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Original Article

Evaluation of Antimicrobial Efficacy of Green Tea, Garlic with Lime, Pomegranate Extract and Chlorhexidine Mouth Rinses in a Group of Egyptian Children (A Randomized clinical trial)

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Abstract:

Background: Herbal mouth rinses have an antimicrobial effect against cariogenic bacteria. This RCT was conducted to evaluate antimicrobial efficacy of the three mouth rinses (Pomegranate,Green tea, Garlic with lime extracts) against mutans bacteria in comparison to Chlorhexidine. **Subjects and methods:** A group of 84 children(5-8 years) were selected randomly from the clinic of pediatric dentistry department of Ain shams University. Children were divided into four equal groups (n=22).Group 1 was(Chlorhexidine),Group 2 was (Green tea extract), Group 3 was (Garlic with lime extract) and Group 4 was (Pomegranate extract). Children were asked to rinse with the prescribed mouth rinse for 1 minute. A baseline plaque sample was collected and tested for the number of CFUs before&after rinsing. Child satisfaction was assessed using likert scale. **Results:** For all groups of the four studied mouth rinses, there was a significant decrease of bacterial count after treatment ($p<0.001$). The highest value of bacterial count was found in Garlic-lime group while the lowest value of bacterial count was found in the Pomegranate group.For all parameters (taste, smell and willing to use), there was a significant difference between different groups with Garlic-lime having significantly lower value than other groups ($p<0.001$),while Green tea having significantly higher value than others. **Conclusion:** Pomegranate showed a better efficacy in controlling bacterial count than others, Garlic had the lowest efficacy. Green tea extract is the most tolerable mouth rinse in terms of taste and smell than other groups while Garlic is the least tolerable. Herbal mouth rinses can be an effective alternative to CHX.

Keywords: Herbal mouth rinse, Pomegranate, Caries prevention, Garlic, Green tea

Introduction:

Dental caries is one of the most common chronic infectious diseases in the world. It is a multifactorial human disease that has widely affected many populations all over the world(Kajfasz *et al.*, 2010).It is a preventable,

localized transmissible, multifactorial disease resulting from the interaction between host, diet, and microflora on the tooth surface over a period of time, resulting in cavitation of inorganic enamel and dentin. The most commonly related bacteria in its etiology are *Streptococcus mutans* for its onset, and lactobacilli spp. for its advancement(Thakur and Habib, 2017).They are highly acidogenic (producing short-chain acids), that dissolve hard tissues of the teeth. Also, it can metabolize the sucrose and synthesize insoluble extracellular polysaccharides that enhance the bacteria's adherence to the tooth surface and form biofilm.

Antimicrobial mouth rinses act as adjuncts of daily home care. They provide an effective means of preventing colonization by micro-organisms(Jauhari, 2015). Chlorhexidine is one of the most commonly used mouth rinses in children. It has a broad spectrum of antibacterial activity. The use of Chlorhexidine rinse for four weeks or more can cause extrinsic tooth staining, bitter taste, altered taste sensation, and enhancing calculus formation which is an adverse side effect of it (Syed, Chopra and Shrivastava, 2016).

Herbal medicine is a therapeutic and preventive approach that treats various diseases by plants and their extracts. Their extracts are used to treat disorders and maintain good health. Green tea has a wide spectrum of medical benefits, it is considered an important source of polyphenols, particularly flavonoids, and catechins. Green tea has numerous pharmacological properties. It has an anti-bacterial effect, anti-cariogenic effects, and anti-oxidative effects(Neturi *et al.*, 2014).

Garlic has antibacterial, antiviral, and antifungal activities(Fani, Kohanteb and Dayaghi, 2007; Owhe-Ureghe, Ehwarieme and Eboh, 2010). It has also shown antioxidant, anti-inflammatory properties, and inhibitory effects due to sulfur-containing compounds in it(Houshmand, Mahjour and Dianat, 2022). The active component of garlic is allicin which is effective against Gram-positive and Gram-negative bacteria equally and is considered bactericidal rather than bacteriostatic.

