Original article

Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet

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Summary Raw and processed foods are open to contamination during their production, sale and distribution. At present, therefore, a wide variety of chemical preservatives are used throughout the food industry to prevent the growth of food spoiling bacteria. However health and economic considerations have led to a search for alternatives, such as essentials oils that can safely be used as substitutes for fungicides and bactericides to partially or completely inhibit the growth of fungi and bacteria. The aim of this work was to determine the effectiveness of the essentials oils from oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum*) on the growth of some bacteria commonly used in the food industry, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* or related to food spoilage *Enterobacter gergoviae*, *Enterobacter amnigenus*. The agar disc diffusion method was used to determine the antibacterial activities of the oils. All six essential oils analysed had an inhibitory effect on the six tested bacteria. Oregano essential oil showed the highest inhibition effect followed by cumin and clove.

Keywords Antibacterial, essential oil, rosemary, sage, thyme.

Introduction

Raw and processed foods are open to contamination during their production, sale and distribution (Deak and Beuchar, 1996). At present, therefore, a wide variety of chemical preservatives are used throughout the food industry to prevent the growth of food spoiling bacterias (Davidson, 2001). However, owing to the economical impact of spoiled foods and consumers' growing concerns over the safety of foods containing synthetic chemicals, much attention has been paid to naturally derived compounds or natural compounds (Alzoreky & Nakahara, 2003).

Essential oils and extracts obtained from many plants have recently gained in popularity and excited scientific interest (Sokmen *et al*., 2004; Tepe *et al*., 2005). However, progress in the application of spice-derived compounds as antimicrobial agents in food products has been slow. The major problems include accurate identification of the active components and the apparent requirement for concentrations that halter the sensory qualities of the food (Nychas & Skandamis, 2003; Roller & Board, 2003). Researchers are interested in biologically active compounds isolated from plant species for eliminating pathogenic micro-organims because of the resistance that micro-organims have built up against antibiotics (Essawi & Srour, 2000). For health and economic considerations, research has been directed at finding some essentials oils that could safely be used as substitutes for fungicides and bactericides to partially or completely inhibit the growth of fungi and bacteria (Soliman & Badeea, 2002). The development of multi-component antimicrobial systems for food products requires a greater understanding of the mechanisms of action of specific agents so that attention can be focused on potentially effective combinations (Gill & Holley, 2004). To resolve the problem of high concentrations, it has been proposed that spice-derived compounds should be utilized in a system of antimicrobial agents in a form of hurdle technology (Nychas & Skandamis, 2003; Roller & Board, 2003).

More than 1340 plants are known to be potential sources of antimicrobial compounds but few have been studied scientifically (Wilkins & Board, 1989). Over 30 000 different components isolated from plant oils compounds containing phenol groups are used in the food industry (Meeker & Linke, 1988). Prindle &

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Wright (1977) mentioned that the effect of the phenolic compounds present in spice essential oils is concentration-dependent. At low concentrations, phenols affected enzyme activity, especially of those enzymes associated with energy production, while at greater concentrations, they caused protein denaturation. Several studies have examined the effect on fungi of compounds isolated from essential oils extracted from plants in the search for natural fungicides and a number of these oil constituents have been shown to be inhibitory (Pitt & Hocking, 1997; Betts *et al*., 1999).

The specific objectives of this work was to determine the effectiveness of the essential oils from oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum*) on the growth of some bacteria usually used in food industry as starter culture, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* and related to food spoilage *Enterobacter gergoviae* and *Enterobacter amnigenus*.

Materials and methods

Essential oils

The essential oil of thyme (*Thymus vulgaris* L.), ref. F71180L, was obtained by steam distillation from leaves, stem and flowers; its density at 20 °C is 0.944 g mL^{-1}, the refraction index at 20 °C is 1.507, while the boiling point is higher than 100 °C. Clove (*Syzygium aromaticum* L.), essential oil ref. F08568L, was obtained by steam extraction from the fruit; its density at 20 °C is 1.093 g mL^{-1}, the refraction index at 20 °C is 1.478, while the boiling point is higher than 70 °C. Oregano (*Oringanum vulgare* L.), essential oil ref. F70900L, was obtained by steam extraction from flowers; its density at 20 °C is 0.938 g mL^{-1}, the refraction index at 20 °C is 1.509 and its boiling point is higher than 100 °C. Cumin (*Cuminum cyminum* L.) essential oil was obtained by steam distillation from seeds, its density at 20 °C is 0.915 g mL^{-1} and the refraction index at 20 °C is 1.503 while the boiling point is 53 °C. Sage (*Salvia officinalis*L.) essential oil ref. F71070L, was obtained by steam distillation from leaves and flowers; its density at 20 °C is 0.915 g mL^{-1} and the refraction index at 20 °C is 1.467, while the boiling point is lower than 100 °C. The essential oil of rosemary (*Rosmarinus officinali* L) ref. F71371R, was obtained by steam distillation of the entire plant; its density at 20 °C is 0.909 g mL^{-1} and the refraction index at 20 °C is 1.467 while the boiling point is 52 °C. Essential oils of thyme, oregano, sage, rosemary and clove were purchased from Ravetllat Aromatics (Barcelona, Spain). Essential oil of cumin was purchased from Ventos (Barcelona, Spain).

