Available online at www.sciencedirect.com
SciVerse ScienceDirect
Procedia Environmental Sciences 12 (2012) 1361 – 1366
Procedia Environmental Sciences

2011 International Conference on Environmental Science and Engineering (ICESE 2011)

Screening of Food Additives and Plant Extracts against Candida Albicans in Vitro for Prevention of Denture Stomatitis

Xia Liu[1], Xiaodong Zheng[2], Weiwen Fang[3], Yanzhen Zhang[4*]

[1]*College of Chemistry and Life Sciences Zhejiang Normal University Jinhua, 321004, PR China*
[2]*Department of Food Science and Nutrition Zhejiang University Hangzhou, 310029, PR China*
Liuxia801209@163.com
[3]*Extension Station of Fruit Tree Technology Center of Agricultural Technology Extension of Chun'an, 311700, PR China*
[4]*Department of Stomatology 2nd Affiliated Hospital School of Medicine Zhejiang University Hangzhou310029, PR China*
zyz85@hotmail.com

Abstract

Denture stomatitis is mainly caused by a fungal species *Candida albicans.* The antifungal agents tested in this experiment are highly secure, especially essential oils, which have no residue after treatment. The agar well diffusion and disc diffusion methods were used to determine the antifungal activity, and the agar dilution method to determine the minimal inhibitory concentrations (MICs) of food additives and plant extracts. Butyl paraben sodium had the lowest MIC value of 0.25 mg • mL^{-1}, followed by chitosan, 0.5 mg • mL^{-1}, among the five food additives tested, and that thyme essential oil exhibited its MIC of 0.25 μL • mL^{-1}, the best among the five plant extracts tested. In view of its strong antifungal activity and user-friendliness (both liquid and fumigation usages), thyme essential oil may be used as a natural disinfectant for the prevention of denture stomatitis.

Keywords: Candida albicans, Denture stomatitis, Food additives, Plant essential oil, Minimum inhibition concentration (MIC).

1. Introduction

Denture stomatitis is defined as pathological changes of the mucosa under partial or complete removable dentures. These changes are characterized by erythema and usually found in both jaws, and are

 doi:10.1016/j.proenv.2012.01.435

commonly associated with angular cheilitis and median rhomboid glossitis [1]. It was further confirmed that the main pathogen of denture stomatitis is *Candida albicans* [2, 3].

Although brushing with soap and/or dentifrice is the method most used by denture-carriers to clean their dentures [4], this treatment neither lessens palatal inflammation nor eliminates mycelium forms. Some methods, such as the ones applying chlorine agents or phenol preparations, easily lead to the deformation of denture or the corrosion of its metal support, while others such as the formaldehyde fumigation have toxicity and irritation to human body. Therefore, effective and safe denture disinfectants are needed.

Plant extracts have been used for a wide variety of purposes for many thousands of years [5], and essential oils are potential sources of novel antimicrobial compounds [6]. They are now attracting attention as useful antimicrobials to be incorporated into mouth rinses. For example, Australian tea tree oil, peppermint, and sage oil were shown to be the most potent against anaerobic oral bacteria [7], and manuka, tea tree, eucalyptus, lavandula, and rosmarinus (rose-mary) oils to inhibit the growth of cariogenic and periodontopathic bacteria [8].

Food additives and preservatives are substances added to food to preserve flavor and improve taste and appearance, and many of them can also prevent or inhibit spoilage of food caused by microorganisms. Commonly used as preservatives and bactericides in personal care products, pharmaceutical preparations and food and beverages, the commercially known parabens are esters of the p-hydroxybenzoic acid [9].

We examined 5 commonly used food additives and 5 plant extracts for their anti *C. albicans* activities by agar well diffusion, disc diffusion, and agar dilution assays. We found that butyl paraben sodium and thyme oil had the strongest antifungal activity against *C. albicans*, with the minimal inhibitory concentrations at 0.25 mg • mL^{-1} and 0.25 μL • mL^{-1}, respectively.

2. Materials and Methods

2.1 Culture and reagents

Fungal species *C. albicans* was obtained from the fungal culture collection in the Second Affiliated Hospital of Zhejiang University Medical School, China. The isolate was maintained at 4°C on nutrient yeast dextrose agar (NYDA) medium containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose, and 20 g agar in 1 L distilled water. The fungal suspension of *C. albicans* was adjusted with sterile water to requisite concentrations before use. Five food additives (ethyl paraben sodium, butyl paraben sodium, potassium sorbate, chitosan, and tea polyphenols) were purchased from Shengxiao Chemicals Co., Hangzhou, China, and five plant extracts (bamboo flavonoids, thyme essential oil, sage essential oil, cinnamon essential oil, and curcuma wenyujin essential oil) from Qinyuan Natural Plant Technology Co., Hangzhou, China, and stored at 4°C.

