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The effect of nonnutritive sweeteners on the antifungal activity of black and green tea aqueous extracts against salivary Candida albicans an (in-vitro study)

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Abstract---Oral diseases, while largely preventable, pose a major health burden for many countries and affect people throughout their lifetime, causing pain, discomfort and disfigurement. Mouth washing agents are an effective vehicle for antimicrobial compounds in reducing oral pathogens, managing dental plaque and maintaining proper oral health. Numerous plant extracts have been successfully incorporated into oral care products due to their antimicrobial properties, availability, and affordability. Tea plants and extracts are one of the most important natural compounds used in dental care products. The problem with tea extracts is their bitter taste, which could compromise their usage by people. So, it needs a kind of supplementation to be palatable, especially for the pediatric and geriatric populations. Hence a nonnutritive sweetener was added to tea extracts to suppress their bitter taste. This study aimed to determine the effect of nonnutritive sweeteners on the antifungal activity of black and green tea aqueous extracts against salivary C. albicans. The results showed that adding 1% of stevia and sucralose to the black and green tea aqueous extracts did not affect the antifungal activity against salivary C. albicans. So, 1% of stevia and sucralose could be successfully used as a taste-masking approach to suppress the bitter taste of tea extracts without interrupting their antifungal activity.

Keywords---Candida albicans, Tea extracts, Antifungal activity, Stevia, Sucralose.
Introduction

Although oral health is widely acknowledged as an important factor in overall quality of life, more focus has been placed on its local clinical effects, even though they might influence people’s everyday lives (Spanemberg et al., 2019). Dental plaque, a biofilm made of microbes and leftover food particles, forms on teeth if proper dental hygiene is not practiced. Brushes, floss, and interdental cleaners are mechanical aids, whereas mouthwashes, dentifrices, and chewing gums are chemotherapeutic agents used in oral hygiene practice (Samaranayake, 2018). Mouthwashes are the vehicles for antimicrobial substances developed to lessen the number of pathogens in the mouth. They provide a pharmacological approach to preventing and treating plaque buildup that is safe and effective (Sugano, 2012). Many commercial oral rinses containing compounds like alcohol, chlorhexidine gluconate and triclosan could cause taste disruption, allergic contact stomatitis and many negative consequences that may be brought on by these compounds (Kaur and Kour, 2020). Herbal mouthwashes, made from harmless herbs and plant extracts, have been developed as a means of avoiding these undesirable effects and considerable research has shown the efficiency of herbal mouthwashes (Qanbar and Al-Mizraaqchi, 2009; Al-Lamy, and Al-Mizraaqchi, 2012; Aldhaher, 2013) in that, the tea extract, in particular, has become quite popular among these herbal supplements (Raju et al., 2020). Aside from water, tea is the most extensively consumed drink in the world (Pavia et al., 2021). Caffeinated non-alcoholic drinks made from the Camellia sinensis plant via an infusion process are the only ones that may properly be called "tea" (Shang et al., 2021). Polyphenols, especially catechins and their derivatives, as well as pigments, alkaloids, amino acids, and saponins, have all been identified as bioactive components in tea and its brewing (Chen et al., 2021). tea and its extracts have antibacterial, antifungal, anti-inflammatory, and immunoregulatory properties (Al-Ezzi et al., 2018; Wu and Brown, 2021).

Nonnutritive sweeteners (NNS), often known as non-caloric sweeteners, are "food additives" that have a sweet flavour comparable to sugar but fewer calories (Schiano et al., 2021). Chemical synthesis, or the industrial production of plant parts, is how they are created. They are non-fermentable and subsequently non-cariogenic; hence Chewing gum and sweets containing them are made to reduce the risk of dental caries by replacing the carbs that would otherwise be eaten by people with diabetes (Purohit and Mishra, 2017).

