



Investigating the impact of non-nutritive sweeteners on the antifungal potential of alcoholic and aqueous Eucalyptus extracts against salivary candida albicans (An in-vitro study)

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Abstract: Background: Eucalyptus extracts and derivatives are natural substances with potent antimicrobial properties. This study investigated the in-vitro effects of non-nutritive sweeteners on the antifungal activity of alcoholic and aqueous Eucalyptus extracts against *Candida albicans*, a common oral pathogen. Materials and Method: Ten isolates of *Candida albicans* were isolated from dental students' salivary samples. The alcoholic and aqueous extracts were prepared from fresh Eucalyptus leaves using maceration. The sensitivity of *Candida albicans* isolates to various concentrations of Eucalyptus extracts ranging from 50 to 250 (mg/mL) was evaluated via agar well diffusion method, while the agar streaking method was used to assess the minimum fungicidal concentration (MFC). In addition, the effect of non-nutritive sweeteners on the MFC of the extracts was investigated. Results: The Eucalyptus extract-sensitive *Candida albicans* isolates showed an increase in inhibitory zone width with increasing extract concentration. Regarding their antifungal effectiveness, clear disparities were observed among extract concentrations. Against *Candida albicans*, the MFC for Eucalyptus alcoholic extract was 75 mg/mL, but the MFC for Eucalyptus aqueous extract was 200 mg/mL. Notably, 15% stevia and 5% sucralose did not affect the antifungal effects of the Eucalyptus alcoholic extract. The antifungal effectiveness of the aqueous Eucalyptus extract against *Candida albicans* was unaffected by stevia and sucralose concentrations of up to 1%. Conclusion: Significant antimicrobial action against *Candida albicans* is shown in Eucalyptus extracts. Results indicated that stevia and sucralose at specific quantities could be utilized as sweeteners for Eucalyptus extracts in an efficient manner without impairing the extracts' antifungal activity.

Keywords: *Candida albicans*, Eucalyptus extract, Non-nutritive sweeteners, Stevia, Sucralose

Introduction

Eucalyptus belongs to the Myrtaceae family and is one of the most significant and widely cultivated genera globally. The family comprises 140 genera and 3800 species and is primarily found in tropical and subtropical regions ⁽¹⁾. Myrtaceae phytochemicals, known for their antimicrobial and anti-inflammatory properties, are valuable for medicinal and cosmetic products, including hair care and skin care ⁽²⁾. Owing to the basic composition of the leaves comprising cineole or eucalyptol, Eucalyptus extracts, which are rich in polyphenols and terpenoids, are successfully employed in different oral hygiene products, such as mouthwash, toothpaste, and antiplaque chewing gum ⁽³⁾. The impact of 1,8-cineole, known for its anti-inflammatory and secretolytic properties, on the airway has attracted interest. Vicks VapoRub and other over-the-counter cough medications often include this substance ⁽⁴⁾. Intense sweeteners, commonly referred to as non-nutritive sweeteners (NNSs), act as sugar substitutes that enhance the sweetness of

meals and drinks without adding calories or sugar. Their appeal in a variety of goods is a result of their increased sweetness when compared with that of normal sugar ⁽⁵⁾.

Owing to its enticing benefits, sucralose is one of the artificial sweeteners recommended for FDA clearance as a generally recognized safe component ⁽⁷⁾. It is stable at all temperatures and pH levels and 600 times sweeter than sugar. It is a noncaloric sweetener that does not degrade in the body ⁽⁸⁾. Artificial sweeteners are popular because of their sweetness and low-calorie content, but they have no nutritional benefits ⁽⁹⁾.

