor of membrane lipid peroxidation (Houghton *et al*., 1995; Jerkovic *et al*., 2001) and the major component (40.2 per cent) among aglycons (Milos *et al*., 2000), as well as pure thymol as the major component (40.4 per cent) of essential oil, were much less active than a mixture of aglycons or essential oil of *O. vulgare*. These results indicated the importance of mixtures and their synergistic power in the antioxidant activity of *O. vulgare*.

In addition to numerous studies, where potent or moderate antioxidant effects of oregano were established in theoretic model systems, practical considerations on the use of oregano as stabilisers of edible oils (vegetable or fish oils) or of finished meat products have been made by several research groups. Generally, authors confirm the protective role of different *Origanum* taxa (*O. vulgare*, *O. compactum*, *O. majorana*) against the autooxidation process over time, although the potencies of oregano antioxidative effects are lower than those reported for rosemary or sage (Özcan and Akgül, 1995; Antoun and Tsimidou, 1997). Özcan and Akgül (1995) studied the antioxidant effects of methanol extracts and essential oils of numerous Turkish spices on sunflower oil, stored at 70 °C, and found that methanol extracts (including *O. vulgare* and *O. majorana*) exhibited higher antioxidant activity compared with essential oils. The increased delay in the onset of autooxidation might be due to the improved preservation of α-tocopherol, an internal antioxidative microcomponent principle of sunflower oil (Yanishlieva and Marinova, 1998; Beddows *et al*., 2000). When compared to hexane and ethyl acetate extracts, the ethanol extracts of several species of the *Lamiaceae* family were the most active in retarding the autooxidation process of sunflower oil exposed to 100 °C. However, *O. vulgare* ethanol extracts (at 0.08 per cent) showed only low antioxidant effect comparable to that of 0.02 per cent BHT (Yanishlieva and Marinova,

1995), or else did not improve the oxidation stability of sunflower oil, as is evident from the later study by Marinova and Yanishlieva (1997). Only a moderate stabilising effect of *O. vulgare* leaves in sunflower oil, exposed to autooxidation at room temperature, was observed also by de Felice *et al*. (1993), who measured the quality characteristics of the oil in the time period of 16 weeks.

By contrast, ground oregano (*O. vulgare*) inhibited lipid oxidation of fish/mackerel (*Scomber scombrus*) oil stored at 40° in dark at concentrations of 0.5 per cent and at 1 per cent as effectively as 200 ppm BHA and 200 ppm TBHQ (tertiary butylhydroquinone), respectively (Tsimidou *et al*., 1995). Dry leaves of *O. vulgare* ssp. *hirtum* (at concentration of 2 per cent) or essential oil of *O. compactum* (at concentrations 0.05 per cent and 0.1 per cent) also showed a high antioxidant activity in olive oil and, besides their stabilising effect, the organoleptic quality of the olive oil was significantly improved by addition of oregano, as assessed by Mediterranean consumer acceptability studies (Antoun and Tsimidou, 1997; Charai *et al*., 1999). A significant increase in the oxidative stability of fried chips, measured as the rate of peroxide formation during storage at 63 °C, was achieved both by addition of ground *O. vulgare* (1 per cent, after frying) or its petroleum ether extracts (1.1 per cent, before frying) (Lolos *et al*., 1999). The oregano antioxidant activity was almost as effective as that of TBHQ up to 6 days of observation, although the peroxide value of cottonseed oil, extracted from oregano-treated potato chips increased after one week. However, the results of this study indicated that ground oregano or its extract might be used to extend the storage life of potato chips as they decrease the oxidative deterioration of the oil absorbed into the chips.

The importance of the testing substrate (bulk oil or o/w emulsion) in the evaluation of oregano antioxidant potency has been shown in the study of Abdalla and Roozen (1999), who found that acetone extract of *O. vulgare* effectively inhibited the autooxidation process of sunflower oil at both 600 ppm and 1200 ppm, but exhibited only moderate antioxidant activity when tested in a 20 per cent sunflower o/w emulsion. It has been also shown that oregano extracts acted as pro-oxidants in both oil and emulsions, when exposed to light (Abdalla *et al*., 1999).

