






Anti-Microbial Activity of Hempseed Oil and Sage Oil against *Streptococcus mutans* and *Candida albicans*: An *In-Vitro* Study

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ABSTRACT

Objective: To assess the anti-microbial activity of sage oil and hempseed oil against *Streptococcus mutans* and *Candida albicans*. **Material and Methods:** The sage oil and hempseed oil in pure extract form were collected from an authorized government organization. The anti-microbial activity from the oils was assessed by Kirby-Bauer disc diffusion method. Standard antibiotics Vancomycin and Fluconazole were taken as a positive control for *Streptococcus mutans* and *Candida albicans*, respectively. The mean zone of inhibition (ZOI) was measured at 24 hours, 48 hours and 72 hours by HI-MEDIAs antibiotic zone scale. One-way Analysis of variance with Tukey's Post Hoc was applied for statistical analysis. **Results:** The mean zone of inhibition of sage oil and hempseed oil at 24 hours, 48 hours and 72 hours against *Streptococcus mutans* was $(7.0 \pm 4.24, 9.1 \pm 3.71$ and $8.4 \pm 3.02)$ and $(0.0, 3.2 \pm 0.75, 1.9 \pm 1.24)$ respectively. The zone of inhibition against *Candida albicans* 24 hours, 48 hours and 72 hours for sage oil $(2.80 \pm 1.151, 6.70 \pm 1.30$ and $6.30 \pm 1.44)$, hempseed oil $(0.0, 3.80 \pm 0.75, 3.10 \pm 0.41)$. Sage oil presented potential anti-microbial activity against *Streptococcus mutans* and *Candida albicans*. Comparing the anti-microbial efficacy, standard antibiotic (Vancomycin/Fluconazole) was more effective than test oils. **Conclusion:** Test oils showed anti-bacterial and anti-fungal activity against *Streptococcus mutans* and *Candida albicans*. Among these, sage oil showed efficacy at 24 hours, while hempseed oil did not show any effect at this time point. Further studies are needed to affirm the same and test their efficacy in different forms and concentrations.

Keywords: Anti-Bacterial Agents; *Salvia officinalis*; Cannabis; Plants, Medicinal.

Introduction

Dental caries is one of the most prevalent dental diseases [1]. *Streptococcus mutans*, a Gram-positive bacteria, play a vital role in progression of caries by producing glucans and acids, which allow them to suppress the activity of the non-cariogenic commensal at low pH in oral cavity [2]. *Candida albicans* commonly colonizes human mucosal surfaces and are prevalent in oral mucosa. Co-adhesion between fungi and bacteria in oral cavity is pivotal for fungal colonization [3].

Various drugs have been tested and are proved to be effective against *Streptococcus mutans* and *Candida albicans*. However, because of increasing resistance to these synthetic drugs, it is necessary to test for newer drugs. Due to the current belief that 'green medicine' is safe and more dependable than expensive synthetic drugs, natural products are being subjected to test their anti-microbial potency [4].

Hemp plant (*Cannabis sativa*) belongs to Family *Cannabinaceae*, popularly known for its preparation like Marijuana, Bhang, Ganja, Charas, etc. [5]. It has been found to be anti-bacterial, anti-ulcerative, anti-inflammatory, and anti-arthritic [6], anti-phlegmatic and also used as an anesthetic agent [7]. The most psychologically active component in *Cannabis* plant is delta-9-tetrahydrocannabinol (THC), which is used to treat side effects in patients undergoing chemotherapy [7,8].

Sage plant (*Salvia officinalis* L.), a *Lamiaceae* family member, is anti-bacterial, anti-fungal, anti-oxidant, anti-spasmodic and anti-diuretic in action [9]. It is also used as a local anesthetic for skin, in aromatherapy and in the treatment of dermatitis [10]. It is also found to be effective in chronic illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer [11]. Even though limited studies were conducted to test the anti-microbial efficacy of these oils against oral pathogens, but the results were promising. Beheshti-Rouy et al. [12] recommended that sage mouth rinse could be used as an adjunct for conventional methods of plaque control against dental caries. Stahl and Vasudevan [13] concluded that Cannabinoids have the potential to be used as an effective anti-bacterial agent against dental plaque-associated bacteria. With this background, the present study was designed and undertaken to assess the anti-microbial activity of sage oil and hempseed oil against *Streptococcus mutans* and *Candida albicans*.