2.2 Antifungal activity tests

The agar well diffusion method [10] with some modification and the disc diffusion method [11] with some modification were used to evaluate the anti *C. albicans* activities of food additives and plant extracts.

In the agar well diffusion method, 20 mL of NYDA was poured into sterilized culture dishes and allowed to solidify. 100 μL of *C. albicans* cell suspension (1×10^5 cfu • mL^{-1}) was added and uniformly spread onto the plates. The excess inoculum was drained and the plates were allowed to dry for 5 minutes. Wells were cut into the agar plates by using a cork borer, and 200 μL of various concentrations of 5 food

additives (ethyl paraben sodium, butyl paraben sodium, potassium sorbate, chitosan, and tea polyphenols) and 1 plant extract (bamboo flavonoids) were placed into each well. In the control plate, the wells were filled with 200 μL of sterile water. After 48 hours of incubation at 28°C, the diameter of the growth inhibition zone was measured. All samples were tested three times and averages were taken.

For the disc diffusion method, a 6-mm filter disc was placed on the surface of each of the plates that were inoculated with *C. albicans*. Essential oils (thyme, sage, cinnamon, and curcuma wenyujin) at various concentrations (1, 2, 4, and 8 μL • plate^{-1}) were applied onto each filter disc, and the plates were sealed with PVC tapes so that the oil volatilizes up onto the surface of the plates. A control plate contained paper discs with sterile water. The plates were incubated at 28°C for 48 hours and photographed by a camera (Canon, Japan), and then the diameter of the inhibition zone was measured. All samples were tested three times and averages were taken.

2.3 Minimal inhibitory concentration (MIC) test

The agar dilution method was used following the instructions of the National Committee for Clinical Laboratory Standards with the modification of the addition of Tween-20 (0.05%, v/v) into the agar after autoclaved to enhance oil solubility. Briefly, NYDA with 0.05% (v/v) Tween-20 containing food additives and plant extracts were serially diluted by two-fold to obtain concentration ranges of 0.063-1.00 mg • mL^{-1} and 0.063-1.00 μl • mL^{-1}, respectively. Plates were dried at 35°C for 30 minutes and then inoculated with 100 μL of *C. albicans* cell suspension (1×10^4 cfu • mL^{-1}). A plate containing 0.05% (v/v) Tween-20 without food additives or extracts was used as a negative control. Inoculated plates were incubated at 28°C for 48 hours. The MICs for *C. albicans* were determined after 48 hours. The MIC was defined as the lowest concentration of a substance that visibly inhibits the growth of an organism on the agar plate. If only one or two colonies were shown up within the plate, they were ignored.

3. Results

3.1 Antifungal activity of food additives and plant extracts against C. albicans

The results of antifungal activity tests by the agar well diffusion method and disc diffusion method are shown in Figures 1, 2 and 3. Among the five food additives, butyl paraben sodium had the highest antifungal activity, 27.1 mm in the zone inhibition at the concentration of 2%, followed by ethyl paraben sodium (25.7 mm, 2%), potassium sorbate (22.0 mm, 2%), chitosan (14.0 mm, 2%), and tea polyphenols (<6 mm, 2%) (Figure 1). In the five plant extracts, only thyme oil showed antifungal activity, with an inhibition zone of 45 mm at 8.0 μl • plate^{-1}, whereas bamboo flavonoids, sage essential oil, cinnamon essential oil, and curcuma wenyujin essential oil produced a mean zone of inhibition less than 6 mm (Figures 2 and 3).

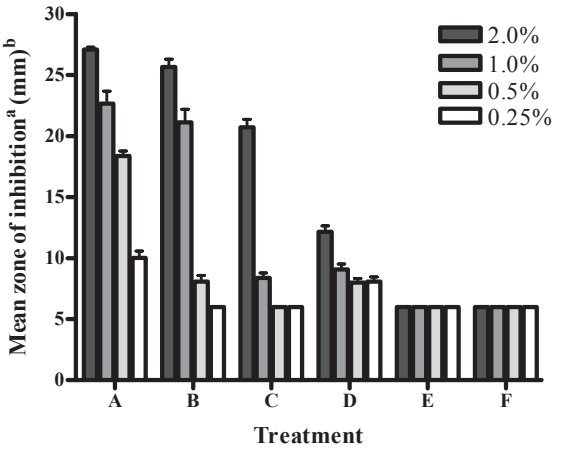

Fig. 1 Mean inhibition zones produced by food additives and bamboo flavonoids against *C. albicans*.
A: Butyl paraben sodium; B: Ethyl paraben sodium; C: Potassium sorbate; D: Chitosan; E: Tea polyphenols; F: Bamboo flavonoids
[a] Diameter of mean zone of inhibition including the well diameter of 6 mm
[b] Mean of three assays ± standard error