When assessing the antioxidative potential of *O. majorana* or *O. vulgare* in preventing rancidity of meat products, only limited practical significance has been documented. El-Alim *et al*. (1999) report that *O. majorana* and *O. vulgare*, and especially their ethanol extracts, had a strong antioxidant activity, inhibiting lipid peroxidation both in fresh chicken meat as well as in heat-treated pork. Because of their shelf time prolonging properties, oregano and oregano- based preparations have been recommended for use in semi-prepared meat products. However, other studies show less optimistic results. Ground *O. majorana* was added at a concentration of 0.2 per cent to the laboratory and industrial prepared sausage model systems, that were exposed to ripening. Only a low antioxidative effect was observed on the basis of the redox potential reduction of the marjoram-supplemented model compared to the control (Palic *et al*., 1993). Korczak and co-workers (1988) have found relative low antioxidant efficacy of *O. majorana* (at 0.5 per cent) in minced meat model systems when compared to those of rosemary or sage. Hence, the pro-oxidising activity of marjoram, which is probably influenced by elevated temperature, diminishes its practical value as a natural additive in meat processing (Korczak *et al*., 1988).

The antioxidative effects of *O. vulgare* drug plant (*Origani herba*) have been studied in the light of both direct use as stabilisers of fat and, indirectly, as feed additives in order to improve the shelf-life of meat and fat-containing food (Vichi *et al*., 2001).

In contrast with the significant antioxidative and stabilising effects of oregano extracts in lard (measured by photochemiluminescence), no effect on the quality or shelf life of the fat obtained from animals fed with oregano additives was observed.

ANALGESIC, ANTIINFLAMMATORY AND ANTISPASMODIC ACTIVITY

Carvacrol-rich (67 per cent) essential oil of *Origanum onites*, collected at the Izmir locality (Turkey), showed a marked analgesic activity as assessed by the tail-flick method in male albino mice. The analgesic activity of *O. onites* essential oil was dose-dependent. When applied at 0.33 ml/kg, the activity of *O. onites* oil was comparable to that of morphine (applied at 1 mg/kg), but at 0.03 ml/kg more potent than the analgesic activity of fenoprofen (at 8 mg/kg) (Aydin *et al*., 1996). The sample of *O. onites*, that originated from the Turkish Antalya region and was found to contain linalool (91 per cent) as a major component, showed no analgesic effects. On the basis of these data and on reports on prostaglandin inhibitory effects of carvacrol (Wagner *et al*., 1986), Aydin and co-workers consider carvacrol content as related to the analgesic activity of essential oil of *O. onites*. The effects of methanol extracts of *O. majorana* on human platelet antiaggregant activity, which is related to the well known mechanism of action of NSAID (non-steroid anti-inflammatory drugs) through inhibition of the prostaglandins' metabolic pathway, have been studied by Okazaki *et al*. (1998). They found that *O. majorana* extracts dose-dependently inhibited platelet aggregation induced by collagen (2.0 μg/ml) or ADP (2.0 μg/ml). Successive fractionation of methanol extracts leads to isolation of an active hydroquinon β-D-glucopyranoside, identified as arbutin. This strongly inhibited platelet aggregation was induced by all tested stimulating agents (collagen, ADP, arachidonic acid, thrombin).

Only a few reports on the topical anti-inflammatory effects of *O. vulgare* refer to oregano-herbal mixtures or their decoctions, used in the treatment of inflammation as supporting therapies (Deryabin, 1990; Deryabin, 1991). Podkolzin *et al*. (1986) report on the favourable local effect of insufflation of fine powder mixture of *Hypericum perforatum* and *O. vulgare* (1:1) on the course of rhinitis, that was induced in an animal (rabbit) experiment. In the control animals the rhinitis symptoms were more pronounced and of longer duration, so the powder was proposed as an adjuvant therapy in treatment of rhinitis.