The most prevalent human fungal pathogen, *Candida albicans*, often lives in various body areas where the immune system of a host controls its development and leads to a variety of illnesses ⁽¹⁰⁾⁽³⁵⁾. It is considered a part of the commensal human microflora. However, *Candida albicans* may quickly become pathogenic if the host's immune system deteriorates or the environment changes ⁽¹¹⁾⁽³⁴⁾. It commonly results in nosocomial infections and can infiltrate superficial and deep organs in humans ⁽¹²⁾. The fungus's pathogenic potential is attributed to a number of virulence characteristics, such as its capacity to form parallel-walled true hyphae or ovoid-shaped budding yeast ⁽¹³⁾. It also possesses specialized proteins that enable it to easily attach to abiotic surfaces, host cells, other microbes, and other *Candida albicans* cells ⁽¹⁴⁾. On biotic and abiotic surfaces, *Candida albicans* may develop biofilms ⁽¹⁵⁾ and secretes hydrolases during hyphal development and adherence to host cell surfaces; the hydrolases facilitate active penetration into cells ⁽¹⁶⁾. Additionally, the fungus has pH sensing and control skills that enable it to adjust to pH variations in the environment ⁽¹⁷⁾.

This study's major goal was to determine how NNSs affect the ability of alcoholic and aqueous Eucalyptus extracts to fight salivary *Candida albicans*.

Materials and Methods:

The scientific committee at the Basic Science Department/College of Dentistry, University of Baghdad, approved the protocol of this study. Additionally, the research ethics committee at the College of Dentistry/University of Baghdad approved the study. In- vitro experiments were performed to examine the influence of NNSs on the antifungal activity of Eucalyptus extracts (alcoholic and aqueous) against salivary *Candida albicans*.

Saliva samples were collected from dental students at the College of Dentistry, University of Baghdad under standard conditions with the method of previous researcher ⁽¹⁸⁾. Healthy dental students, aged 18–23, volunteered, and stimulated saliva was obtained after the students chewed Arabic chewing gum for three minutes. From the saliva, 15 fungal isolates of *Candida albicans* were obtained using tenfold serial dilutions and spread on Sabouraud dextrose agar plates, which are selective media for isolating *Candida albicans*. The isolates were incubated aerobically for 48 hours. Identification involved testing colonies from the agar plates with gram staining and germ tube formation in human sera.

Fresh Eucalyptus leaves were collected from a local tree in Baghdad and identified by a botanist. The leaves were thoroughly cleaned and rinsed before the preparation of alcoholic and aqueous extracts with the method of Richardson and Harborne (1985). A stock solution of 300 mg/ml was prepared for each extract, and final concentrations of 50, 100, 150, 200, and 250 mg/ml were used for testing. The NNSs stevia (in powder form) and sucralose (12.5% aqueous compound) were obtained from a local market. Final concentrations of 1%, 5%, and 10% for stevia and 1%, 2%, and 3% for sucralose were prepared by dissolving the sweeteners in distilled water and sterilized using a Millipore filter.

To study the antifungal effects of the Eucalyptus extracts, stevia, and sucralose, we employed the agar well diffusion technique on brain heart infusion agar. Ten isolates of *Candida albicans* were used McFarland standard turbidity to approximate microbial cell density (1.5×10^8 CFU/ml) by adding more microbes or more sterile saline ⁽²⁰⁾. Equally sized and deep wells were created in the agar plates, and

different concentrations of the extracts were added to the wells. Distilled water and DMSO were used as the controls for the aqueous and alcoholic extracts, respectively. After incubation, the diameters of the inhibition zones were measured. The minimum fungicidal concentration (MFC) of the Eucalyptus extracts was determined using the agar streaking method described by AL-Mizraqchi (1998). The MFC represents the lowest extract concentration that effectively killed the microorganisms.

Once the MFC values for the extracts were established, the specified concentrations of stevia and sucralose were added to the MFC of the extracts for the assessment of the effects of NNSs on the antifungal activity of alcoholic and aqueous Eucalyptus extracts against salivary *Candida albicans*.

Statistical analysis was performed using the general linear model (Univariate Factorial ANOVA) to determine the effects of categorical independent variables on quantitative dependent variables. The Tukey's honestly significant difference (Tukey's HSD) post hoc test was also applied. Data were represented with minimum, maximum, mean, and standard deviation (SD). The level of significance was determined as "not significant" ($P > 0.05$) or "significant" ($P < 0.05$).