Pomegranate has a pharmacological properties, as well as powerful antioxidant properties as it contains tannins and polyphenols which can disrupt the cell wall of the bacteria and the cytoplasmic membrane, and damage the membrane proteins leading to cell death(Al-obaidi, Muhsin and Ibrahim, 2017). Its antimicrobial activity is attributed to its ability to suppress microorganisms to adhere to the surface of the tooth(Subramaniam *et al.*, 2012).

There is a deficiency of evidence in the literature concerning the use of the previously mentioned naturally derived mouth rinses in the prevention of caries in children so the aim of the study was to estimate the antimicrobial and anticariogenic effect of these mouth rinses (Green tea, Garlic, Pomegranate) compared with Chlorhexidine mouth rinse.

Subjects and Methods:

A group of 84 children were selected randomly according to certain inclusion criteria from the clinic of the pediatric and dental public health department, Faculty of Dentistry, Ain shams University. Demographic data was also taken from patients and their caregivers.

Study design

Randomized clinical trial in which each child rinsed with the prescribed group of mouth rinse.

- Randomization was performed using an online software application (sealed Envelope https://www.sealedenvelope.com).
- Each child had an equal chance of being assigned to the control group or the study group.
- Each number was written on a piece of paper that was placed and concealed in an opaque sealed envelope and the dental operator was unaware of the allocation sequence.
- The randomization list was with the senior investigator throughout the study.

Blinding

- There was no blinding, due to the nature of the study and the materials used.
- Only the statistician was blinded.

Ethical consideration

The study protocol granted ethical approval from Ain Shams University Research Ethics Committee with approval code (FDASU-RecIM021835).

Sample size estimation

Sample size calculation was calculated using power and sample size calculation program version 3, mean diff. =44 , S.E=50 (**Thomas, et al, 2017).** P-value= 0.05 and the power of the study was 80%(Vilela *et al.*, 2020).The final sample size was 84 children as all children and their parents who accepted to consent were allocated in the study.

Informed consent and assent

A signed informed consent to the parents and also an assent to the child outlined by the Ethical Committee, Faculty of Dentistry, AinShams University were obtained before the start of the study.

Inclusion criteria:

1- Age 5-8 years.

2- No history of systemic disease.

3- Patients didn't receive antibiotics for at least 3 months before and during the study.

Exclusion criteria:

1- Children who can't expectorate completely or brush their teeth on their own.
2- Fixed or removable orthodontic appliances.
3- Children with intra-oral abscesses, oral lesions or gingival diseases.
4- Parental consent was not acquired.

Study procedures:

Preparation of the mouth rinses:

-The extracts were prepared in the Faculty of Medicine in the Microbiology Department at Al-Azhar University.

-The extracts were stored in the deep freezer until they were used. Their expiry date was about one year.

-Green tea extract: Green tea leaves were dried then grounded to desirable size using an electrical mill then diluted to a concentration of 0.5% phenolic compound using double distilled water. Some additives such as peppermint flavor, and a sweetening agent such as sodium saccharine were used to formulate the mouth rinse(Thakur and Habib, 2017).

-Garlic extract: fresh garlic cloves were washed, peeled, cut into pieces, and ground in the grinder. Then water was added to it to obtain a homogenate mix. About 100 mL of lime juice was extracted from fresh lemons and added to the garlic extract (Owhe-Ureghe, Ehwarieme and Eboh, 2010). Other additives as peppermint flavor, sodium saccharine which is a sweetening agent, and sodium bicarbonate as a preservative agent were added.

-Pomegranate extract: Fruits of pomegranate were handpicked washed and peeled, and the arils with seeds were hand crushed and squeezed to obtain the juice. The peel was air-dried a few days and then pulverized. The samples were stored at −20°C. The juice was defrosted at room temperature. The same procedure was carried out for the peel powder. Each sample was mixed for 30 minutes and then the extracts were filtered. Pomegranate juice was freshly prepared using the sterilized grinder in the laboratory, then was heated for 1 hour to get a heavy concentrate, and then the 300 mg/ml concentration of pomegranate peel extract was used to prepare the mouth rinse(Umar and Dilshad, 2016).

Assessment of the Antibacterial efficacy:

Children were randomly allocated into four equal groups. Each group was 21 children as follows: **(Figure 1)**

Group (I) was given Chlorhexidine mouth rinse.

