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<p><b>LITERATURE DATA</b></p> <p>Summary</p> <p>Thyme extract</p>
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## *1. Antiseptic effects of thyme*

A high number of natural substances including thyme possess an antiseptic effect. The phenols, mainly thymol and carvacrol are known to be the principal components for its action. Most studies regarding the antiseptic effects have been performed on the essential oil but a similar antiseptic effect has been observed for the thyme extract.

### *A. Antibacterial effects of thyme*

In general thymol and carvacrol are more efficient against Gram+ bacteria than against Gram- bacteria, because of the presence of an outer membrane for the Gram- bacteria. Except in some particular cases (e.g. *Pseudomonas* spp) they can nevertheless insert into the outer membrane of Gram- bacteria: they affect the structure and stability of the lipid bilayer and alter the physicochemical properties of the membrane [HELANDER, 1998]. Thymol and carvacrol increase the permeability of the cytoplasmic membrane.

In *Bacillus cereus*, the potassium efflux causes the decrease of the pH gradient and the disappearance of the membrane potential; the ATP production is interrupted and the cell dies [ULTEE, 1998; 1999]. A molecule as carvacrol, sufficiently hydrophobic, penetrates through the cytoplasmic membrane of *B. cereus* and, within the cell, will exchange its proton against a cation (potassium) before crossing the membrane again in the other direction and share its cation against a proton and so on. The presence of a system of delocalized electrons allowing dissociation (i.e. of a phenol) is therefore considered to determine the activity: menthol, an alcoholic counterpart, is only slightly antiseptic [ULTEE, 2002].

We must nevertheless emphasize that p-cymene - often present in significant amounts in the essential oil and thyme extracts - is itself antibacterial: the presence of the phenolic function would therefore not be indispensable [VELDHUIZEN, 2006]. However the available data on this molecule p-cymene are discordant and the activity spectrum seems to be very limited [DORMAN AND DAENS, 2000].

In *Escherichia coli* and *Listeria monocytogenes*, carvacrol destroys the membrane, inhibits the ATPase membrane [GILL AND HOLLEY, 2006a,b]. Carvacrol also changes the composition of the membrane fatty acids [DI PASQUA, 2006; 2007]. The difference in sensitivity of bacteria to thymol and carvacrol, denoted by some authors, seems to depend on the lipid composition of the membrane and the surface charge thereof [CRISTANI, 2007].

The antibacterial effect of thymol and carvacrol and of the thyme extract has been studied in a large number of studies. In a review [BRUNETON, 2011] an antibacterial activity has been shown against the following species:

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- *Bacillus cereus*
- *Campylobacter jejuni*
- *Enterobacter cloacae*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Listeria innocua*
- *Listeria monocytogenes*
- *Micrococcus flavus*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Salmonella enteritidis*
- *Salmonella typhi*
- *Salmonella typhimurium*
- *Shigella sonnei* and *flexneri*
- *Staphylococcus aureus*
- *Yersinia enterocolitica*
- *Aeromonas hydrophila*
- *Brochothrix thermospata*
- *Clostridium perfringens*
- *Lactobacillus acidophilus*
- *Photobacterium phosphoreum*
- *Shewanella putrefaciens*
- *Vibrio parahaemolyticus*
- *Bacillus subtilis*
- *Micrococcus flavus*
- *Sarcinia lutea*
- *Enterococcus* spp.
- *Haemophilus influenzae*,
- *Serratia marcescens*,
- *Streptococcus pneumoniae*
- *Vibrio vulnificus*

All studies show that the thyme extract, and its principal components thymol and carvacrol have a clear antibacterial effect against a wide range of bacteria. Several studies have compared the antimicrobial activity of different essential oils and plant extracts:

<i>Efficacy of thyme extract on microorganisms</i>	
HAMMER, 1999 In-vitro study	In-vitro study comparing the antimicrobial activity of 52 plant oils and extracts including thyme oil

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DORMAN AND DAENS, 2000 In-vitro study	In-vitro study comparing the antibacterial activity of different plants including thyme against 25 different genera of bacteria
GUTIERREZ, 2009	In-vitro study comparing the antimicrobial activity of different plants oils including thyme against <i>Listeria</i> spp. and spoilage bacteria

In a review of KALEMEBA AND KUNICKA regarding the antibacterial and antifungal properties of essential oils, it was found that the essential oils of spices and herbs like thyme possess the strongest antimicrobial properties among many tested [KALEMBA AND KUNICKA, 2003].