Results:

The findings demonstrated that *Candida albicans* isolates were sensitive to alcoholic and aqueous Eucalyptus extracts, respectively, and the width of the inhibitory zone against the test microorganisms increased with extract concentration. Significant differences were observed among the various doses of each Eucalyptus extract ($P < 0.05$; Table 1). Additionally, analysis of the antifungal activity of the two extracts against the test microorganisms revealed a significant difference between them ($P < 0.05$; Table 3).

Table 1: An investigation of the width of inhibition zones against isolates of *Candida albicans* at different *Eucalyptus* extract doses. Descriptive statistics and the findings of statistical analyses performed to compare inhibition zone sizes were included in the data.

Extract	Concentration (mg/ml)	Minimum	Maximum	Mean	±SD	F	P value*	Effect size
Eucalyptus alcoholic	50	10.500	11.500	10.800	0.350	1199.305	0.000	0.982
	100	14.000	15.000	14.600	0.459			
	150	18.000	19.000	18.550	0.438			
	200	21.000	22.000	21.600	0.459			
	250	23.000	25.000	24.150	0.669			
Eucalyptus aqueous	50	8.500	9.000	8.800	0.258	897.988	0.00000	0.976
	100	10.500	11.500	11.000	0.408			
	150	13.000	14.500	13.700	0.537			
	200	16.000	17.000	16.600	0.459			
	250	20.000	22.000	20.550	0.685			

*Significant at $P < 0.05$.

Table 2: The Tukey HSD test was used to compare the concentrations of *Eucalyptus* extracts against 10 isolates of *Candida albicans*. The results are shown in Table 2, which provides useful information on the notable variations in antifungal activity among extract concentrations.

Concentration		Extract			
		Eucalyptus alcoholic		Eucalyptus aqueous	
		Mean deference	p value*	Mean deference	p value*
50	100	-3.800	0.000	-2.200	0.000
	150	-7.750	0.000	-4.900	0.000
	200	-10.800	0.000	-7.800	0.000
	250	-13.350	0.000	-11.750	0.000
100	150	-3.950	0.000	-2.700	0.000
	200	-7.000	0.000	-5.600	0.000
	250	-9.550	0.000	-9.550	0.000
150	200	-3.050	0.000	-2.900	0.000
	250	-5.600	0.000	-6.850	0.000
	200	-2.550	0.000	-3.950	0.000

*Significant at P <0.05.

Table 3: Descriptive and statistical test of diameter of inhibition zone of *Candida albicans* between extracts concentrations.

Concentration	Minimum	Maximum	Mean	±SD	±SE	F	P value*	Effect size	
50	Eucalyptus alcoholic	10.50	11.50	10.800	0.350	0.111	83.818	0.00000	0.482
	Eucalyptus aqueous	8.50	9.00	8.800	0.258	0.082			
100	Eucalyptus alcoholic	14.00	15.00	14.600	0.459	0.145	271.572	0.00000	0.751
	Eucalyptus aqueous	10.50	11.50	11.000	0.408	0.129			
150	Eucalyptus alcoholic	18.00	19.00	18.550	0.438	0.138	492.905	0.00000	0.846
	Eucalyptus aqueous	13.00	14.50	13.700	0.537	0.170			
200	Eucalyptus alcoholic	21.00	22.00	21.600	0.459	0.145	523.865	0.00000	0.853
	Eucalyptus aqueous	16.00	17.00	16.600	0.459	0.145			
250	Eucalyptus alcoholic	23.00	25.00	24.150	0.669	0.211	271.572	0.00000	0.751

*Significant at P <0.05.