Origanum compactum Benth., a species native to North Africa and locally named '*za'atar*', was used traditionally against affections of the respiratory organs as an antispasmodic and anticatarrhal drug and, especially in Morocco, as a spasmolytic drug in the gastrointestinal tract, as antacid, antidiarrhoeal agent, vermifuge and aphrodisiac (van den Brouke and Lemli, 1980; Bellakhdar *et al*., 1988; Hmamouchi *et al*., 2000). In order to scientifically validate the traditional medicine data, van den Brouke and Lemli (1980) surveyed extracts of *O. compactum* on antispasmodic effects in different smooth muscle preparations *in vitro*. It was found that water macerates of *O. compactum* significantly inhibited smooth muscle response induced by any of the tested spasmogens (acetylcholine, histamine, serotonine, $BaCl_2$, nicotine . . .) in the guinea-pig ileum. The structure–activity relationship revealed that the antispasmodic effect of *O. compactum* was almost completely explained by its essential oil content. Moreover, in the pharmacological inhibition of smooth muscular activity, non-specific and non-competitive mechanism of action was attributed to thymol and carvacrol: they caused

both direct musculotropic (muscle relaxant activity) and indirect neurotropic action (inhibition of the nerve action potential) on the smooth muscle (van den Brouke and Lemli, 1980). The same results were obtained by testing the antispasmodic effects of pure active components, i.e. thymol ($ED_{50\ per\ cent} = 0.86 \times 10^{-4}$ M) and carvacrol ($ED_{50\ per\ cent} = 1.0 \times 10^{-4}$ M). It was concluded that both phenols act as non-competitive Ca^{2+} antagonists, which block nerve fibre conduction and induce musculotropic and neurotropic spasmolyse (van den Brouke and Lemli, 1982).

IMMUNOSTIMULANT, ANTIMUTAGENIC AND ANTICANCER ACTIVITY

Some studies have shown that oregano extracts or herbal mixtures with *Origanum* spp. possess *in vitro* antiviral activity or have immunostimulating effects both *in vitro* and *in vivo*. However, little knowledge has been attained so far on mechanisms of immunomodulating activity or underlying active compounds. It has been shown that ethanol extracts of *O. vulgare* inhibited intracellular propagation of $ECHO_9$ Hill virus and also showed interferon inducing activity *in vitro* (Skwarek *et al*., 1994). Flavonoid luteoline, a constituent of *Origani herba*, has been considered as responsible for the induction of an interferon-like substance. A mixture of herbal preparation containing rosemary, sage, thyme and oregano (*O. vulgare*) showed radical scavenging activity and inhibition of the human immunodeficiency virus (HIV) infection at very low concentrations (Aruoma *et al*., 1996). It was suggested that the main active compounds of herbal preparations were carnosol, carnosic acid, carvacrol and thymol. Significant inhibitory effects of *O. vulgare* extracts against HIV-1 induced cytopathogenicity in MT-4 cells were also observed by Yamasaki *et al*. (1998). According to Krukowski *et al*. (1998), an increase in immunoglobulin (IgG) levels was observed in reared calves, fed with a conventional concentrate supplemented by a mineral-herbal mixture containing *O. majorana*.

A strong and dose-dependent capacity of inactivating dietary mutagen Trp-P-1 in the *Salmonella typhimurium* TA 98 assay was observed in *O. vulgare* water extracts, that exhibited significant antimutagenic effects *in vitro* (Ueda *et al*., 1991). *Origanum majorana* aqueous extracts were also able to suppress the mutagenicity of liver-specific carcinogen Trp-P-2 (Natake *et al*., 1989). When studying the mechanism of suppressing the mutagenicity of Trp-P-2 in *O. vulgare*, it was found that two flavonoids, galangin and quercetin acted as Trp-P-2 specific desmutagens, which neutralised this mutagen during or before mutating the bacteria (*Salmonella typhimurium* TA 98) (Kanazawa *et al*., 1995). The amounts of galangin and quercetin required for 50 per cent inhibition (IC_{50}) against 20 ng of Trp-P-2 were 0.12 µg and 0.81 µg, respectively. It was also found that quercetin acted as a mutagen at high concentrations (>10 µg/plate), but was a desmutagen when applied at low (>0.1 <10 µg/plate) concentrations. Milic and Milic (1998) have found that isolated phenolic compounds from different spice plants, including *O. vulgare*, strongly inhibited pyrazine cation free radical formation in the Maillard reaction and the formation of mutagenic and carcinogenic amino-imidazoazarene in creatinine containing model systems.