Antimicrobial activity

Microbial strains

The essentials oils were individually tested against a panel of bacteria: *Staphylococcus xylosus* CECT 237, *Staphylococcus carnosus* CECT 4491, *Lactobacillus sakei*, CECT 4808, *Lactobacillus curvatus* CECT 904, *Enterobacter gergoviae* CECT 857 and *Enterobacter amnigenus* CECT 4078. All these species were supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia.

Agar disc diffusion method

The agar disc diffusion method described by Tepe *et al*. (2005) with some modifications was used to determine the antibacterial capacity of the essential oils. Briefly, a suspension (0.1 mL of 10^6 CFU mL^{-1}) of each microorganism was spread on the solid medium plates (Nutrient Agar I; Oxoid, Basingstoke, Hampshire, England) in the case of *S. xylosus*, *S. carnosus*, *E. gergoviae* and *E. amnigenus*; de Mann Rogosa Sharpe (MRS) agar (Sharlau, Barcelona, Spain) for *L. sakei* and *L. curvatus*). Filter paper discs, 9 mm in diameter (Schlinder & Schuell, Dassel, Germany) were impregnated with 40 μL of the oil and placed on the inoculated plates; these plates were incubated at 37 °C for 48 h in the case of *Staphylococcus* spp. and *Enterobacter* spp. and at 30 °C for 48 h in the case of *Lactobacillus* spp. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate.

Determination of concentration effect

The concentration effect (CE) was studied for to ascertain which doses of essential oil had an inhibitory effect on bacterial growth in the disc diffusion assay. The culture techniques used were those described in the previous paragraph (Agar disc diffusion method), but adding 40, 20, 10, 4 and 2 μL of essential oil which meant doses of 100%, 50%, 25%, 10% and 5% of the initial volume (Viuda *et al*., 2005). All tests were performed in triplicate.

Statistical analysis

Each parameter was tested in triplicate. Conventional statistical methods were used to calculate means and standard deviations, while ANOVA was applied to the data to determine differences ($P < 0.05$). To ascertain significant differences between the levels of the main factor, Tukey's test was applied between means (Afifi & Azen, 1979). ANOVA was made with the following factors: doses (five levels; 40, 20, 10, 4 and 2 μL) for each essential oil. Statistical data analysis was undertaken using the statistical package Statgraphics plus 2.0

Results and discussion

Antibacterial activity

The *in vitro* antibacterial activities of thyme, sage, cumin, rosemary, clove and oregano essential oils against the micro-organisms and their activity potentials were qualitatively and quantitatively assessed for the presence or absence of inhibition zones (Table 1).

The essential oils of thyme, sage, rosemary, oregano, cumin and clove showed inhibitory effects ($P < 0.05$) on the six tested bacteria. The agar disc diffusion method indicated that oregano essential oil showed the highest ($P < 0.05$) antibacterial activity against the six bacteria tested, with inhibition zones ranging from 35.29 mm on *S. xylosus* to 57.90 mm on *E. amnigenus*. In the case of *E. gergoviae*, thyme was the most ($P < 0.05$) potent inhibitor. The next most ($P < 0.05$) effective essential oil in this respect was cumin, which showed inhibition zones between 31.23 mm on *L. sakei* and 38.17 mm on *E. gergoviae*. Rosemary essential oil performed the worst ($P < 0.05$) in the inhibition assays with all six bacteria, while the other oils showed similar antibacterial activities.