Originally from South America, stevia (Stevia rebaudiana Bertoni) is a perennial plant in the Asteraceae family. The plant is grown in many different regions, including the Middle East. Because it contains several Steviol glycosides in its leaves, it has a sweetening efficacy 200–300 times higher than sucrose. Therefore, it might be used as a sugar replacement in the food and pharmaceutical industries (Ghaheri et al., 2018). In addition to these glycosides, stevia is a good source of essential amino acids, fatty acids, and vitamins, including niacin, thiamine, and ascorbic acid may be derived from this plant (Ahmad et al., 2020).
Sucralose, sold under the trade name Splenda, is a non-caloric sweetener authorized for use in foods and drinks; it has a sweetening power of around 600 times that of sugar (Martyn et al., 2018).

The human mouth is home to a unique and very unstable environment. In rare circumstances, the equilibrium may be tipped toward an elevated risk of oral illnesses due to disrupting the usual commensal connection between the host and the resident oral microbiota (Willis and Gabaldón, 2020). *C. albicans* is the most common fungal human pathogen. However, it is also a commensal human microflora member that colonizes many regions of the human body where the host's immune system limits its development and causes a wide variety of illnesses (Byadarahally and Rajappa, 2011; Tsui et al., 2016). If the host's immune system is compromised or the host's environment is disturbed, *C. albicans* may rapidly morph into a pathogen (Pappas et al., 2018). The pathogenic potential of this fungus may be attributed to several virulence factors and activities, including polymorphism which allows the fungus to develop into either ovoid budding yeast or genuine linear hyphae (Lohse et al., 2017). Aside from that, it possesses proteins that let it stick to things like other *C. albicans* cells, other microbes, abiotic surfaces, and host cells (Naglik et al., 2017). However, biofilm formation by *C. albicans* is possible on both abiotic and biotic surfaces (Atriwal et al., 2021). It is hypothesized that after adhering to host cell surfaces and expanding through hyphae, it secretes hydrolases that aid in its active penetration into the host cells (Arendrup et al., 2019).

This study aimed to determine the effect of nonnutritive sweeteners on the antifungal activity of black and green tea aqueous extracts against salivary *Candida albicans*

**Materials and methods**

This study was an *in-vitro* approach to examine how adding nonnutritive sweeteners affected the antifungal activity of black and green tea aqueous extracts against *C. albicans* isolated from saliva. Saliva samples were taken under normal settings, with stimulation, as described by Tenovuo and Lagerlöf (1994), and were collected from healthy dental students aged 18 to 23 who participated in the research. Each participant chewed on a piece of Arabic chewing gum (0.45g) for three minutes to increase salivation, and then their saliva was collected in screw-capped, sterilized bottles. Two minutes were spent in a vortex mixer, bringing consistency to the saliva. Ten-fold serial dilutions were performed using phosphate buffer saline (PBS). Sabouraud dextrose agar (SDA) plates were used to disseminate 0.1 ml of each sample at dilutions of $10^{-1}$ and $10^{-3}$ before incubating them aerobically for 48 hours. These plates are the selective medium for isolating *C. albicans* (Murray et al., 2004). Under sterile circumstances, a colony was extracted from agar plates and stained with Gram stain to determine its identity. Germ tube development was also evaluated during a 37°C incubation of 0.5 ml human serum containing fungal inoculum.

According to Cowans (1999), the aqueous tea extracts were prepared by infusing 100 g of dried black and green tea leaves in 500 ml of boiling distilled water and letting them cool before storing them for 24 hours. Once the infusion had been
poured over filter paper (Wattman No. 1), the leftover sediment was thrown away. The infusion was allowed to air-dry in a glass Petri plate at room temperature. The powdery byproduct of the reaction was collected and kept in the dark glass container at room temperature until it was required to create different concentrations. Then concentrations of (50mg/ml, 100mg/ml, 200mg/ml, 300mg/ml, and 500mg/ml) were made by adding the powder to different volumes of deionized, sterile, distilled water. on the other hand, two varieties of nonnutritive sweeteners: stevia in powder form and sucralose in the form of an aqueous compound with a 12.5% concentration, were sold from a local supermarket. Stevia final concentrations of 1%, 5%, and 10% were prepared by dissolving the powder in distilled water, and final sucralose concentrations of 1%, 2%, and 3% were prepared from the stock compound after dilution in distilled water. All the extracts and the nonnutritive stock compounds were sterilized by filtration through Millipore filter 0.22µm.

