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# Efficacy of herbal-based mouth rinses against oral microorganisms in a group of children - a randomized controlled trial

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***Abstract:***
***Background:*** *Dental caries, gingival and periodontal diseases remain major public health problems worldwide. The burdens of oral diseases are higher in poor populations in both developed and developing countries. This study was conducted to enhance the preventive oral health care and increase the motivation in children by using natural herbal-based mouth rinses to decrease dental caries.*
***Materials and methods:*** *In this prospective randomized controlled study, the sample consisted of 28 children range 7 to 12 years old where they were randomly allocated into four groups of 7 patients each, Group A received licorice extract mouthwash, Group B received stevia extract mouthwash, Group C received thyme extract mouthwash and Group D received Chlorohexidine mouthwash as a control group. A baseline pre-rinse saliva samples were collected for evaluation of pH value and streptococcus mutans colony counts. Post -rinse saliva samples were taken after 1 ,15, 30 minutes for evaluation of changes in pH value. Post-rinse saliva samples were collected for streptococcus mutans colony counts at 2 weeks and one month follow up.*
***Results:*** *There was a statistically significant difference in mean change in Strep. Mutans count within different mouthwashes (P=0.002 in licorice group, P=0.047 in stevia group, P=0.001 in thyme group and P<0.001 in chlorhexidine group). There was a statistically significant difference in salivary pH between different evaluation times within licorice group (P<0.001), stevia group (P=0.002), thyme group (P<0.001) and chlorhexidine group (P<0.001) groups.*
***Conclusion:*** *licorice extract, stevia extract and thyme extract mouth rinses significantly decreased salivary Streptococcus Mutans count like chlorohexidine mouthwash*
***Key Word:*** *Chlorhexidine; Dental caries; Licorice; Stevia; Thyme; Streptococcus Mutans; Mouth rinse.*

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## I. Introduction

Dental caries has become one of the most common oral diseases in adults as well as children worldwide. It results in demineralization of the hard tooth structure, due to bacterial acid activity.[1] Nowadays Plaque control becomes necessary for prevention of dental caries and inflammatory periodontal diseases, but most people do not reach the aim of the total plaque removal. Studies reveal that proper mechanical plaque control measures as tooth brushing are not achieved well leaving more than half of plaque in the oral cavity after brushing. This acts as a reservoir, leads to regrowth of plaque rapidly.[2] Dental caries pathogenesis comes from the metabolism pathway of S.mutans.[3] The pathogenicity of S. mutans comes from the continuous acid production which results in demineralization and destruction of enamel. The mature biofilm withstands the low pH acidic environment due more colonization of other various species of bacteria. As a result, an acid- tolerant flora emerges which further promotes the progression of dental caries.[4] Using antimicrobial agents limits the growth of cariogenic microorganisms and prevents dental caries. Chlorhexidine is the most commonly used mouth rinse. Its effect appears in control of gingivitis in long-term studies. Due to the main side effects of chlorohexidine which are teeth and tongue staining, taste alteration and mucosal erosion [5] , natural herbal extracts are introduced in the field of dentistry nowadays. In our study we used herbal based mouth rinses licorice extract, stevia extract and thyme extract, that have received special attention nowadays because of being natural, non-synthetic without any chemical additives or markedly side effects, in comparison to the chlorohexidine mouthwash. Moreover, it has been resulted their efficacy in suppressing the bacterial colonies of streptococcus mutans.[6 7 8]

## II. Material And Methods

This study was a randomized controlled clinical trial following the CONSORT standards, children were enrolled from the outpatient clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
Recruitment of participants for the study started on the first of June 2019

**Study Design**: double blinded randomized controlled trial
**Study Location**: the outpatient clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
**Study Duration**: June 2019 to November 2019
**Sample size**: 28 participants, 7 in each group
**Sample size calculation**: A total number of 28 children (7 in each group) were recruited to evaluate the effects of *Licorice* extract mouth rinse, *Thymus Vulgaris extract* mouth rinse and *Stevia extract* mouth rinse in comparison to *Chlorohexidine* mouthwash as a control on S.mutans bacterial count, based on Power and Sample Size Calculation Software Version 3.1.2 (Nashville, USA) based on previous data (Sajadi et al, 2014) reporting a mean difference of $5.14x10^6$ in S. mutans count after rinsing with Chlorhexidine mouthwash for 2 weeks, with difference in standard deviation of $1.0x10^7$. A sample size of 5 subjects per group was needed to be able to reject the null hypothesis with probability of 0.05 and 80% power.[9]
Sample size was increased by 30% to 7 subjects per group for a total of 28 subjects per 4 groups to compensate for dropouts.