The antimicrobial activity of essential oils is assigned to a number of small terpenoid and phenolic compounds (Conner, 1993). Chemical analysis of these oils have shown that the principal active compounds of these oils are principally carvacrol, thymol, citral, eugenol, 1–8 cineole, limonene, pinene, linalool and their precursors (Viuda *et al.*, 2006). Differences in the antimicrobial activity should be attributed to their chemical composition and relative proportions of the individual constituents in the essential oils. Several authors (Arnold *et al.*, 2000; Veres *et al.*, 2003) have claimed that the major component of oregano essential oil is carvacrol, and the antimicrobial activity of this compound has been confirmed on bacteria such as *Escherichia coli*, *Salmonella typhimurum*, *Listeria monocytogenes* (Kim *et al.*, 1995; Cosentino *et al.*, 1999), *Staphylococcus aureus* (Cosentino *et al.*, 1999; Lambert *et al.*, 2001) and *Bacillus cereus* (Cosentino *et al.*, 1999; Ultee *et al.*, 2000).

The inhibition action mechanism has not been studied in great detail (Lambert *et al.*, 2001). Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not because of one specific mechanism but that there are several targets in the cell (Skandamis *et al.*, 2001; Carson *et al.*, 2002). Not all of these mechanisms are separate targets; some are affected as a consequence of another mechanism being targeted (Burt, 2004).

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable (Sikkema *et al.*, 1995). The leakage of ions and other cell contents can then occur (Lambert *et al.*, 2001; Carson *et al.*, 2002). Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death (Denyer & Hugo, 1991).

The effect of phenolic antioxidants on microbial growth and toxin production could be the result of the ability of phenolic compounds to alter microbial cell permeability, leading to the loss of macromolecules from the interior. They could also interact with membrane proteins, causing a deformation in structure and functionality (Fung *et al.*, 1977). Lis-Balchin & Deans (1997) reported that strong antimicrobial activity was associated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamic aldehyde and thymol. Davidson (2001) reported that the exact cause-effect relation for the mode of action of phenolic compounds, such as thymol, eugenol and carvacrol, has not been determined, but that they may inactivate essential enzymes, react with the cell membrane or disturb genetic material.

Components of essential oils also appear to act on cell proteins embedded in the cytoplasmic membrane (Knobloch *et al.*, 1989). Most studies investigating the action of whole essential oils against food spoilage organisms and food-borne pathogens agree that, in general, essential oils are slightly more active against

Table 1 Antimicrobial activity of thyme, sage, cumin, rosemary, clove and oregano essential oils using disc diffusion method

	Diameter (mean and SD) of inhibition zone (mm) including disc diameter of 9 mm					
Essential oil	***Staphylococcus xylosus***	***Staphylococcus carnosus***	***Enterobacter gergoviae***	***Enterobacter amnigenus***	***Lactobacillus sakei***	***Lactobacillus curvatus***
Thyme	21.60 ± 0.78	28.57 ± 0.81	53.85 ± 1.28	21.61 ± 0.86	24.05 ± 0.86	23.64 ± 1.17
Sage	28.76 ± 1.04	27.08 ± 0.94	29.68 ± 0.75	18.77 ± 1.07	23.05 ± 0.49	21.55 ± 0.95
Oregano	35.29 ± 0.88	38.47 ± 1.16	38.92 ± 0.53	57.90 ± 0.95	40.29 ± 1.05	45.20 ± 1.64
Rosemary	17.23 ± 0.91	23.53 ± 0.79	28.47 ± 1.67	18.07 ± 0.83	20.17 ± 0.79	18.82 ± 0.73
Clove	22.37 ± 0.59	24.39 ± 0.88	29.5 ± 0.71	21.96 ± 0.91	26.03 ± 1.12	23.45 ± 0.91
Cumin	34.34 ± 1.23	37.22 ± 1.21	38.17 ± 0.78	35.04 ± 1.01	31.23 ± 0.52	32.65 ± 0.83

gram-positive than gram-negative bacteria (Cosentino *et al*., 1999; Ruberto *et al*., 2000; Cimanga *et al*., 2002; Harpaz *et al*., 2003; Karaman *et al*., 2003). However, these results show that spice essential oil did not possess any selective antimicrobial activity on the basis of the cell wall differences of bacteria. These results are in accordance with those described by Sokmen *et al*. (2004), who affirmed that the essential oils of spices show no selectivity as regards the cell walls of bacteria.

Determination of CE

The CE values for the bacterial strains can be seen in Table 2. The essential oils of oregano, cumin and clove showed inhibitory effects ($P < 0.05$) on all six tested bacteria in all added doses.