Using the agar well diffusion technique, the antifungal effects of both types of tea extract, as well as the antifungal potential of stevia and sucralose, were examined against isolates spread on Brain Heart Infusion Agar (BHI-A) according to (Valgas et al., 2007). Ten different C. albicans isolates were employed in this investigation. The microbial inoculum density was adjusted to match the McFarland standard turbidity (0.5 for fungal isolates) to achieve the target microbial cell density of 1.5 x 10⁶ CFU/ml. Moreover, using a 6mm Kork porer, uniformly sized and shaped wells were carved out of the agar. Each well received 0.1ml of extract, with distilled water as a control.

Further, the plates were kept in an incubator at 37 degrees Celsius for 24 hours. The agar streaking technique was used to determine the minimum fungicidal concentration of black and green tea extract. Tube dilutions of tea extract in BHI-B at several concentrations were made, inoculated with 0.1 ml of freshly activated fungal inoculum, and then incubated aerobically for 24 hours. Then the next day, dipping a sterile microbiological lobe in these bottles and then streaking on BHI-A to determine the MFC of the extract. And then, all these Petri dishes were incubated for 24 hours at 37°C to gather with the control plates (negative control, BHI-A streaked with microbial inoculums without adding the extract) and the positive control plates (BHI-A streaked with different concentrations of tea extracts without microbial inoculums). Microbial growth was observed in each petri dish. Moreover, after the determination of the MFC value for the black and green tea against the test microbes, the previously mentioned concentrations of stevia and sucralose were added to the MFC according to Al- Mizraqchi (1998) to conduct the effect of adding nonnutritive sweeteners on the antifungal activity of black and green tea aqueous extracts against salivary C. albicans.

**Statistical Analysis**

The recorded data were examined by using General linear Model (Univariate Factorial ANOVA): statistical test used to find the effect of two or more than two categorical variables (independent) on the quantitative dependent variable and measure the main and interaction effect of those independent variables and using Tukey’s Honestly Significant Difference (Tukey’s HSD) post hoc test. and the data were represented as minimum, maximum, mean, and Standard Deviation (SD).
Level of significance as: Not significant $P>0.05$, Significant $P<0.05$.

**Results**

The results showed that *C. albicans* isolates were sensitive to the aqueous extracts of black and green tea, respectively, and the diameter of the inhibition zone was found to increase as the concentrations of the extracts increased (Figure 1). Furthermore, there was a significant difference between the concentration of both of the tea extracts in their antifungal activity ($P$ value $<0.05$), as illustrated in Tables (1, 2 and 3). In this experiment, the minimum fungicidal concentration of black tea aqueous extract against *C. albicans* isolates was 250 mg/ml. For green tea aqueous extract, the MFC against *C. albicans* isolates was 225 mg/ml (Figure 2). And adding 1% of stevia or sucralose to the MFC of the experimental extracts resulted in the antifungal activity of these extracts did not affect. Also, adding 5% of stevia aqueous compound to the MFC of black tea will not affect the antifungal activity of black tea aqueous extracts against *C. albicans* isolates. While adding 5% of stevia to green tea aqueous extract interfered with the antifungal activity of the extract. On the other hand, the antifungal activity of both tea extracts was affected by an increase in the concentration of the added sucralose beyond 1%.

![Figure 1: Mean diameter of inhibition zone of different concentrations of black and green tea aqueous extracts against *C. albicans.*](image-url)
Figure 2: Minimum fungicidal concentration of tea extracts against *C. albicans* (A) Black tea aqueous extract (B) Green tea aqueous extract

Table 1: Descriptive and statistical analysis of diameter of inhibition zone (mm) of different concentrations of black and green tea aqueous extract against *C. albicans* (10 isolates)

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>±SD</th>
<th>F</th>
<th>P value*</th>
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<td>8.500</td>
<td>7.750</td>
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<td></td>
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<td>12.000</td>
<td>11.700</td>
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<td>14.000</td>
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</tr>
<tr>
<td></td>
<td>300</td>
<td>16.000</td>
<td>17.500</td>
<td>16.750</td>
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<td></td>
</tr>
<tr>
<td></td>
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<td>21.000</td>
<td>20.650</td>
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<tr>
<td>Green tea</td>
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<td>9.750</td>
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<tr>
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<td>23.000</td>
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</table>

*=significant at P<0.05.