**All these studies show that thyme has an antimicrobial effect equivalent or stronger than other plants. This antimicrobial effect is mainly due to an increased permeability of the cytoplasmic membrane. The sensitivity of bacteria to the antimicrobial activity of thyme is species dependent and depends on the lipid composition of the membrane and its surface charge. Gram- bacteria are less sensitive to the antimicrobial effects of thyme because of the presence of an outer membrane.**

### B. Antiviral effect of thyme

In literature, thyme is also known to have an antiviral effect. The number of studies concerning the antiviral effect is considerable lower than for the antibacterial effect but nevertheless, different independent studies clearly indicate that thyme has an antiviral effect:

- Thymol and carvacrol were found to be effective in reducing the titers of norovirus surrogates in a dose-dependent manner [SANCHEZ AND AZNAR, 2015; SANCHEZ, 2015]. Thymol and carvacrol possessed significant antiviral activity against herpes simplex virus type 1 [LAI, 2012].
- Boubaker-Elandalousi et al concluded that Thyme capitata extracts inhibit the viral replication by interfering with the early stages of viral adsorption and replication and are therefore effective against bovine herpesvirus-1 [BOUBAKER-ELANDALOUSI, 2014].
- Thymus vulgaris extracts were found effective against Newcastle disease virus [REZATOFIGHI, 2014].
- Astani compared the antiviral activity of the essential oils of different plants including thyme against herpes simplex virus type 1. All plants exhibited clear anti-HSV-1 activity [ASTANI, 2010]. This effect was also seen in a publication studying the effect of extracts from Lamiaceae plants including thyme against HSV [REICHLING, 2008] and several other publications [RAJBHANDARI, 2009; NOLKEMPER, 2006].

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- *Thymus linearis* was found to have a remarkable antiviral activity against HSV-1 and influenza virus A [RAJBHANDARI, 2009].
- In a plaque reduction assay, thyme oil reduced significantly plaque formation and was thus effective against HSV-2, probably by interacting with the viral envelope [KOCH, 2008].

**In conclusion, the studies mentioned above clearly demonstrate the antiviral activity of thyme.**

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## 2. Safety of thyme

Thyme is a frequently used herb. The most common variety is *Thymus vulgaris*. The phenols, mainly thymol and carvacrol are the principal components for its action (monoterpene compounds).

Thymol is FDA approved when used as a synthetic flavoring (21 CFR 172.515), a preservative, and indirect food additive of adhesives (21 CFR 175.105). Additionally, the source plant (thyme), from which thymol is extracted is acknowledged by FDA as generally recognized as safe (GRAS) (21 CFR 182.10, 21 CFR 182.20) [Federal register, thymol, 2009].

Based on all the reliable available information the Agency reviewed on thymol, the Agency EPA concludes that there are no residual uncertainties for prenatal/postnatal toxicity resulting from thymol and that thymol has relatively low toxicity to mammals from a dietary standpoint, including infants and children [Federal register, thymol, 2009].

Thymol is commonly used as flavoring agent from 2.13 to 78.05 ppm i.e. 0.00021 to 0.0078% [HSDB Thymol, 2015].

Thymol is used in cosmetics, foods, and pharmaceutical applications [ROWE, 2009].

Thymol is GRAS listed and included in

- FDA Inactive Ingredients Database (inhalation, liquid; oral, powder for solution)
- Non-parenteral medicines (topical creams and ointments) licensed in the UK
- Canadian List of Acceptable Nonmedicinal Ingredients [ROWE, 2009].

Thymol may be irritating when inhaled or following contact with the skin or eyes; rare sensitization could occur in humans.

A respiratory arrest, attributed to acute nasal congestion and edema, has been reported in a 3-week-old patient due to the erroneous intranasal application of Karvol decongestant drops, a combination product including levomenthol (7.90% w/v), Pine oil sylvestris (2%), chlorbutanol (0.5%), terpineol (14.80%), pumilio pine oil (22.90%) and thymol (0.70%) [ROWE, 2009].

The patient recovered and Karvol decongestant drops are normally contra-indicated in infants below 3 months [Karvol decongestant drops SPC, 2010].

**The adverse event was attributed to the combination containing high dose levels of essential oils and levomenthol. The quantity of thymol was also rather high.**

Carvacrol is also authorized as flavoring agents and related substances by FDA [US FDA CFR 21, 2015]. The reported uses: in non-alcoholic beverages 26 ppm; ice cream, ices, etc. 34

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ppm; candy 92 ppm; baked goods 120 ppm; condiments 37 ppm i.e. from 0.00026% to 0.00120% [HSDB Carvacrol, 2002].

In a double-blind randomized controlled clinical trial, the effect of a thyme honey nasal spray (thyme honey contains also thymol and carvacrol) as an adjunctive medication on chronic rhinosinusitis after endoscopic sinus surgery was tested [HASHEMIAN, 2015]. 64 patients with chronic rhinosinusitis undergoing functional endoscopic sinus surgery were enrolled in this study. Patients were randomized and blinded to receive either placebo or thyme honey nasal spray in addition to the standard regimen postoperatively. Patients were visited on postoperative days 7, 30 and 60.

Significant improvement was observed in both treatment groups. However, a greater reduction in endoscopic scores was shown in thyme honey group. The incidence of adverse effects was not significantly different between the groups, but synechia formation and epistaxis were lower in treatment group. **The authors concluded that a thyme honey nasal spray seems to be a low-priced potential adjuvant remedy with excellent safety profile, to reduce inflammation and polyp formation and also fostering mucosal healing for patients suffering from chronic rhinosinusitis.**

<i>Thyme and nasal administration</i>		
HASHEMIAN, 2015 Double-blind randomized controlled study	64 patients with chronic rhinosinusitis undergoing functional endoscopic sinus surgery.	Thyme honey nasal spray versus placebo : synechia formation and epistaxis were lower in treatment group

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