In a literature survey, referring to the anticancer activity of *Origanum* genus, different approaches, testing systems and cell lines have been used by different authors when assessing the carcinogenic potential of plants or their isolated compounds. However, there are no available data on practical/clinical use of oregano in cancer prevention. In 1966

an international project was performed with the aim of screening the native plants of former Yugoslavia for their potential agricultural use in the USA and Yugoslavia (Mayer *et al*., 1971). In the frame of this project 1466 samples of 754 plant species were analysed for chemical and antitumour activity. According to the results of the Cancer Chemotherapy National Service Center Screening Laboratories (Washington, DC) a high carvacrol (60–85 per cent) containing *O. heracleoticum* (= *O. vulgare* spp. *hirtum* (Link) Ietswaart) was reported to show high antitumour activity. Lam and Zheng (1991) have found that essential oil of *O. vulgare* fed to mice, induced the activity of glutathione *S*-transferase (GST) in various tissues. The GST enzyme system is involved in detoxification of chemical carcinogens and plays an important role in prevention of carcinogenesis, what would explain the anticancer potential of *O. vulgare* essential oil. This oil exhibited high levels of cytotoxicity (at dilutions of up to 1:10000) against four permanent eukaryotic cell lines including two derived from human cancers (epidermoid larynx carcinoma: Hep-2 and epitheloid cervix carcinoma: HeLa) (Sivropoulou *et al*., 1996). Other studies, that refer to *in vitro* cytotoxic and/or anti-proliferative effects of *O. vulgare* extracts or isolated compounds (carvacrol, thymol) include those of Bocharova *et al*. (1999) and He *et al*. (1997), who observed moderate suppressing activities of *O. vulgare* extracts (CE_{50} = 220 mg/ml) on human ovarian carcinoma cells (CaOv), or of isolated carvacrol and thymol (IC_{50} = 120 μmol/l) on Murine B 16(F10) melanoma cells – a tumour cell line with high metastatic potential.

Antitumour-promoting activity or *in vitro* cytotoxic effects towards different tumour cell lines were attributed also to *O. majorana* extracts or their constituents (Assaf *et al*., 1987; Okuyama *et al*., 1995; Hirobe *et al*., 1998). When studying cytotoxic activity of *O. majorana* water–alcoholic extracts and of isolated compounds (arbutin, methylarbutin and their aglycons – hydroquinone and hydroquinone monomethyl ether) towards cultured rat hepatoma cells (HTC line), a high dose-dependent HTC cytotoxicity of hydroquinone was observed, whilst arbutin was not active (Assaf *et al*., 1987). At 300 μM hydroquinone caused 40 per cent cellular mortality after 24 h of incubation, but no cells remained viable after 72 h. It has been established that this well known antiseptic of the urinary tract was a more potent cytotoxic compound towards rat hepatoma cells than many classic antitumour agents like azauridin or colchicin, but less than valtrate, a monoterpenic ester of *Valeriana* spp.

INSECT-POLLINATING AND ANTIPARASITIC ACTIVITY

Origanum taxa, especially those that are rich in essential oils, have been extensively studied for their insect-pollinating (Ricciurdelli d'Albore, 1983; Beker *et al*., 1989) or nectar yielding (Kucherov and Siraeva, 1981; Jovančević *et al*., 1984; Jablonski, 1986) effects. Although scientifically poorly understood, the traditional knowledge on attracting effects *Origanum* spp. for pollinating insects, especially honeybee (*Apis mellifera*), has been practically exploited since 1877, when the idea of culturing the bee forages with additional but non-marketable values was born (Ayers and Ayers, 1997). Interesting findings, that reveal the very complex mechanism underlying the communication between insects and attracting plants, were reported by Beker *et al*. (1989). They have observed that honeybees are capable of discriminating between different blends of odours and behave selectively to different parts (leaves, inflorescence) or chemotype (thymol, carvacrol) of *O. syriacum* due to perceiving distinct olfactory stimuli. It was assumed that

the aroma blend from the whole plant serves as a long-distance olfactory cue, while the final short-range orientation is dependent on floral odour signals.