**Subjects and selection method**: forty children were examined for eligibility in the outpatient clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University. Out of these 40 children, 12 were eligible for the study. The participants were randomly allocated into 4 groups.
Group A (N=7) -10 ml of Licorice extract mouthwash
Group B (N=7) -10 ml of Stevia extract mouthwash
Group C (N=7) -10 ml of Thyme extract mouthwash
Group D (N=7) -10 ml of Chlorohexidine 0.2% mouthwash

**Inclusion criteria:**
1. Normal children medically free with no systemic disease.
2. Their age ranged from 7 to 12 years old.
3. Children who scored either (0, 1,2) in gingival index proposed by Silness and Loe.
4. Free from any severe dental signs and symptoms (pain, periapical abscess, swelling).
5. Should not have used mouthwashes for the last month or any professionally applied fluoride.

**Exclusion criteria:**
Children of following criteria were excluded from this study:
1. Uncooperative children according to Frankle scale
2. Suffering from any physical disability, systemic disease, or with special health care needs
3. Children who received any antimicrobial agents last two weeks.
4. Children who used to wear any orthodontic, fixed, or removable appliance.
5. History of allergy to any of the materials used in preparation of tested mouth rinses.

**Procedure methodology:**
**Preparation pf herbal extracts:**
*Licorice, Stevia, and Thymes* dried leaves were grinded into powdered form. Ethanolic solution of the three extracts were prepared by suspending for each 50 grams of dry powder in 100 mL of 70% (w/v) ethyl alcohol. Suspended powder was incubated with regular shaking at speed 200 rpm / min for 7 days at 37oC.

The suspensions were filtered, cold centrifuged and dried using Rotary evaporator. The herbal extracts precipitated on the walls of the flask was scrapped. the residues were and stored at 4°C for further preparation of the mouth rinses. Finally, the herbal extract mouthwashes were prepared (0.5 g of extract in 100 ml distilled water) and poured into sterile amber dark bottles under the laminar air flow and kept at 4°C till use. [6 10 11 12]

**Clinical steps:**
The study was explained to all the parents' participants, and they signed informed consent included their agreement to participate in this study. Verbal assent from the children was obtained according to the rules of the ethical committee. Ethical approval was obtained from the Research Ethical Committee, Faculty of Dentistry, Ain Shams University.

Children involved in the study were instructed to take breakfast two hours prior to the collection of the saliva sample. Primary stimulated saliva samples were collected before using the mouth-rinse in sterile disposable plastic containers to record the baseline for pH and microbial counts. Children were instructed to rinse with 10 ml of each mouth rinse over a period of one minute according to the study group. saliva samples were collected again from each child after 1, 15 and 30 minutes for recording the pH value by pH digital meter (**ADwa AD31** Waterproof Conductivity-TDS-Temp Pocket Testers with replaceable electrode), The baseline

saliva samples were transported in an ice box to the laboratory within one hour. After 2 weeks saliva samples were collected for microbial counts then one month. In the laboratory 0.1 ml from each sample was spread on Mitis salivaris agar **(**Difco Co. USA**)** as a selective media for S.mutans,

Information regarding the taste of solution or any discomfort following the rinse was recorded to assess and compare the acceptability of the introduced mouth rinses.

**Follow up:**

Data on the set outcomes were initially recorded at the beginning of the study prior to any interventions. Parents were instructed to ensure frequent uptake of rinses (twice daily) by their children and then same measurements were undertaken 2 weeks and 1 month.


Baseline pre-rinse sample one month follow up

Figure (1): Licorice Group:S.mutans colony count

**Statistical analysis:**

Statistical analysis was performed using IBM SPSS Statistics Version 20 for Windows. Data was presented as mean, standard deviation (SD) and percentages. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess data normality of continuous data. The significance level was set at $P \leq 0.05$.

One-way ANOVA followed by Tukey's post-hoc test was conducted for inter- and intragroup comparisons of minimum inhibitory concentration. One-way ANOVA followed by Tukey's post-hoc test was performed to compare change in bacterial count, salivary pH of different mouthwashes at each evaluation time. Repeated measures ANOVA was used for comparisons of change in bacterial count, salivary pH of different evaluation times within each mouthwash.