The inhibitory effect of each oil was seen to be proportional to its doses. The disks impregnated with 4 and 2 μL of essential oils of sage and rosemary and the disk impregnated with 2 μL of essential oil of thyme did not have inhibitory effects ($P > 0.05$) on any of the six tested bacteria. The discs impregnated with 10 μL of sage essential oil had no inhibitory effect ($P > 0.05$) on *S. carnosus* or *E. gergoviae*.

As regards thyme essential oil, significant differences ($P < 0.05$) were found between the 10%, 25%, 50% and 100% doses in the case of *S. xylosus*, *S. carnosus*, *E. gergoviae* and *L. sakei*. In the case of *E. amnigenus*, differences were not significantly different ($P > 0.05$) between 10% and 25%, but were ($P < 0.05$) between the 50% and 100% doses. The same was true in the case of *L. curvatus*.

Table 2 The concentration effect of thyme, sage, cumin, rosemary, clove and oregano essential oils

		Diameter (mean and SD) of inhibition zone (mm) including disc diameter of 9 mm					
Essential oil	**Doses* (%)**	***Staphylococcus xylosus***	***Staphylococcus carnosus***	***Enterobacter gergoviae***	***Enterobacter amnigenus***	***Lactobacillus sakei***	***Lactobacillus curvatus***
Thyme	5	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	10	11.30 ± 0.04^{a}	13.51 ± 0.45^{a}	11.68 ± 0.51^{a}	12.27 ± 0.56^{a}	11.04 ± 0.41^{a}	10.78 ± 0.93^{a}
	25	17.08 ± 0.62^{b}	19.84 ± 0.41^{b}	21.95 ± 0.34^{b}	13.21 ± 0.71^{a}	13.92 ± 0.86^{b}	12.98 ± 0.51^{a}
	50	19.24 ± 0.62^{c}	23.51 ± 0.81^{c}	37.56 ± 0.71^{c}	17.55 ± 1.04^{b}	16.32 ± 0.53^{c}	17.03 ± 0.68^{b}
	100	21.60 ± 0.78^{d}	28.57 ± 0.81^{d}	53.85 ± 1.28^{d}	21.61 ± 0.86^{c}	24.05 ± 0.86^{d}	23.64 ± 1.17^{c}
Sage	5	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	10	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	25	11.41 ± 0.83^{a}	N.A.	N.A.	11.44 ± 0.81^{a}	13.01 ± 0.32^{a}	12.42 ± 0.51^{a}
	50	20.98 ± 0.69^{b}	21.87 ± 1.03^{a}	22.28 ± 1.10^{a}	16.14 ± 0.69^{b}	18.32 ± 0.51^{b}	17.21 ± 0.55^{b}
	100	28.76 ± 1.04^{c}	27.08 ± 0.94^{b}	29.68 ± 0.75^{b}	18.77 ± 1.07^{c}	23.05 ± 0.49^{c}	21.55 ± 0.95^{c}
Oregano	5	20.08 ± 0.35^{a}	13.56 ± 0.66^{a}	25.88 ± 0.41^{a}	25.91 ± 0.74^{a}	18.09 ± 0.87^{a}	21.31 ± 0.83^{a}
	10	25.09 ± 1.06^{b}	15.61 ± 0.19^{b}	30.77 ± 0.77^{b}	29.86 ± 0.68^{b}	20.77 ± 1.04^{b}	25.99 ± 0.42^{b}
	25	31.56 ± 0.82^{c}	22.89 ± 1.00^{c}	33.33 ± 0.91^{c}	33.46 ± 0.85^{c}	26.52 ± 0.54^{c}	31.06 ± 0.87^{c}
	50	32.10 ± 0.06^{c}	33.73 ± 0.76^{d}	37.44 ± 0.51^{d}	36.52 ± 0.51^{d}	34.81 ± 0.75^{d}	36.12 ± 1.05^{d}
	100	35.29 ± 0.88^{d}	38.47 ± 1.16^{e}	38.92 ± 0.53^{e}	57.90 ± 0.95^{e}	40.29 ± 1.05^{e}	45.20 ± 1.64^{e}
Rosemary	5	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	10	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	25	10.88 ± 0.03^{a}	12.51 ± 0.87^{a}	11.69 ± 0.55^{a}	11.75 ± 0.58^{a}	12.32 ± 0.77^{a}	11.94 ± 0.29^{a}
	50	15.81 ± 0.21^{b}	17.26 ± 0.61^{b}	21.19 ± 0.39^{b}	12.93 ± 0.71^{b}	16.45 ± 0.50^{b}	15.61 ± 0.74^{b}
	100	17.23 ± 0.91^{c}	23.53 ± 0.79^{c}	28.47 ± 1.67^{c}	18.07 ± 0.83^{c}	20.17 ± 0.79^{c}	18.82 ± 0.73^{c}
Clove	5	11.37 ± 0.09^{a}	12.78 ± 0.14^{a}	18.13 ± 0.48^{a}	11.33 ± 0.87^{a}	12.94 ± 1.00^{a}	12.73 ± 0.36^{a}
	10	13.22 ± 0.15^{b}	18.26 ± 0.59^{b}	19.67 ± 0.71^{b}	12.80 ± 0.48^{b}	14.04 ± 0.73^{a}	13.44 ± 0.72^{a}
	25	14.77 ± 0.43^{c}	21.03 ± 0.39^{c}	23.89 ± 0.62^{c}	13.32 ± 0.87^{c}	16.81 ± 0.59^{b}	15.25 ± 0.53^{b}
	50	17.74 ± 0.52^{d}	23.79 ± 0.73^{d}	25.12 ± 0.87^{d}	18.38 ± 1.04^{d}	19.11 ± 0.87^{c}	17.97 ± 0.86^{c}
	100	22.37 ± 0.59^{e}	24.39 ± 0.88^{e}	29.5 ± 0.71^{e}	21.96 ± 0.91^{e}	26.03 ± 1.12^{d}	23.45 ± 0.91^{d}
Cumin	5	11.06 ± 0.09^{a}	18.55 ± 0.35^{a}	12.93 ± 0.12^{a}	11.30 ± 0.59^{a}	12.54 ± 0.67^{a}	13.76 ± 0.78^{a}
	10	21.91 ± 0.21^{b}	27.78 ± 1.04^{b}	21.75 ± 0.46^{b}	14.02 ± 0.71^{b}	16.03 ± 0.29^{b}	17.04 ± 1.02^{b}
	25	25.51 ± 0.33^{c}	33.58 ± 0.23^{c}	27.59 ± 0.69^{c}	22.25 ± 0.56^{c}	21.91 ± 82^{c}	23.19 ± 0.54^{c}
	50	32.00 ± 0.39^{d}	34.89 ± 0.27^{d}	33.03 ± 0.7^{d}	31.07 ± 0.42^{d}	27.46 ± 0.92^{d}	29.51 ± 0.94^{d}
	100	34.34 ± 1.23^{e}	37.22 ± 1.21^{e}	38.17 ± 0.78^{e}	35.04 ± 1.01^{e}	31.23 ± 0.52^{e}	32.65 ± 0.83^{e}

