

## Impact of Salvia and Peppermint Oil on the *In Vitro* Survival of *Demodex* Mites

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### Abstract

Demodicosis is a medical condition caused by presence of *Demodex* mites. Mites may cause ocular demodicosis with symptoms such as burning and itching of eyelids. Currently, several drugs are available for the treatment of demodicosis. However, their use carries a risk of serious side effects. According to recent studies, substances contained in some plant-derived essential oils kill *Demodex* mites. Good efficacy of tea tree oil against *Demodex* sp. has been reported. However, some patients develop allergic reactions and ocular irritation in the course of tea tree oil treatment. Tests with essential oils showed that salvia and peppermint oils rapidly kill *Demodex* in 7 and 11 minutes, respectively. Salvia is known as a valuable herb and is used to treat eye disease. Therefore, salvia essential oil could be an alternative treatment for demodicosis.

**Keywords:** *Demodex*; Salvia oil; Peppermint oil; Essential oils

### Introduction

The Demodicidae family includes strictly specialized parasitic mites living in the skin, hair follicles or outer epidermal layers. *Demodex* mites show strong host-species specificity. Up to now, two human-specific *Demodex* species have been described: *Demodex folliculorum* and *Demodex brevis*. These mites may cause ocular demodicosis with symptoms such as burning and itching of eyelids. *Demodex* plays an important role in *Demodex blepharitis*, meibomian gland dysfunction, conjunctival inflammation or corneal lesions [1].

Demodicosis is a major medical problem in dermatology and ophthalmology because it is chronic, its symptoms vary in intensity, early diagnosis is difficult, and inappropriate treatment (e.g. with steroids) leads to unwanted consequences. Currently, there are several drugs available for treating demodicosis. However, most of them are applied in veterinary medicine, and their use carries the risk of serious side effects.

Gao et al. [2] reported good effects of tea tree oil (TTO) on *Demodex* sp., both in vitro and in vivo. The same group [3] tested an ointment containing 5% tea tree oil in *Demodex*-infested patients with chronic anterior blepharitis. The authors indicated that daily massage with the use of the ointment can partially or completely relieve subjective symptoms of ocular demodicosis. Koo et al. [4] described TTO as safe and acceptable for clinical use in patients with *Demodex* mites but they mentioned allergic reactions and ocular irritation as complications of TTO treatment.

Development of new, alternative substances with demodicidal activity could contribute to greater efficacy and tolerance of skin and ocular demodicosis therapy.

### Materials and Methods

The study material consisted of vital *Demodex* mites collected from eyelashes of patients who were subject to examination for presence of the mites. Four eyelashes were collected from each eye, by means of tweezers. Immediately after collection, the eyelashes were placed on a slide and examined under 200-400x magnification [5]. Identified *Demodex* mites were assessed for motility on the glass slide under 200-400x magnification. Only adult forms of *Demodex* mites were tested [6]. Vital mites were placed in a drop of tested substance, and closed in a humid chamber to prevent the applied substance from evaporating. Motility of the mites was observed at the different time intervals: continuously from 0 to minute 15; every 5 minutes from minute 15 to minute 180; from minute 180 onwards, every 2 hours. When the mites' motility was decreasing clearly, the observation was more frequent. Movements of the legs were used to judge whether mites were alive and the time when they ceased to show vital signs was recorded [6].

Survival time of *Demodex* was measured starting from application of the substance on mites and ending when no movement of *Demodex* mites was observed. Survival time of the mites in saline was used as control. Effects of salvia (100% *Salvia hispanica*, Avicenna-Oil) and peppermint essential oils (100% *Mentha piperita*, Avicenna-Oil) were studied. Also salvia oil with liquid paraffin and triglycerides of caprylic and capric acids (Croda) were tested.

Statistical analysis of obtained results was performed with the use of Microsoft Excel 2010. Mean survival time and standard deviation (SD) was calculated.

### Results

*Demodex in vitro* survival time in studied substances is presented in Table 1.

Agent	Number of tested Mites	mean survival time (± SD)	minimum survival time	maximum survival time
saline	15	82 h (± 35 h)	35 h	156 h
100% salvia oil	21	7 min (± 3, 2 min)	2 min	14 min
50% salvia oil	18	32 min (± 4, 2 min)	30 min	45 min
25% salvia oil	15	85 min (± 30, 4 min)	31 min	150 min
12,5% salvia oil	11	119 min (± 35, 5 min)	61 min	175 min
3% salvia oil	5	60 h (± 9, 4 h)	48 h	72 h
1% salvia oil with paraffin	9	135 h (± 7, 9 h)	120 h	144 h
1% salvia oil with triglycerides	8	80 h (± 7, 9 h)	72 h	92 h
salvia extract	8	48 h (± 6, 6 h)	32 h	52 h
100% peppermint oil	18	11 min (± 3, 8 min)	6 min	19 min
50% peppermint oil	9	43 min (± 14, 5 min)	28 min	70 min
25% peppermint oil	11	110 min (± 44, 4 min)	40 min	183 min
12,5% peppermint oil	12	157 min (± 45, 5 min)	77min	211min

**Table 1:** Effects of salvia and peppermint oil on *Demodex* survival time.

## Discussion

Tests of essential oils revealed that salvia and peppermint oils killed *Demodex* mites in a short time - 100% salvia oil kills mites in 7 minutes and 100% peppermint oil in 11 minutes. For comparison, tea tree oil kills in 3,7 minutes [2]. However, some patients present allergic reactions after using TTO. Therefore, examining and testing other essential oils could help in developing alternative products for treating demodicosis. Especially salvia is known as a valuable herb, which produces useful secondary metabolites like terpenes [7]. In TTO, Terpinen-4-ol was found to be the most active ingredient that kills *Demodex* mites [6]. Both salvia essential oil and salvia extracts were studied. Salvia oil from the chia plant eliminated *Demodex* effectively and rapidly, while salvia extract proved to be less effective in that the mean survival time was as long as 2 days. Salvia extracts contain only 2% to 3% essential oils. This suggests that it is solely the terpenes, contained in essential oils, that decrease vitality of *Demodex* mites.

Liquid paraffin and triglycerides of caprylic and capric acids are one of the basic components of the base emulsion systems. Ointment composition may additionally strengthen or weaken the effect of

acaricides therefore triglycerides and paraffin were tested. *Demodex* mites survived longer in 1% salvia oil with paraffin (135 h) than in triglycerides (80 h). This suggests that an ointment for patients with demodicosis should contain appropriate excipients.

Salvia is used to treat eye disease, acne, headache and oral or pharyngeal inflammation. Recently, other uses have been suggested. Salvia or chia essential oils have mosquito larvicidal activity [7,8]. Salvia essential oils were able to inhibit the growth of the cancer cells [9]. Also *Salvia officinalis* aroma was found to significantly enhance memory in cognitive tasks [10]. Jeong et al. used a topical formulation containing 4% chia seed oil for 8 weeks. They observed that topical chia seed oil is effective for pruritus and xerosis treatment and is also beneficial for skin moisturization in healthy volunteers with xerotic pruritus [11].

Peppermint oil killed *Demodex* mites less effectively compared to salvia oil. This shows that the efficacy of essential oils from different plant species is not equal.

Salvia essential oil shows promising effects and requires further investigations to prove its usefulness in demodicosis treatment.

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