Material and Methods

Study Design and Ethical Clearance

This *in-vitro* study was conducted in the Department of Microbiology, Rungta College of Dental Sciences and Research, Bhilai, India. The ethical clearance was obtained from the institutional review board of the institute (Protocol No. 18/023).

Materials

Sage plant (*Salvia officinalis* L.) and Hemp plant (*Cannabis sativa*) were used as sources of anti-microbial agents. *Streptococcus mutans* (MTCC 497) and *Candida albicans* (MTCC 3958) were used as test microorganisms.

Essential Oils Acquisition

Sage oil and hemp oil were collected from Chhattisgarh State Minor Forest Produce Federation (Sanjeevani Retail Outlet, Durg, India). The package showed that sage oil and hempseed oil were extracted from the steam distillation and cold expression methods.

Preparation of Antibiotic and Test Oils Sensitivity Testing Disc

The Kirby-Bauer disc diffusion technique [14] was used to determine the anti-microbial activity of the two oils. Sterile Whatman No. 1 paper was punched into 5 mm diameter disc size. The discs were placed in a glass Petri plate and oven; sterilized at 170° C for 1 hour. Five separate sterile discs were soaked in the test oil, antibiotic, and distilled water (negative control) for 24 hours for proper absorption, after which it was allowed to dry for 24 hours.

Preparation of Culture Agar Plates

Mitis Salivarius agar and Sabouraud Dextrose agar was used as selective media for culture of *Streptococcus mutans* and *Candida albicans*, respectively. Agar was poured into sterile Petri dishes (Borosil Glass Works Ltd., Mumbai, India) of size 90 mm, in a sterile lamellar airflow chamber which was equipped with ultraviolet light and high-efficiency particulate air filter, sterile Petri-dishes were placed and about 20 ml of molten agar were poured in these Petri-dishes. Appropriate precautions were taken to avoid contamination to culture plates. The growth of freshly sub-cultured isolates was matched with a turbidity of 0.5 on McFarland scale [15].

Disc Diffusion Method

Each test plate comprised of 4 discs viz., one positive control (Vancomycin disc/Fluconazole disc), one negative control and two discs treated with tests oils. Five such plates were prepared and incubated for 24 hours, 48 hours, and 72 hours. A similar procedure was carried out for both test microorganisms separately. After incubation, the plates were examined for the zone of inhibition (ZOI) using HI-MEDIA's antibiotic zone scale.

Statistical Analysis

The data obtained were analyzed using SPSS (Statistical Package for Social Sciences), version 21.0 (IBM SPSS, Chicago, IL, USA). Descriptive statistics in terms of mean and standard deviation were calculated. For Multiple group comparison of the mean zones of inhibition between the test oils and standard antibiotic one-way Analysis of variance was used and for group-wise comparison, Tukey's post hoc was performed. A p-value ≤ 0.05 was considered statistically significant.

Results

The zone of inhibition around the discs against anti-microbial was calculated using HI-antibiotic zone scale. The mean zone of inhibition of sage oil and hempseed oil at 24 hours, 48 hours and 72 hours against *Streptococcus mutans* was $(7.0 \pm 4.24, 9.1 \pm 3.71$ and $8.4 \pm 3.02)$ and $(0.0, 3.2 \pm 0.75, 1.9 \pm 1.24)$, respectively. The mean zone of inhibition of both the test oils was less than standard antibiotic Vancomycin at 24 hours, 48 hours, and 72 hours which was $20.0 \pm 4.8, 27.9 \pm 1.34$ and 24.4 ± 0.96 , respectively (Figure 1).

The zone of inhibition against *Candida albicans* 24 hours, 48 hours, and 72 hours for sage oil $(2.80 \pm 1.151, 6.70 \pm 1.30$ and $6.30 \pm 1.44)$, hempseed oil $(0.0, 3.80 \pm 0.75, 3.10 \pm 0.41)$ and standard anti-fungal agent fluconazole $(25.60 \pm 1.14, 26.30 \pm 1.25, 24.80 \pm 1.64)$, respectively. The ZOI measured for anti-fungal agent fluconazole was markedly high compared to both the test oils, i.e., sage oil and hempseed oil (Figure 2).