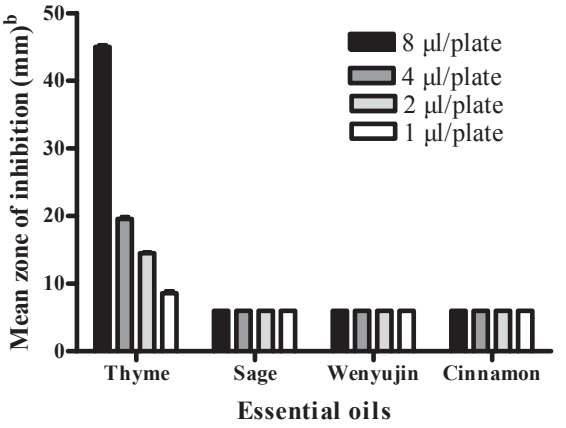

Fig. 2 Mean inhibition zones produced by essential oils against *C. albicans*.
[a] Diameter of mean zone of inhibition including the well diameter of 6 mm
[b] Mean of three assays ± standard error.

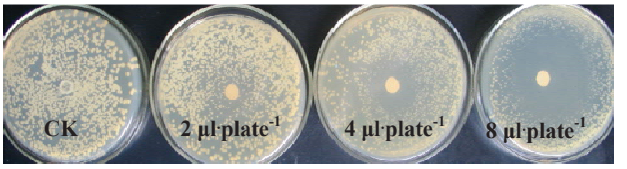

Fig. 3 Antifungal activity of thyme essential oil against *C. albicans* using disc diffusion method.

3.2 The minimum inhibition concentration (MIC)

Table 1 show MICs of 5 food additives and 5 plant extracts determined by the agar dilution method, respectively. Butyl paraben sodium had high activity against *C. albicans*, with the lowest MIC value of 0.25 mg • mL^{-1}, compared with 0.5 mg • mL^{-1} for chitosan, 1.0 mg • mL^{-1} for ethyl paraben sodium, 1.0 mg • mL^{-1} for potassium sorbate, and >1.0 mg • mL^{-1} for tea polyphenols (Table 1). For plant extracts, thyme essential oil had the lowest MIC of 0.25 μl • mL^{-1}, followed by bamboo flavonoids, sage essential oil, cinnamon essential oil, and curcuma wenyujin essential oil (>1.0 mg • mL^{-1}) (Table 1).

Table. 1 Average MIC values for food additives and plant extracts

Additives and plant extracts	Average MIC values ($mg \cdot mL^{-1}$ or $\mu L \cdot mL^{-1}$)
Butyl paraben sodium	0.25
Ethyl paraben sodium	1.00
Potassium sorbate	1.00
Chitosan	0.50
Tea polyphenols	>1.00
Bamboo flavonoids	>1.00
Thyme oil	0.25
Sage oil	>1.00
Cinnamon oil	>1.00
Curcuma wenyujin oil	>1.00

4. Discussion

According to the experiments carried on in vitro by Edgerton [12], yeasts and *C. albicans* were able to firmly attach to the surface of resin, glass, ceramics, and metal. At present, the disinfectants chlorhexidine and formalin, commonly used in cleaning the denture, are effective and inexpensive [13]. However, it is suggested that they may cause side effects, including staining, bleaching, and being accompanied by unpleasant odor [14]. In contrast, the antifungal agents tested in this experiment are highly secure, especially essential oils, which have no residue after treatment.

Butyl paraben sodium and ethyl paraben sodium and potassium sorbate are the most soluble forms of paraben and sorbate, respectively, and are well-known for their potent antifungal activity. In food systems, they are the most widely used compounds to prevent the growth of molds and thus extend the shelf life of products [15]. Our experimental results indicate that butyl paraben sodium, ethyl paraben sodium, and potassium sorbate had higher antifungal activity with MIC values of 0.25-1.0 mg • mL^{-1} against *C. albicans* in vitro.