Observations from studies on *Origanum* benefit effects in parasite-control in pollinating insects show promising results (Abou Zaid *et al*., 1987; Mazeed, 1987; Kraus *et al*., 1994; Gal, 1997; Long *et al*., 1997), although some authors were sceptical towards the practical significance of essential oils in treatment of parasite infestation (Koeniger, 1991). In laboratory tests *Origanum* oils showed a high acaricidal effect (80–90 per cent mortality) on *Varroa jacobsoni*. Under the subtropical climatic conditions of Israel, a high mortality (85 per cent and 91 per cent) of *Varroa* mites was observed after spring treatment with 20 per cent and 33 per cent oregano oil impregnated in cardboard, respectively. *Origanum* treatment in autumn as well as the use of pure origanum oil during summer was harmful to the bee colonies (Gal *et al*., 1992; Lensky *et al*., 1996). The use of essential oil of *O. majorana* in treatment of *V. jacobsoni* has attracted much practical attention from several authors. A combination of formic acid and of essential oil of marjoram has been shown to be very effective in treatment of *Varroa* mites both in laboratory trials and in field experiments (Long *et al*., 1997). In field experiments, that were carried out under tropical (Vietnam) and temperate (Germany) climatic conditions, formic acid was applied to a tray covered by gauze and placed on the bottom board of the hive while *O. majorana* essential oil was applied to two wood pieces (1.5 ml per piece), that were placed on the top bars of the combs. The combination of essential oil and formic acid, applied at 15 per cent concentration, resulted in 96.24–99.68 per cent mite mortality in tropical climate and in 97.56–99.92 per cent in temperate climatic conditions. Due to the relatively low concentrations of formic acid this combination did not affect bee mortality and was proposed as a promising practical method in the control of *V. jacobsoni*. The highly significant repellent activity and antiparasitic effects of essential oil of marjoram were observed towards *Varroa* mites, which were exposed to test wax tubes with incorporated essential oil at 0.1 per cent and 1 per cent (Kraus *et al*., 1994). These concentrations of *O. majorana* essential oil were not noxious to honeybees.

Effective antiparasitic activity was observed also when essential oil of *O. majorana* was sprayed onto bees in colonies infested with *V. jacobsoni* at concentration (100 ppm), that was found non toxic to bees (Fathy and Fouly, 1997). *Origanum majorana* essential oil has been shown as a potent acaricidal agent against *Acarapis woodi* (Renie), the acarine disease-causing parasite that invades the tracheal system of the honeybees during winter and early spring. Infestation percentage in the bee colonies, treated with *O. majorana* essential oil (10 drops of oil per piece of cotton wool in a Petri dish, that was put under the combs of infested colonies) was significantly reduced already after 15 days of treatment, and after 30 days of treatment no infestation was found among the tested bees (Abou Zaid *et al*., 1987; Mazeed, 1987).

In respect of the control of human parasites or parasite-related diseases both *in vitro* and *in vivo* studies were conducted. *O. vulgare* essential oil was studied for its *in vitro* antimalarial activity on *Plasmodium falciparum* (Milhau *et al*., 1997). It displayed only moderate (IC_{50} = 516 μg/ml after 24 h and 355 μg/ml after 72 h) antiparasitic effects against chloroquine resistant strains of *Plasmodium falciparum* when compared to the more active oils of *Rosmarinus officinalis* or *Myrtus communis* (IC_{50} = 267 μg/ml after 24 h and 149 μg/ml after 72 h). However, the observed efficacy of the tested oils against both chloroquine-resistant and -sensitive strains allowed it to be deduced that the oils could interfere with *P. falciparum* growth by different mechanisms than chloroquine. This preliminary screening of activity together with concomitant analysis of essential

oils set the direction toward selection of major components, like carvacrol, that were proposed for future investigations for antimalarial potential.

A clinical study, done by Force *et al*. (2000), showed that emulsified *O. vulgare* diet (600 mg daily) for 6 weeks considerably affected the enteric parasites (*Blastocystis hominis*, *Entamoeba hartmanni*, *Endolimax nana*) and significantly improved the gastrointestinal symptoms in seven of 11 patients, who were positive for *Blastocystis hominis*.