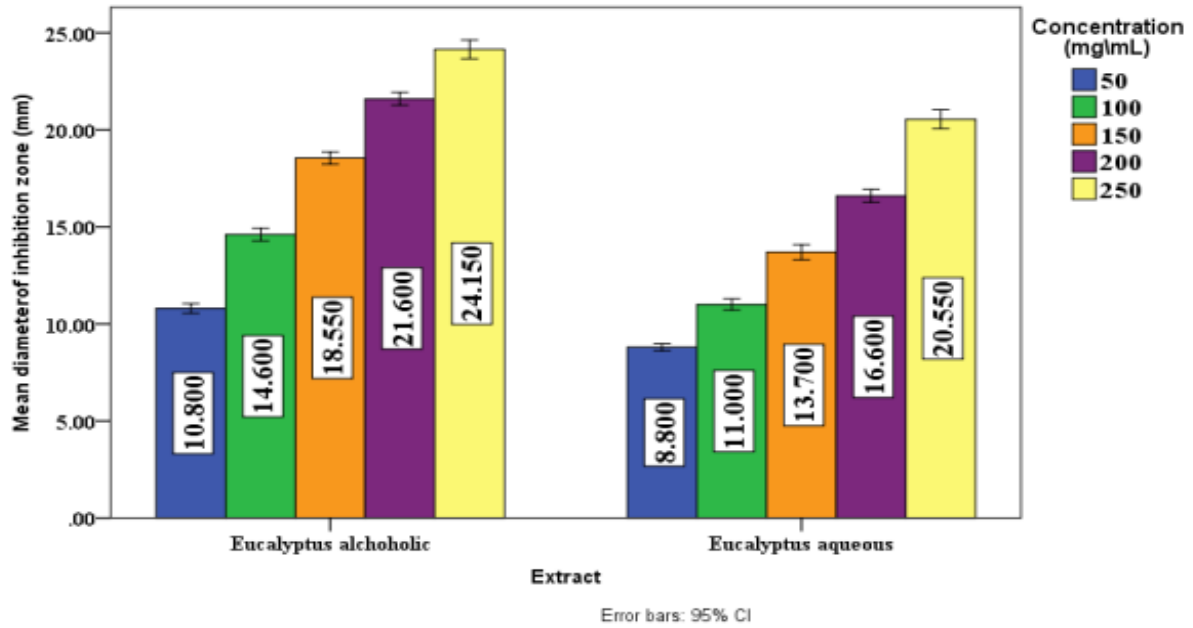


Figure 1: Mean diameter of inhibition zone of alcoholic and aqueous Eucalyptus extracts against *Candida albicans*

The MFC of the Eucalyptus alcoholic extract against *Candida albicans* was 75 mg/ml, whereas the MFC of the aqueous Eucalyptus extract was 200 mg/ml (Figure 2). *Candida albicans* isolates exhibited greater sensitivity to the alcoholic extract. In addition, the antifungal activity of the extracts was unaffected by the addition of 1% stevia or sucralose at MFC.

Moreover, increasing the stevia concentration to up to 15% had no effect on the antifungal activity of the alcoholic extract against *Candida albicans*. Similarly, increasing the sucralose concentration to 5% had no effect on the antifungal activity of the alcohol extract. By contrast, when the concentrations of stevia or sucralose were greater than 1% in Eucalyptus aqueous extract at MFC, antifungal activity was disrupted.