A wide range of aromatic substances, or phytochemicals, can be found in plants, and many of them have antimicrobial activity. Sokovié and van Griensven indicated that thyme essential oil, composed of p-cymene and thymol, can strongly inhibit important fungal plant and foodborne pathogens [16]. In the current study, we observed that thyme oil had very low MIC (0.25 μL • mL^{-1}) by the agar dilution method, consistent with the finding of Hammer, who reported that thyme oil had a MIC of 0.03% (v/v) against *C. albicans*, the lowest in 20 plant extracts investigated [17].

In conclusion, we found that 3 food additives including butyl paraben sodium, ethyl paraben sodium, and potassium sorbate and 1 essential oil, the thyme oil, possessed pretty strong antifungal activity against *C. albicans* in vitro. In view of its broad activity and user-friendliness (both liquid and fumigation usages), thyme essential oil may be used as a natural disinfectant for the prevention of denture stomatitis.

Acknowledgment

This research was supported by grants from the Science and Technology Project of Zhejiang Province (2007C33016) andAuthor of National Excellent Doctoral Dissertation of PR China (FANEDD201061). The authors thank Mr. Lou for critical reading of the manuscript.

References

[1] T. Arendorf and D. Walker, "Denture stomatitis: a review," *J Oral Rehabil*, vol. 14, pp. 217-27, 1987.

[2] C. Dong and F. Zhang, "Influence of removable partial denture on oral microecosystem," *Int J Stomatology*, vol. 3, pp. 71-3, 2008.

[3] Y. Liu and Y. Mou, "The research of denture stomatitis," *Chin J Prosthodontics*, vol. 8, pp. 151-3, 2007.

[4] C. Coelho, Y. Sousa and A. Dare, "Denture-related oral mucosal lesions in a Brazilian school of dentistry," *J Oral Rehabil*, vol. 31, pp. 135-9, 2004.

[5] F. Jones, "Herbs-useful plants. Their role in history and today," *Eur J Gastroenterol Hepatol*, vol. 8, pp. 1227-31, 1996.

[6] S. Prabuseenivasan, M. Jayakumar and S. Ignacimuthu, "In vitro antibacterial activity of some plant essential oils," *BMC Complement Altern Med*, vol. 6, pp. 39, 2006.

[7] S. Shapiro, A. Meier and B. Guggenheim, "The antimicrobial activity of essential oils and essential oil components towards oral bacteria," *Oral Microbiol Immunol*, vol. 9, pp. 202-8, 1994.

[8] K. Takarada, et al, "A comparison of the antibacterial efficacies of essential oils against oral pathogens," *Oral Microbiol Immunol*, vol. 19, pp. 61-4, 2004

[9] L. Nunez, J. Tadeo, A. Garcia-Valcarcel, and E. Turiel, "Determination of parabens in environmental solid samples by ultrasonic-assisted extraction and liquid chromatography with triple quadrupole mass spectrometry," *J Chromatogr A*, vol. 1214, pp. 178-82, 2008.

[10] U. Schillinger and F. Lucke, "Antibacterial activity of Lactobacillus sake isolated from meat," *Appl Environ Microbiol*, vol. 55, pp. 1901-61989, 1989.

[11] V. Berghe and A. Vlietinck, "Screening methods for antibacterial and antiviral agents from higher plants," London Press, 1991.

[12] M. Edgerton and M. Levine, "Characterization of acquired denture pellicle from healthy and stomatitis patients," *J Prosthet Dent*, vol. 68, pp. 683-91, 1992.

[13] D. Jagger and Harrison A, "Denture cleaning-the best approach," *Br Dent J*, vol. 178, pp. 413-7, 1995.

[14] M. Fang, J. Cheng and X. Xu, "Evaluation the ability of inorganic antibacterial agents against oral pathogenic bacteria: a method comparison," *J Oral Sci Res*, vol. 1, pp. 38-40, 2006.

[15] S. Valencia-Chamorro, Palou L, M. Del Rio and M. Perez-Gago, "Inhibition of *Penicillium digitatum* and *Penicillium italicum* by hydroxypropyl methylcellulose-lipid edible composite films containing food additives with antifungal properties," *J Agric Food Chem*, vol. 56, pp. 11270-8, 2008.

[16] M. Sokovié and V. Griensven, "Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*," *Euro J Plant Pathology*, vol. 116, pp. 211-24, 2006.

[17] K. Hammer, C. Carson and T. Riley, "Antimicrobial activity of essential oils and other plant extracts," *J Appl Microbiol*, vol. 86, pp. 985-90, 1999.