## III. Result

**I-Antibacterial activity:**

**Effect of mouthwashes on change in Streptococcus Mutans count at different time intervals:**

One-way ANOVA followed by Tukey's post-hoc test (Table (1) and Figure (2)) showed that there was no statistically significant difference between different mouthwashes in mean change in Strep. Mutans count at different time intervals (P=0.877 at baseline – 2 weeks, P=0.591 at 2 weeks – 1 month and P=0.331 at baseline – 1 month).

Repeated measures ANOVA (Table (1) and Figure (2)) showed that there was a statistically significant difference in mean change in Strep. Mutans count within different mouthwashes (P=0.002 in liquorice group, P=0.047 in stevia group, P=0.001 in thyme group and P<0.001 in chlorhexidine group).

Within liquorice and chlorhexidine groups, mean change in Strep. Mutans count was significantly highest from baseline – 1 month; followed by that recorded from baseline – 2 weeks; then from 2 weeks – 1 month. Within stevia group, mean change in Strep. Mutans count from baseline – 1 month was significantly higher than that from 2 weeks – 1 month; while mean change recorded from baseline – 2 weeks did not differ significantly from other time intervals. Within thyme group, mean change in Strep. Mutans count from baseline – 1 month was significantly higher than that recorded from 2 weeks – 1 month and baseline – 2 weeks which were statistically similar.

**Table (1):** Mean difference and percentage change in Streptococcus Mutans count (CFU) within each mouthwash at different time intervals.

| | **Baseline – 2 weeks** | **2 weeks – 1 month** | **Baseline – 1 month** | **P-value** |
|---|---|---|---|---|
| **Liquorice** | $30.6x10^3 \pm 26.5x10^3$ $(79.7\%)^B$ | $7.3x10^3 \pm 7.4x10^3$ $(93.7\%)^C$ | $38.0x10^3 \pm 3.2x10^3$ $(98.7\%)^A$ | 0.002* |
| **Stevia** | $28.2x10^3 \pm 26.4x10^3$ $(68.4\%)^{AB}$ | $13.0x10^3 \pm 21.8x10^3$ $(99.5\%)^B$ | $41.2x10^3 \pm 37.7x10^3$ $(99.8\%)^A$ | 0.047* |
| **Thyme** | $23.4x10^3 \pm 12.3x10^3$ $(63.9\%)^B$ | $10.8x10^3 \pm 7.8x10^3$ $(82.3\%)^B$ | $34.2x10^3 \pm 14.2x10^3$ $(93.6\%)^A$ | 0.001* |

| | | | | |
|---|---|---|---|---|
| **Chlorhexidine** | $32.4x10^3 \pm 18.5x10^3$ $(65.5\%)^B$ | $16.5x10^3 \pm 10.4x10^3$ $(96.8\%)^C$ | $49.0x10^3 \pm 26.1x10^3$ $(98.9\%)^A$ | <0.001* |
| **P-value** | 0.877NS | 0.591NS | 0.331NS | |

**: significant at P≤0.05; NS: non-significant at P>0.05*
*Means with different superscript uppercase letters within each row are statistically significantly different at P≤0.05*