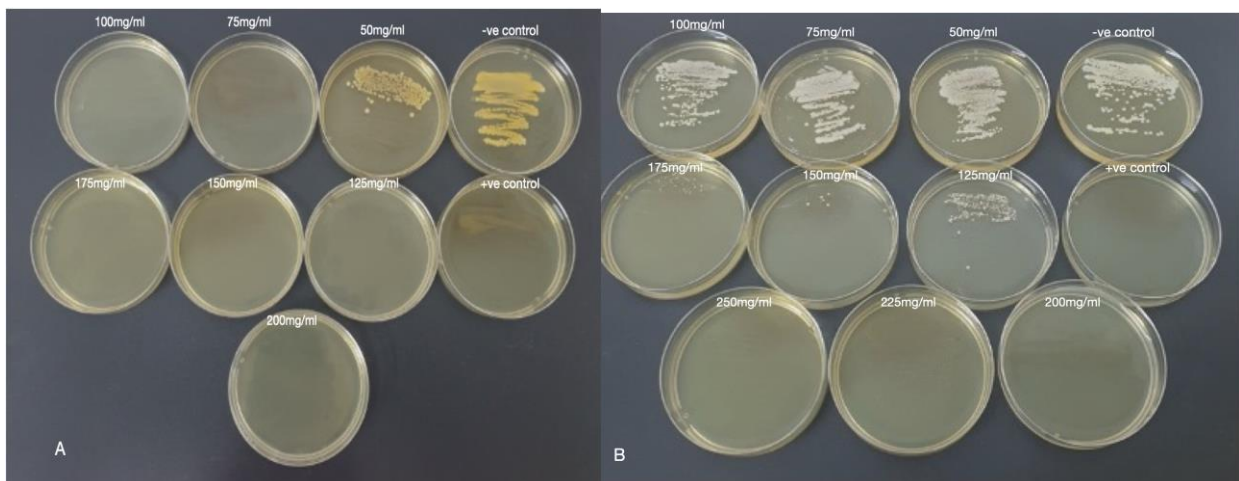


Figure 2: Minimal fungicidal concentration of: A: Eucalyptus alcoholic extract against *Candida albicans*. B: Eucalyptus aqueous extract against *Candida albicans*.

Discussion

Oral diseases are widespread globally and have significant health and economic implications for affected individuals, leading to a reduced quality of life. Dental infections and periodontal diseases are the most prevalent and consequential oral disorders worldwide ⁽²²⁾, and oral hygiene practices plays a crucial role in their prevention ⁽²³⁾. Dentists traditionally use various tools and compounds to maintain the optimal oral health of patients. To explore novel antimicrobial compounds, scientists have investigated alternative sources, such as medicinal plants ⁽²⁴⁾.

Candida albicans is a part of the human oral microbiota. It possesses various virulence factors that can cause severe damage to oral soft tissues under some conditions ⁽²⁵⁾. In this study, the alcoholic extract of *Eucalyptus* resulted in the more substantial inhibition zone against experimental microorganisms. This finding is consistent with that of previous research on the antimicrobial activity of *Eucalyptus camaldulensis* leaves; the ethanolic extracts were found to be more effective than water extracts against specific microorganisms ⁽²⁶⁾.

Differences in antifungal activity among different concentrations and among different extracts (alcoholic and aqueous) may be attributed to the presence of oxygenated monoterpenes, such as 1,8-cineole, and monoterpene hydrocarbons, such as α -pinene, β -pinene, and limonene. These compounds are insoluble in water but can be mixed with organic solvents, including ethanol. The presence of these compound may explain why the alcoholic extract exhibited higher sensitivity. Meanwhile, the aqueous extract contained water-soluble compounds, such as flavonoids (ellagic acid, quercitrin, and kaempferol 3-O-glucoside) ⁽²⁷⁾⁽²⁸⁾.

Before the addition of NNSs, all microbial isolates were eliminated at the same MFC. No significant interactions between NNSs at concentrations of 1% or less and primary antimicrobial molecules, including total polyphenols, catechins, tannins, and flavonoids, in these extracts were observed. Similar results were found in studies on the effect of adding NNSs to tea, where no significant effect was observed on the total phenolic compounds and radical scavenging activity of phenolic compounds ⁽²⁹⁾.

Moreover, the addition of higher concentrations of stevia (up to 15%) and sucralose (up to 5%) did not alter the antifungal activity of alcoholic *Eucalyptus* extract against salivary *Candida albicans*. Potent compounds, such as limonene and 1,8-cineole ⁽³⁰⁾, which are insoluble in water but miscible with ether, ethanol, and chloroform ⁽³¹⁾, may have contributed to the antimicrobial activity of the alcoholic extract. However, concentrations of NNSs exceeding 15% (stevia) or 5% (sucralose) seemed to have counteracted the antimicrobial activity of the alcoholic *Eucalyptus* extract against salivary *Candida albicans* beyond certain limits. The possible reason was that the alcoholic *Eucalyptus* extract became saturated at high concentrations of stevia and sucralose and antimicrobial effectiveness subsequently decreased.