Group (II) was given Green tea extract mouth rinse.

Group (III) was given Garlic with lime extract mouth rinse.

Group (IV) was given Pomegranate extract mouth rinse.

Examination was done using Silness and Loe plaque index, the plaque was seen by using the probe on the tooth surface. No special instructions were given to the patients except rinsing with the prescribed mouth rinse. Baseline plaque samples were collected from each child using sterile buds, the sample was taken from the lingual side of the lower molars before rinsing then 10ml of each solution was given to the respective groups to rinse for one minute(Neturi *et al.*, 2014).

Five minutes after rinsing, plaque samples were collected, then transferred to a sterile test tube containing 10ml of thioglycolate that acts as a transport media. These plaque samples were sent to the laboratory of the Microbiology Department, Faculty of Medicine, AinShams University within half an hour of the sample collection in an ice box.

The plaque samples were diluted with sterile saline in a ratio of 1:100 and then cultured on selective agar plates, the plates were incubated for 48-96 hours at 37°C.The number of bacterial colonies before and after rinsing was counted.

The morphology, size, color, and counting of CFUs were done by hand-held digital colony counter. The number of colonies in each plate was counted three times by the same observer on different days under the same consistent environmental conditions and constant conditions to overcome intra-observer bias(Thakur and Habib, 2017).

Assessment of the patient satisfaction: (figure 2)

Child satisfaction was measured using a 5-point pictorial likert scale which is graded from 1 to 5 (1.Excellent.2.Good.3 Average 4.Poor. 5. Very poor)(Abbasi et al., 2021).This Facial image scale was a suitable and easy way to evaluate patient satisfaction with taste, smell, and willingness to use the mouth rinse.

Statistical analysis

Numerical data were presented as mean, standard deviation, median and range values and were explored for normality by checking the data distribution and using Shapiro-Wilk test.

Results

For all groups of the four studied mouth rinses, there was a significant decrease in bacterial count after treatment ($p<0.001$). The highest value of bacterial count was found in the Garlic-lime group (9.12±2.00) while the lowest value was found in the Pomegranate group (1.91±2.45). **(Table 1).** In comparison with Chlorhexidine, there was no significant difference between CHX and Green tea and Garlic with lime, while there was a significant difference between CHX and Pomegranate. **(Table 2).**

For all parameters (taste, smell and willing to use), there was a significant difference between different groups with Garlic-lime having significantly lower value than other groups ($p<0.001$).while Green tea having significantly higher value than other groups. **(Table 3)**

Discussion

Most of the mouth rinses available in the market contain alcohol and other chemicals such as Chlorhexidine gluconate. These chemicals cause many side effects like taste disturbance, burning sensation, and discoloration(Reshawn and Muralidharan, 2021). Some of the cariogenic microorganisms have shown resistance to some types of chemical antimicrobials, so to overcome these side effects, nontoxic herbal mouth rinses are introduced, as they possess numerous useful effects with the least side effects in addition to their bioactive components which can promote better oral hygiene and health. So they can be an appropriate substitute(Tusi *et al.*, 2020).