INSECTICIDAL, NEMATICIDAL AND MOLLUSCICIDAL ACTIVITY

Higher plants, especially medicinal and aromatic plants (MAP), are a potential source of new insecticides, and many research groups are trying to prove their activity against noxious pests. Some natural compounds, isolated from these MAP (such as rotenon, pyretrins, and azadirachtin) are already commercially available on the market. A range of active compounds, including terpenoids, flavonoids, tannins, essential oils or their components (like carvacrol), that are present in *O. vulgare* in relative high amounts, were considered as a potential source of natural biocides (Duke, 1992). Investigations on the activity of aromatic plants against stored product- or plant-noxious pests gave diverse results when considering different insect species, their developmental stages (eggs and adults) or the way of application (fumigant, contact) (Regnault-Roger and Hamraoui, 1993a; Shaaya *et al*., 1993; Regnault-Roger and Hamraoui, 1995; Kalinović *et al*., 1997; Mateeva *et al*., 1997; Rakowski and Ignatowicz, 1997; Baricevic *et al*., 2001). Generally, plants or their essential oils showed more potent activities when applied directly to the insect surface than after fumigant application. Among different aromatic species, the plant essential oils from the Lamiaceae family have the best insecticidal effects against bean weevil *A. canthoscelides obtectus* (Regnault-Roger and Hamraoui, 1993a). Oregano (*O. vulgare* L.) is one of the plants, used traditionally in southern France, to control bean weevil (*A. obtectus* Say) in stored kidney beans (*Phaseolus vulgaris* L.) (Regnault and Hamraoui, 1993b; Bernath and Badulosi, 1997). A high carvacrol containing *O. vulgare* ssp. *hirtum* essential oil showed both fumigant and contact toxicities to bean weevil (*A. obtectus* Say) in laboratory trials (Baricevic *et al*., 2001). When considering fumigant toxicity, insecticidal effect (mortality rate 82.5 per cent) was observed 6 days after application of high concentrations of oregano essential oil (150 µl per 55 g of beans). When considering contact toxicity, both oregano drug plant and essential oil at all tested concentrations significantly increased the bean weevils' mortality rates in comparison to the controls. Essential oils (5 µl, 15 µl and 30 µl per 55 g of beans) induced 100 per cent mortality of the bean weevil population when applied directly to the surface of the beans (55 g) in Petri dishes. Also, egg laying and hatching was inhibited after treatment of bean weevil with powdered drug plant (0.33 g, 0.66 g, 1.0 g and 2.0 g) or with essential oil at all tested concentrations.

O. vulgare L. susbsp. *hirtum* essential oil with high carvacrol content showed insecticidal activity also against *Drosophila melanogaster* (Karpouhtsis *et al*., 1998) and a strong ovicidal activity against the eggs of stored product insects *Tribolium confusum* and *Ephestia cautella* (Shaaya *et al*., 1993) already at low concentrations (2 µl/l or 4 µl/l air), but showed very low fumigant toxicity against adult insects (*Tribolium confusum*, *Tribolium castaneum*, *Ephestia cautella*, *Sitophilus oryzae*) (Shaaya *et al*., 1993; Shaaya *et al*., 1997). The exposure to vapours of essential oil from *O. syriacum* var. *bevanii* Ietswaart resulted in 77 per cent and 89 per cent mortality of the eggs of the confused flour beetle (*Tribolium*

confusum) and the Mediterranean flour moth (*Ephestia kuehniella*), respectively (Tunç *et al*., 2000). This oil (1 µl/l air) also showed high fumigant toxicity against females of two greenhouse pests, i.e. carmine spider mite (*Tetranychus cinnabarinus*) and cotton aphid (*Aphis gossypii*) (100 per cent mortality after 48 h and 96 h exposure, respectively) (Tunç and Şahinkaya, 1998). By contrast, only limited insecticidal potential of essential oils of *Origanum creticum*, which showed only moderate contact toxicity ($LD_{>90}$ = 100 µg/larva) against tobacco cutworm (*Spodoptera litura*), was reported by Isman *et al*. (2001).

Origanum vulgare essential oil (15 per cent) was also tested for its repellent activity against *Culicoides imicola* Kieffer, the vector of the African horse sickness virus. When applied at concentration of 4 mg/m^2 on farm animals (horses), essential oil showed only non-significant repellency for 2 h and was far less effective than synthetic repellent di-ethyl toluamide (DEET), and was not recommended for its use in order to prevent the spread of *Culicoides*-borne pathogens (Braverman and Chizov-Ginzburg, 1997, 1998).