Conclusion

Alcoholic and aqueous *Eucalyptus* extracts had antimicrobial activity against salivary *Candida albicans*. *Eucalyptus* extracts can be sweetened and fortified successfully with specific concentrations of stevia and sucralose without interfering with the extracts' antifungal activity.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

DMA; study conception and design, data collection, methodology. ASA and DMA; statistical analysis and interpretation of results. DMA; original draft manuscript preparation. DMA and ASA; Writing -

review & editing. Supervision; DMA and ASA. All authors reviewed the results and approved the final version of the manuscript to be published.

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Informed consent

Informed consent was obtained from all individuals or their guardians included in this study.

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العنوان: التحقيق في تأثير المحليات غير الغذائية على القدرة المضادة للفطريات لمستخلصات اليوكالبتوس المائية والكحولية ضد فطريات المبيضات البيضاء (دراسة مختبرية)
الباحثون: طيبة الإنسان داليا مصطفى القرعة لوسي , أستاذة دكتور عباس صبري المزرقجي
المستخلص:

الخلفية: يعتبر تجويف المدخل مرحلة حرجة في علاج قناة الجذر وقد يؤثر على الخطوات اللاحقة للعلاج. يهدف المفهوم الجديد لتحضير تجويف اللبنة طفيف التوغل إلى الحفاظ على بنية الأسنان السليمة من خلال الحفاظ على عاج الأسنان السليم قدر الإمكان بما في ذلك سقف حجرة اللب ، للحفاظ على الأسنان من الكسر أثناء وبعد المعالجة اللبنة. في حين أن هناك اهتمامًا كبيرًا يمثل هذه التصاميم الافتتاحية للقبول في العديد من المنشورات ، لا يزال هناك نقص في الأدلة العلمية لدعم تطبيق تصاميم تجويف الوصول الحديثة في الممارسة السريرية. تهدف إعادة العرض هذه إلى فحص الأدبيات الخاصة بالحد الأدنى من تحضيرات تجويف الوصول بشكل نقدي ، وإبراز تأثير تصميمات تجويف الوصول طفيفة التوغل على جوانب مختلفة من معالجة قناة الجذر ، وتحديد المجالات التي تتطلب بحثًا إضافيًا.

البيانات: تم إجراء بحث إلكتروني عن مقالات باللغة الإنجليزية باستخدام قواعد البيانات التالية Google Scholar و PubMed و Research Gate. تم استخدام الكلمات الرئيسية التالية: "تجويف الوصول الأقل بضعًا"، "تجويف اللبنة المحافظ"، و "تصنيف تجويف الوصول".

اختيار الدراسة: تم اختيار 64 ورقة بحثية كانت الأكثر صلة بالموضوعات في هذه المراجعة بين عامي 1969 و 26 فبراير 2022. الاستنتاجات: يمكن تصنيف تجاويف الوصول طفيفة التوغل إلى تجاويف محافظة ، شديدة التحفظ ، وصول تروس ، تسوس ، تجاويف مدفوعة تصالحية. هناك نقص في الدليل على أن تجويف الوصول طفيف التوغل يحافظ على مقاومة الكسر في الأسنان المعالجة بشكل أسنان أكبر من تجاويف الوصول التقليدية. لم يكن هناك فرق في النسبة المئوية للجدران التي لم تمسها وإزالة الحطام في الأسنان ذات التجاويف المحافظة مقابل التجاويف التقليدية ، ومع ذلك ، نتج عن تجاويف تروس وتجاويف الوصول شديدة التحفظ ضعف كفاءة الري مقارنة بالآليات التقليدية. أيضًا ، ارتبطت مقاومة الإجهاد الدوري السفلية للأدوات الدوارة وسد قناة الجذر بالفراغات بتجاويف وصول طفيفة التوغل. لا تزال الدراسات حول تجاويف الوصول طفيفة التوغل تحتوي على مجموعة واسعة من العيوب المنهجية أو تسجل نتائج غير مرضية أو غير قاطعة. لذلك ، هناك حاجة إلى مزيد من البحث حول هذا الموضوع خاصة مع التقدم اليومي للتقنيات والأسلحة المستخدمة في علاج جذور الأسنان.