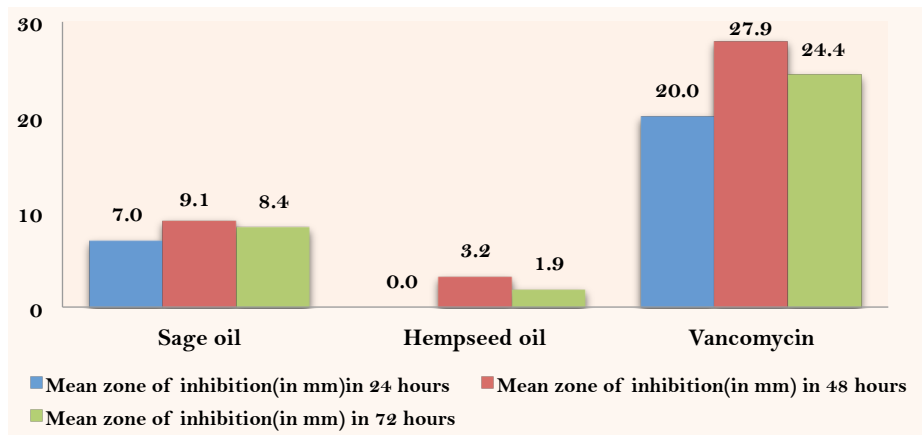


Figure 1. Anti-microbial activity of the sage oil, hempseed oil and vancomycin against *Streptococcus mutans* at 24 hours, 48 hours and 72 hours.

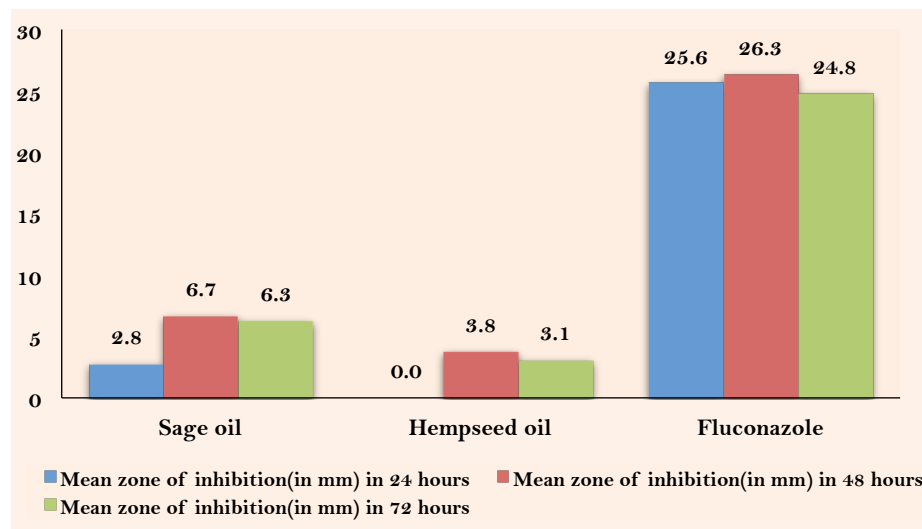


Figure 2. Anti-microbial activity of the sage oil, hempseed oil and fluconazole against *Candida albicans* at 24 hours, 48 hours and 72 hours.

Hempseed oil and sage oil were active against both the microorganisms, i.e., *Streptococcus mutans* and *Candida albicans*. Sage oil was more effective against *Streptococcus mutans* and *Candida albicans* than Hemp oil and standard antibiotics, i.e., Vancomycin (*Streptococcus mutans*) and Fluconazole (*Candida albicans*) were more effective against both the test organism compared to test oils. Analysis of variance with post-hoc showed a statistically significant difference in anti-microbial activity between sage oil, hempseed oil and vancomycin for *Streptococcus mutans* at 24, 48, and 72 hours ($p < 0.00$). Post-hoc also showed a statistically significant difference between the test oils at 24 hours, 48 hours, and 72 hours ($p = 0.01$, $p = 0.004$, and $p = 0.005$), respectively (Table 1).

Similarly, against *Candida albicans*, analysis of variance test showed a statistically significant difference between standard anti-fungal agent fluconazole and the test oils ($p < 0.00$). Post-hoc also showed a statistically significant difference between both the test oils at 24 hours, 48 hours and 72 hours ($p = 0.042$, $p = 0.004$, and $p = 0.001$), respectively (Table 2).

Table 1. Intergroup comparisons of the sage oil, hempseed oil and vancomycin against *Streptococcus mutans* at 24 hours, 48 hours and 72 hours.