*Doses of essential oil referred to initial volume (40 μL).

$^{a-e}$For the same essential oil, values followed by different letters within the same column are significantly different ($P < 0.05$) according to Tukey's multiple range test.

N.A., non-active.

As regards sage essential oil, significant differences existed ($P < 0.05$) between the 25%, 50% and 100% doses in the case of *S. carnosus* and *E. gergoviae*, and between 10%, 25%, 50% and 100% in the case of *E. amnigenus*, *S. xylosus*, *L. curvatus* and *L. sakei*.

When oregano essential oil was used on *S. carnosus*, *E. gergoviae*, *E. amnigenus*, *L. curvatus* and *L. sakei*, there were significant differences ($P < 0.05$) between all the concentrations assayed, while on *S. xylosus* the differences were not significantly different ($P > 0.05$) between the 25% and 50% doses.

As regards rosemary, significant differences ($P < 0.05$) were observed between the 25%, 50%and 100% doses for all six bacteria analysed.

When clove essential oil was analysed statistically, significant differences ($P < 0.05$) existed between all five doses used on all six bacteria. The same was true for cumin essential oil.

As can be seen, these essential oils showed antibacterial activity not only against food spoilage microbiota but also against microbiota used in food processing (fermentation process). This is very important because a lot of food elaboration process include a fermentation stage. If some essentials oils are going to be used in this type of foods, the antibacterial activity against the microorganism used in the fermentation stage must be checked.

It can be concluded that these essential oils (from oregano, thyme, rosemary, sage, cumin and clove) posses *in vitro* antibacterial activity against *L. curvatus*, *L. sakei*, *S. carnosus*, *S. xylosus*, *E. gergoviae* and *E. ammigenus*, although, the effects of thyme, rosemary and sage essential oils are dose-dependent. However, if essential oils were to be more widely applied as antibacterials in foods, it must be taken into account that the antibacterial efficiency is diminished when they are added to more complex materials (such as food products) and the organoleptic impact would be important and also that issues of safety and toxicity will need to be addressed.

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