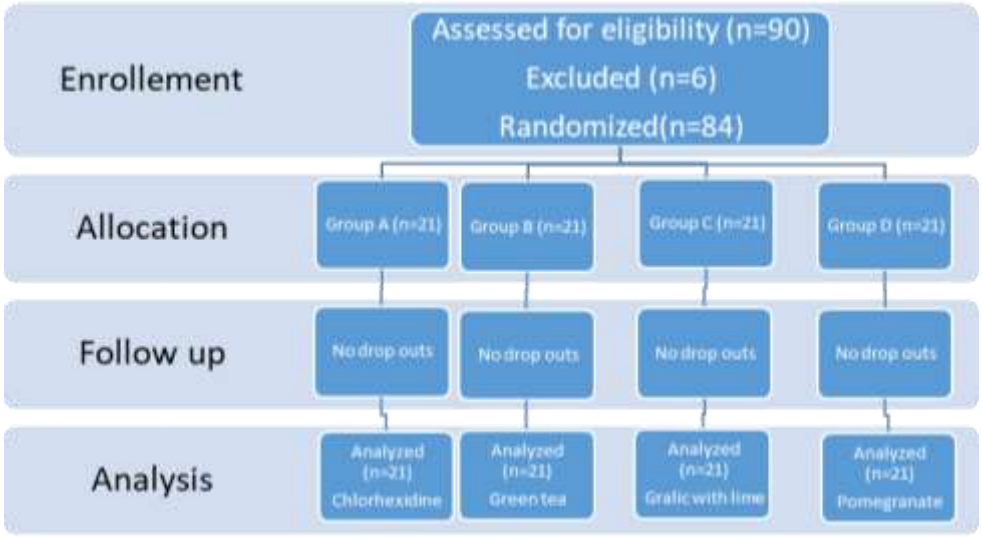

Figure 1: flow chart

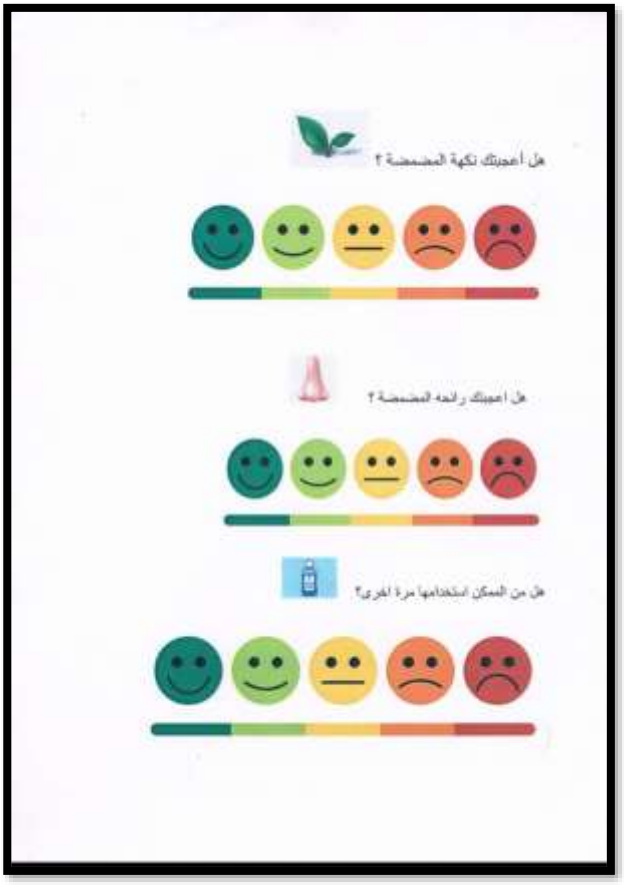

Figure 2: Facial image scale

Table (1): Mean ± standard deviation (SD) of log bacterial count (CFU/ml) for different intervals.

Groups	Log bacterial count (mean±SD)		p-value
	Before	After	
Chlorhexidine	12.19±2.11	6.50±4.47	**<0.001***
Green tea	11.87±2.35	4.99±4.03	**<0.001***
Garlic-lime	13.38±2.31	9.12±2.00	**<0.001***
Pomegranate	9.95±2.57	1.91±2.45	**<0.001***

**; significant ($p \leq 0.05$)*

Table (2): Mean ± standard deviation (SD) of log bacterial count (CFU/ml) for different groups.

Log bacterial count (mean±SD)				p-value
Chlorhexidine	**Green tea**	**Garlic-lime**	**Pomegranate**	
6.50±4.47^{AB}	4.99±4.03^{BC}	9.12±2.00^{A}	1.91±2.45^{C}	**<0.001***

Means with different superscript letters are statistically significantly different; significant ($p \leq 0.05$).*

Table (3): Mean ± standard deviation (SD) of patient satisfaction score for different groups.

Parameter	Patient satisfaction score (mean±SD)				p-value
	Chlorhexidine	**Green tea**	**Garlic-lime**	**Pomegranate**	
Smell	3.86±0.73^{A}	4.24±0.54^{A}	2.71±1.15^{B}	3.95±0.74^{A}	**<0.001***
Taste	4.05±0.59^{A}	4.43±0.60^{A}	2.76±1.09^{B}	3.86±0.79^{A}	**<0.001***
Willingness	3.86±0.73^{A}	4.19±0.75^{A}	2.67±1.02^{B}	3.90±0.77^{A}	**<0.001***

Means with different superscript letters are statistically significantly different; significant ($p \leq 0.05$)*

There is a deficiency of literature about herbal mouth rinses so herbal extracts mouth rinses were used in this study to evaluate their antimicrobial effect against Chlorhexidine.