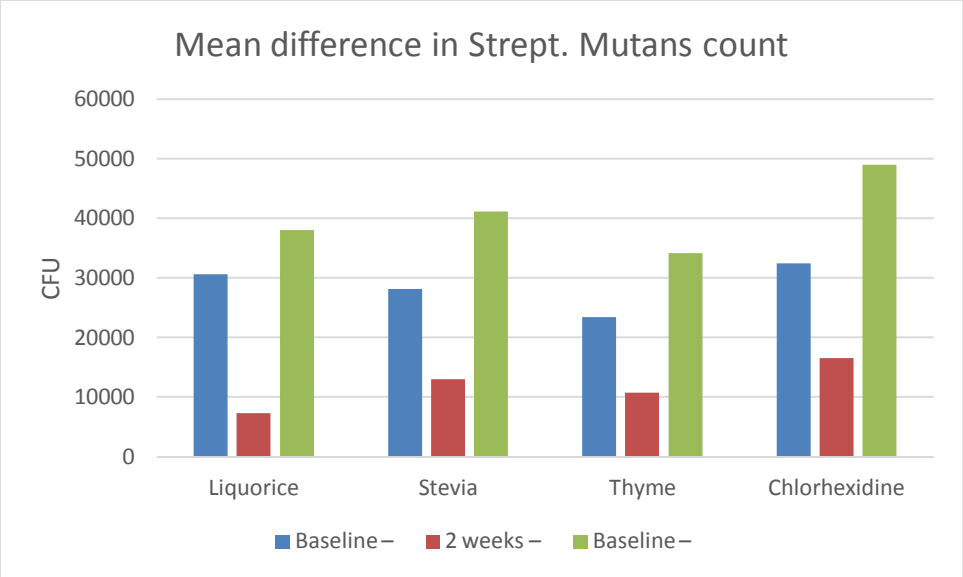


**Figure (2):** Bar chart showing mean difference in Streptococcus Mutans count (CFU) at different time intervals after each mouthwash**.**

**II- Salivary pH:**

One-way ANOVA followed by Tukey's post-hoc test (Table (2) and Figure (3) showed that there was no statistically significant difference in salivary pH at different evaluation times (P=0.837 at baseline, P=0.919 at 1 min, P=0.753 at 15 min and P=0.729 at 30 min).

Repeated measures ANOVA (Table (2) and Figure (3) showed that there was a statistically significant difference in salivary pH between different evaluation times within licorice group (P<0.001), stevia group (P=0.002), thyme group (P<0.001) and chlorhexidine group (P<0.001) groups. With each mouthwash group, pH readings were significantly highest at 30 min; followed by that recorded at 15 min; then at 1 min. While pH readings were significantly lowest at baseline.

**Table (2):** Mean±SD and P-value for the effect of mouthwashes on salivary pH at different evaluation times**.**

| | **Baseline** | **1 min** | **15 min** | **30 min** | **P-value** |
|---|---|---|---|---|---|
| **Licorice** | $7.75\pm0.32^D$ | $8.0\pm0.27^C$ | $8.20\pm0.32^B$ | $8.27\pm0.33^A$ | <0.001* |
| **Stevia** | $7.88\pm0.30^D$ | $8.12\pm0.28^C$ | $8.35\pm0.25^B$ | $8.42\pm0.23^A$ | 0.002* |
| **Thyme** | $7.87\pm0.31^D$ | $8.10\pm0.30^C$ | $8.21\pm0.30^B$ | $8.31\pm0.33^A$ | <0.001* |
| **Chlorhexidine** | $7.85\pm0.28^D$ | $8.14\pm0.28^C$ | $8.25\pm0.29^B$ | $8.35\pm0.29^A$ | <0.001* |
| **P-value** | 0.837NS | 0.919NS | 0.753NS | 0.792NS | |

**: significant at P≤0.05; NS: non-significant at P>0.05*
*Means with different superscript uppercase letters within each row are statistically significantly different at P≤0.05*