Toxicity and resistancy toward nematodes count as important attributes of aromatic plants, that offer new applications in the field of plant health care programmes of sensitive crops, especially when nematicides or resistant cultivars are not available. A high level of resistancy against infestation with root-knot nematode (*Meloidogyne inconita* Chitwood) was observed in *O. vulgare* and *O. majorana* plants, which were free of root galls even after exposure to initial nematode populations of 15 eggs/cm^3 of soil medium in greenhouse experimental conditions (Walker, 1995). However, the root-knot nematodes caused a significant decrease in dry weight of *O. vulgare* but not that of *O. majorana*. Essential oil of *O. majorana*, with terpinen-4-ol (41.6 per cent) as the major compound, affected the soil stages of phytonematodes (*Rotylenchulus reniformis*, *Criconemella* spp., *Hoplolaimus* spp.) and inhibited more than 80 per cent of *Meloidogyne incognita* juvenile hatching compared to about 3.5 per cent at the control (Abd-Elgawad and Omer, 1995). Laboratory trials carried out with *O. vulgare*, *O. majorana* and *O. syriacum* leaf extracts or essential oils showed, that these plants considerably affected the spread of *Meloidogyne* nematodes, either by inhibition of egg hatching (Ramraj *et al*., 1991; Oka *et al*., 2000) or by immobilisation and exhibiting toxicity to nematode juveniles (Hashim *et al*., 1999; Oka *et al*., 2000). The toxicity increased with increasing concentration and exposure time. *Origanum* extracts or essential oils showed protective effects against root galling also when applied to nematode-sensitive crops. Alagumalai *et al*. (1997) observed that water extracts of *O. vulgare* dose-dependently diminished the population of *M. incognita* around chickpeas. Oka *et al*. (2000), who studied the nematicidal effects against *Meloidogyne javanica in vitro* and in pot experiments, found that *O. vulgare* and *O. syriacum* essential oils, when mixed in sandy soil at concentration of 200 mg/kg, reduced the root galling of cucumber seedlings. Similar effects were obtained by carvacrol and thymol at concentration of 150 mg/kg soil.

A strong molluscicidal effect of *O. compactum* ethyl acetate extracts (LC_{90} = 2.00 mg/l) against the schistosomiasis-transmitting snail *Bulinus truncatus* was attributed to the content of flavonoids and terpenoids, that are known to have molluscicidal potential (Hmamouchi *et al*., 2000). Interesting findings were observed by Vokou *et al*. (1998), who studied the effects of two subspecies of *O. vulgare* (ssp. *hirtum* and ssp. *vulgare*) on the behaviour of three snail species, native in Greece (*Helix lucorum*, *H. aspersa*, and *Eobania vermiculata*) during the different stages of the foraging cycle. *O. vulgare* ssp. *hirtum*, which contained much higher amounts of essential and was rich in phenolic compounds, considerably affected the snail feeding behaviour, while no significant effects were observed in ssp. *vulgare*. During the encounter stage, a repellent activity of

O. vulgare ssp. *hirtum* was observed. During the acceptance stage, all snail species tended to reject food types that contained high concentrations of subsp. *hirtum* essential oil, but at the feeding stage, subsp. hirtum essential oil caused a reduction of daily consumption rates. This is in agreement with Barone and Frank (1999), who found that polar (methanol) extracts of *O. vulgare* showed only scarce repellent effects on slug (*Arion lusitanicus*) feeding on rape.

TOXICITY

Besides toxicological data, referring to toxicities of single components of essential oils – such as limonene (carcinogenic in male rats) from *O. majorana* (de Vincenzi and Mancini 1997) – several clinical studies confirmed the allergenic potential of *Origanum* spp. On the basis of clinical history, and of *in vitro* and *in vivo* studies, *O. vulgare* showed cross-sensitivity with other plants of the Lamiaceae family. The potential allergic response, that could be evoked in sensitive patients after the ingestion of food seasoned with *O. vulgare*, comprises an increased serum level of specific IgE and induced systemic allergic reactions (Benito *et al*., 1996). Similarly, perioral dermatitis has been reported to be induced by *O. majorana* food flavouring (Farkas, 1981). *O. vulgare* has been shown also to induce allergic contact dermatitis, as clinically evaluated by patch test (Futrell and Rietschel, 1993).

Due to empirically proven emmenagogue and abortifacient effects, excessive use of *O. vulgare* or *O. majorana* should be avoided during pregnancy (Brinker, 1998).

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