Plaque samples were collected from the children before and after rinsing with the prescribed group of mouth rinse. They were collected from children by sterile cotton swab in order to evaluate the bacterial counts by swab technique from gingival 1/3 of the lingual side of lower molars. The samples were transferred to a sterile test tube containing thioglycolate which acted as a transport medium for determination of the oxygen requirements of microorganisms(Neturi *et al.*, 2014). The samples were cultured on selective agar plates (Mitis Salivarius Agar). It was an elective and selective media for the detection and counting of colonies of

streptococcus mutans. Bacitracin was added to this media to allow Streptococcus mutans to grow and form colonies in addition to inhibiting the growth of other oral bacteria(El-sharkawy, Mostafa and Malt, 2019).

The current study showed that for all groups of the mouth rinses, there was a significant decrease in bacterial count after treatment with the mouth rinses ($p<0.001$), in which Pomegranate extract showed the highest antimicrobial efficacy. The Results also showed that for parameters (smell, taste, and willingness to use again), there was a significant difference between different groups in which green tea is the most tolerable herbal mouth rinse.

The current study goes in consistence with the results of a clinical study conducted by **Umar and Dilshad, 2016** who stated that there was a reduction in the number of S. mutans bacteria when used Pomegranate extract mouth rinse, including an inhibitory effect on streptococcus mutans growth when compared to Chlorhexidine mouth rinse. So pomegranate extract may be considered as a potential anticariogenic mouth rinse. This study was consistent with the results of the clinical study conducted by **Naggar *et al.*, 2021** who stated that the pomegranate mouth rinse has a similar antibacterial effect as Chlorhexidine mouth rinse on short and long terms of usage.

Concurring with our study **Salama and Alsughier, 2019** found that the green tea extract mouth rinse showed promising results in reducing the streptococcus mutans count. Consequently, it was suggested that the green tea mouth rinse inhibits the growth of the streptococcus mutans bacteria due to the presence of active phenolic ingredients or other nutritional components in it. Our findings were in accordance with other investigators **Ajay Rao *et al.*, 2014** who also showed that garlic extract can be an alternative to Chlorhexidine mouth rinse. As garlic has an antimicrobial activity with no side effects such as dental staining when compared to Chlorhexidine mouth rinse.

On the other hand, the findings of the present study were not in consistence with that of **Thakur and Habib, 2017** that stated that no significant difference against *Streptococcus mutans* between green tea extract and garlic extract. However, both green tea and garlic extracts can be an alternative to Chlorhexidine mouth rinse.

Child satisfaction was measured using a 5-point pictorial Likert scale. This Facial image scale was a suitable and easy way to evaluate patient satisfaction about taste, smell, and willingness to use the mouth rinse. Its format was graded from 1 to 5 while 1 is the most satisfied and 5 is the least satisfied.

It is worth noting that the limitations of this study were that due to the mouth rinses being administered in a hospital setting under the supervision of the dentist, consequently patient compliance and long-term efficacy of the mouth rinses were not tested. Another limitation was that the morphology, size, color and counting of CFUs was done by hand- held digital colony counter, this might affect the accuracy of the detection of the bacterial count.

This study showed the benefit of herbal extracts mouth rinses in the prevention of dental caries as they have an antimicrobial efficacy as Chlorhexidine with a more acceptable taste and smell.

Conclusion

Pomegranate extract showed a better efficacy in controlling bacterial count than Chlorhexidine and Green tea extract mouth rinse while Garlic extract had the lowest efficacy. Green tea extract mouth rinse is the most tolerable herbal mouth rinse in terms of taste and smell than the other groups while Garlic is the least tolerable mouth rinse. Pomegranate and Green tea mouth rinses can be an effective alternative to Chlorhexidine.

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Conflict of Interest

The authors declare no conflict of interest.

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