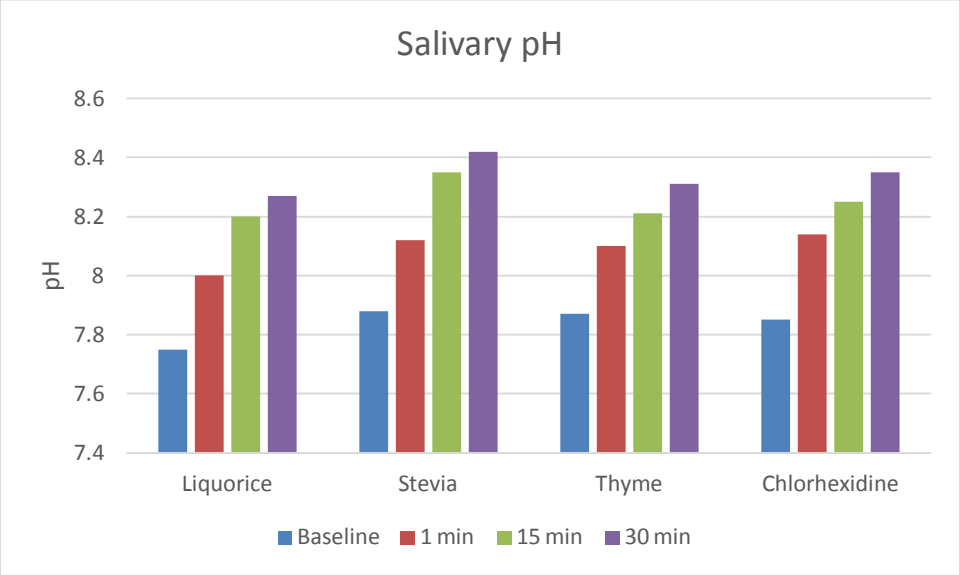


**Figure (3):** Bar chart showing salivary pH at different evaluation times after using each mouthwash.

## IV. Discussion

Dental caries is considered as one of the most common chronic infectious diseases in children.[13] Although many preventive measures have been made to decrease dental caries in children, its prevalence is still high. Mechanical tooth brushing is the main base for oral care. However, it is not enough for most children due to lack of their parents supervision, and impairment hand skills.[14] The aim of this study was to assess and compare the effectiveness of three herbal extracts intraorally licorice, stevia and thymus vulgaris mouthwashes and using of chlorohexidine 0.2% as a control group on the bacterial count of streptococcus mutans.

Licorice, stevia, and thyme have been used as natural antimicrobials with excellent biocompatibility. Herbal mouthwashes contain naturally occurring ingredients called phytochemicals which give the desired antimicrobial effect.[15] Although, the wide range of antimicrobial effects of Chlorohexidine mouthwash, its unfavorable side effects in teeth discoloration and burning sensation [16] limit its long term use especially in children.

This study was conducted on children with mixed dentition (7-12 years old) to avoid the swallowing of the mouthwash during rinsing at children below 6 years. Children with mixed dentition are also liable to gingival diseases and dental caries.

In this study during preparation of Licorice, Stevia and Thyme extracts, 70% ethanol alcohol was used as a solvent because of being safe to use as well as its effectiveness in the extraction of the bioactive antimicrobial molecules of plants.[17 18 12]

Since The pathogenicity of S. mutans comes from the continuous acid production which cause demineralization of the tooth structure. The study aim was to decrease the low pH acidic environment by using these herbal mouth-rinses which had the ability to modify the salivary pH and stimulate the salivary buffering action. This was carried out by monitoring the salivary pH value using digital pH meter for each child before the rinsing as a baseline and after 1 minute, 15 minutes, and 30 minutes post rinse.

Using a hand-held digital pH meter showed more accurate results than the colourimetric strip paper test. As it is a quantitative test and gives more accurate scores.[19]

On comparing the 4 groups in this study there was a significant increase in salivary pH value reaching its maximum after 30 minutes post rinse. Thus, Alkalinity increased which might serve as a preventive me3asure to reduce dental caries.

The rise in pH value by licorice extract mouthwash was consistent with those **Söderling et al. (2006)** [20] and, **Eisha Jain et al, (2013)**[10] who concluded that liquorice extracts inhibit acid production.

The significant increase in pH in Stevia group was in agreement with **Tanushri et al.** who stated that there was a significant rise in the salivary pH values in Stevia at 20 minutes after mouth rinsing.[21]

The effect of Thyme in pH increase was in agreement with ***Eman A. Al-Timimi et al.*** who stated that the increase pH with Thymus Vulgaris and chlorhexidine mouth rinses could be due to a buffering capacity of the Thymus Vulgaris extract, salivary stimulation due to Thyme taste, and/or antibacterial activity against acid producing bacteria.[22]

As for the bacterial count of S.Mutans, there was a significant reduction of bacterial counts for all groups without statistically significant differences between them.

The effect of Licorice extract mouthwash results in reduction of streptococcus mutans count was consistent with **D.Kamal, et al (2021)**[6], who showed an antibacterial effectiveness against oral streptococci by licorice mouthwash at different time intervals.

The result of this study was in agreement with **Carounanidy Usha et al (2017)** [7]who showed that 0.5% stevia mouthwash was effective in prevention of dental caries as a significant reduction in the colony count of streptococcus mutans was observed.

Thymus mouthwash inhibited the growth of streptococcus mutans significantly and this might be due to the presence of polyphenolic substances as thymol and carvacrol that prevented the attachment of streptococcus mutans to the tooth structure. The result of his study was consistent with studies conducted by **R. Abdel Hameed , et al (2020)** [8]and **F Sajadi ,et al (2021)**[23]**.**

**Ethical approval:**

Ethical approval was obtained from the Research Ethical Committee, Faculty of Dentistry, Ain Shams University.

**Conflict of interest:**

The authors declare that they have no conflict of interest.

## V. Conclusion

Licorice, Stevia, and Thyme showed an antimicrobial efficacy on streptococcus mutans in children compared to cholorhexidine on long term use.

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