Dependent Variables	Comparison	24 Hours	48 Hours	72 Hours
		p-value	p-value	p-value
Sage Oil	Hempseed Oil	0.001	0.004	0.005
	Antibiotic	0.000	0.000	0.000
Hempseed Oil	Sage Oil	0.001	0.004	0.005
	Antibiotic	0.000	0.000	0.000
Fluconazole	Sage Oil	0.000	0.000	0.000
	Hempseed Oil	0.000	0.000	0.000

Post-hoc Tukey HSD.

Table 2. Intergroup comparisons of the sage oil, hempseed oil and vancomycin against *Candida albicans* at 24 hours, 48 hours and 72 hours.

Dependent Variables	Comparison	24 Hours	48 Hours	72 Hours
		p-value	p-value	p-value
Sage Oil	Hempseed oil	0.042	0.004	0.001
	Antibiotic	0.000	0.000	0.000
Hempseed Oil	Sage oil	0.042	0.004	0.001
	Antibiotic	0.000	0.000	0.000
Fluconazole	Sage oil	0.000	0.000	0.000
	Hempseed oil	0.000	0.000	0.000

Post-hoc Tukey HSD.

Discussion

Streptococcus mutans is a prime etiologic agent for dental caries and having high prevalence in dental biofilms, where *Candida albicans* also resides, and interaction between the two mediate cariogenic developments. Main purpose of this study was to test the medicinal plants which can be used to inhibit the growth of oral pathogens namely *Streptococcus mutans* and *Candida albicans*.

The genus *Salvia* L. is mainly cultivated in Mediterranean, Southeast Africa, and Central and South America. It is cultivated for culinary, medicinal, and ornamental purposes. Its anti-microbial activity against several microorganisms has been reported for decades. Some major compounds are present in oil like 1,8-cineole, β -thujone, camphor, borneol and p-cymene [16]. In the present study, mean ZOI (in mm) by sage oil against *Streptococcus mutans* was found to be 7.0 ± 4.24 mm at 24 hours which is in corroboration with the study conducted by Pierozan et al. [17], (8.7 mm at 24 hours). Anti-microbial activity of sage oil at 24 hours against *Candida albicans* was found to be 2.80 ± 1.151 mm, which is in contrast with the study conducted by Sookto et al. [18], who observed ZOI of 31.5 mm at 24 hours. This difference may be because of the concentration of oil used in this study and the process of extraction of oil from the plants. One more reason may be the strains of *Candida albicans* in both the study may be different. The sage oil anti-microbial activity was maximum at 48 hours and then declined at 72 hours.







In the present study, hempseed oil showed no anti-microbial activity at 24 hours against both the test organism. At 48 hours, hempseed oil showed ZOI of 3.2 ± 0.75 mm against *Streptococcus mutans*. A similar finding is seen in a study conducted by Verma et al. [7], which shows 8.4 mm of ZOI against *Streptococcus mutans*. Against *Candida albicans*, hemp oil showed slight anti-microbial activity (ZOI= 3.80 ± 0.75 mm) at 48 hours, which contrasts with the study conducted by Ali et al. [5]. The authors noted a mean ZOI of 13 mm on Methanol extract of whole plant and the reason for this difference may be the use of whole plant extract in their study [5], while in the present study, oil was extracted by seeds of whole plant by cold expression method.

The main limitation of the present study is that test oils were not tested at different concentrations. Secondly, the purity of the test oil could not be assessed as it was obtained in oil form.

Conclusion

Test oils showed anti-bacterial and anti-fungal activity against *Streptococcus mutans* and *Candida albicans*, respectively. Among these, sage oil showed efficacy at 24 hours, while hempseed oil did not show any effect at this time point. Further studies are needed to affirm the same and test their efficacy in different forms and concentrations. These oils can be used to prevent oral diseases and can act as a natural adjunct to synthetic antibiotics. These oils can also be used as chemical plaque control agents and is a cost-effective and efficient means of plaque control.

Authors' Contributions

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NN		---	Visualization and Supervision.
ABHI		https://orcid.org/0000-0003-2650-6993	Formal Analysis and Writing - Original Draft.
RK		https://orcid.org/0000-0001-6493-170X	Methodology and Investigation.
SRP		https://orcid.org/0000-0003-0715-497X	Conceptualization, Data Curation and Writing - Review and Editing.
All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.			

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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