Table 2: Multiple Comparisons between concentrations of tea extracts against *C. albicans* (10 isolates) by Tukey Honestly Significant Difference (Tukey HSD).

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Extract</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Black tea</td>
</tr>
<tr>
<td>MD</td>
<td>p value*</td>
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</tbody>
</table>


### Table 3: Descriptive and statistical analysis of diameter of inhibition zone (mm) against *C. albicans* (10 isolates) between tea extract's concentrations.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
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<th>Green tea</th>
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<th>Black tea</th>
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*=significant at P<0.05.

### Discussion

Herbs and plant extracts have been used in oral hygiene products for many years because of their effectiveness, availability, low cost, and safety. In addition, many studies revealed the antimicrobial activity of tea against oral microbes. As a result, tea extracts have been successfully incorporated into numerous oral care
products (Mageed et al., 2015; Janakiram et al., 2020). Because tea is commonly administered as an aqueous infusion (tea infusion), this research examined the impact of aqueous tea extracts on salivary *C. albicans*. This experiment used two types of dried leaves of tea: black tea, which represented the fermented type, and green tea, which was the unfermented one (Zhang et al., 2020). It is difficult to employ tea in dental products as a mouth care agent because of its bitterness test. Hence some supplementation is required to make it more palatable, particularly for pediatric and geriatric populations. In order to combat the bitter aftertaste of the tea extracts, this research used the addition of a nonnutritive sweetener (NNS) as a taste masking technique.

The results showed that the antifungal activity of both tea extracts against salivary *C. albicans* was not affected by the addition of stevia and sucralose at concentrations up to 1%, at which all these microbial isolates were killed at the same MFC of the extracts prior to the addition of NNS. Perhaps there were no appreciable interactions between the NNS and the extracts at concentrations up to 1% that may affect the extracts' potent antimicrobial compounds, such as their overall polyphenol, catechin, tannin, flavonoid, and other chemical components; this finding is agreed with Korir et al. (2013) who found that adding stevia at a concentration of 0.1g to 100ml of green and black tea aqueous extract separately had no significant influence on the total phenolic components in the tea. Also, this finding was consistent with that of Shalaby et al. (2016). They proposed a mechanism by which sweeteners influence the radical-scavenging activity of phenolic compounds in black and green tea. In addition, they found no statistically significant interactions between aspartame glycosides and phenolic compounds in the analyzed tea samples.

Moreover, the addition of 5% stevia to black tea aqueous extract did not affect the extract's antifungal activity against salivary *C. albicans*, while adding 5% stevia to green tea aqueous extract interfered with the extract's antifungal activity, this discrepancy may be attributable to differences in the total polyphenols (the primary antimicrobial agents) between black and green tea, which result from their respective processing methods (Zhang et al., 2020).

On the other hand, sucralose concentrations over 1% compromised the antifungal properties of both black and green tea extracts. The precise mechanism through which NNS in high concentrations might inhibit the antifungal action of extracts is not easy to explain. It has been hypothesized that the ionized form of the extracts is primarily responsible for their antimicrobial effects (Bark et al., 2011; Ben Abid et al., 2015). NNS may react with phenolic compounds' functional sites, preventing such compounds from carrying out their bioactive roles (Kesinger and Stevens, 2009). Also, the stevia compound may contain ascorbic acid (Mlambo et al., 2022), which may interact with the polyphenolic compound in the extracts, subsequently affecting their antifungal activity (Chen et al., 2020).

**Conclusion**

Black and green tea aqueous extracts have antifungal activity against salivary *C. albicans*. 
Stevia and sucralose in specific concentrations could be successfully used to sweeten the black and green tea aqueous extracts without compromising their antifungal activity.

**Ethical Approval**

This study was approved by the Ethical Committee of the College of Dentistry/University of Baghdad.

**Conflict of interest:** None

**References**


Different Harvested and Processing Conditions. Antioxidants, 10(2), 183. https://doi.org/10.3390/antiox10020183

