ORIGINAL RESEARCH

The Efficacy of *Populus euphratica* and *Myrtus communis* Herbal Mouthwash on Gingivitis in Pediatric Patients Diagnosed Clinically and with Photometric CIELab Analysis: An *In Vivo* Randomized Double-blind Clinical Study

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Abstract

Background: Children are more prone to develop gingivitis as a result of deficient oral hygiene, morphological variance, and a diet that encourages the growth of pathogenic oral bacteria.

Aim: The purpose of this randomized, double-blind clinical study is to compare the therapeutic effect of an herbal mouthwash made of decoction and ethanolic extract of *Populus euphratica* and *Myrtus communis* to that of chlorhexidine (CHX) mouthrinse.

Materials and methods: In this study, 60 patients aged between 8 and 10 years were divided into four groups, each with 15 patients. The first group was treated with a mouthwash made of ethanolic herbal extract, the second group was treated with a mouthwash made of herbal decoction, the third group was treated with CHX mouthwash (0.12%) (positive control), and the fourth group was treated with a placebo made with colored distilled water (negative control). Clinical parameters gingival index (GI) and plaque index (PI) were evaluated at the start, 1, and 2 weeks from ensuing the treatment. Preoperative and postoperative photographs of the maxillary anterior sextant were recorded to evaluate CIELab coordinates to monitor color changes.

Statistical analysis: One-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) *post hoc* tests were used for data assessment.

Results: Significant reductions in GI and PI were found among the tested groups ($p < 0.05$). A significant reduction in a* value was observed, while L* value showed a significant increase between baseline and posttreatment ($p < 0.05$).

Conclusion: Herbal-based mouthwash of *Populus euphratica* and *Myrtus communis*, whether prepared by boiling or ethanolic extraction, reduced gingival inflammation significantly and has a lot of potential for treating and preventing periodontal disease in young children. Digital photography is a reliable supplementary approach for diagnosing and monitoring gingival inflammation in pediatric patients.

Keywords: Chlorhexidine, Gingivitis, Herbal, Mouthwash, *Myrtus communis*, *Populus euphratica*.

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Introduction

One of the main effects of bacterial plaque buildup on tooth surfaces during childhood is periodontal disease that ravages the gingiva and tooth-supporting bone structures. Furthermore, children are more susceptible to gingivitis due to plaque.[1] Low appreciation of maintaining good oral hygiene, combined with the presence of anatomical variations in the structure of the periodontium in children compared to adults, such as the width of the attached gingiva as well as the presence of spaces between the teeth that provide a favorable location for bacterial accumulation, contributes to increased susceptibility to periodontal issues in children.[2] Oral hygiene is the practice of maintaining the mouth in a sanitary condition as a means of preventing dental caries, gingivitis, periodontal disease, bad breath, and other dental maladies. Mechanical oral hygiene techniques, including tooth brushing, interdental brushing, and dental floss, are the primary means of controlling plaque. However, population-based studies and clinical experience indicate that many individuals are not employing these techniques appropriately.[3] Plaque removal is challenging for youngsters, necessitating the use of antiplaque mouthwash with antibacterial action, as these are more manageable for children than mechanical methods.[4,5] Chlorhexidine (CHX) mouthwash is renowned as the most extensively used positive control against plaque and is regarded as the ultimate standard against which

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the efficacy of various antiplaque medications is tested.[6,7] Long-term usage of CHX mouthwash may result in a number of adverse effects, including a bad taste and teeth discoloration, which has steered the search for safer alternatives that young children can use for extended periods without experiencing negative effects.[8] CHX mouthwash available in two concentrations 0.2 and 0.12%.[9] In addition to providing the equivalent clinical effect as a 0.2% CHX solution, the 0.12% CHX helps in diminishing the stains and harsh taste associated with CHX, making it more child-friendly.[10,11] For many centuries, people have extensively utilized plants as a source of therapeutic agents. Many bioactive chemicals are thought to be responsible for these qualities. Furthermore, herbal products are not only affordable but also well-recognized for their antimicrobial, antioxidant, anti-inflammatory, and anticariogenic capabilities with few side effects.[12–17] These qualities have been attributed to a diverse spectrum of phytochemicals, which include phenolic compounds, polyacetylenes, steroids, terpenoids, alkaloids, polysaccharides, fatty acids, glycosidic derivatives, and essential oils (EO). The American Dental Association has approved the use of EO and CHX as antiseptics in mouthwashes to treat laryngitis and other oral ulcers and inflammations, such as gingivitis.[18,19] The *Salicaceae* family, which includes the *Populus euphratica* tree, is rich in phenols and glycosides such as populin and salicin. Previous research on *Populus euphratica* has revealed that this plant contains volatile oils and several recognized phenolic chemicals. A thorough chemical analysis of *Populus euphratica* leaves was conducted by Ezghayer and Kadhim, in a previous study yielded 13 compounds, two of which were novel: 6-O-cis-cinnamoylsalicin and 6-O-benzoylsalicortinol. Some peasants in certain parts of Iraq have long utilized *Populus euphratica* extract as a pain reliever for carious teeth, inflamed gums, mucous membranes, eczema and various skin conditions.[20] *Myrtus* is a genus of one or two species of flowering plants in the *Myrtaceae* family. It is a shrub with dark green leaves, large flowers, and small bluish-black fruits. The plant contains a wide range of biologically active compounds, such as tannins, flavonoids, coumarins, essential oil, fixed oil, fibers, sugars, citric acid, malic acid, and antioxidants. Different parts of *Myrtus communis* have therapeutic properties and have been used traditionally as an antiseptic, disinfectant, and antihypertensive agent.[21] It has been shown that *Myrtus communis* exhibits antimicrobial activity against both gram-positive and gram-negative bacteria. However, the antibacterial effect of *Myrtus communis* on oral gram-negative pathogens has not yet been determined. The aim of this study was to examine the antimicrobial effect of *Myrtus communis* on *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.[22–24] Clinical and radiographic evaluations are part of a routine periodontal examination. The presence or absence of inflammation is assessed by various gingival indicators. Based on a subjective evaluation of a combination of visual signs of inflammation (color, texture, and size), the severity of gingival inflammation is scored. Digital photography was utilized to evaluate shade selection and gingival color, as it is reproducible, inexpensive, and noninvasive. Subjectivity associated with individuals' variable capability to discern colors is minimized with methodical color assessment using mathematical equations of color spaces to define colors. The CIELab color system is extensively utilized in tooth-color investigations for calculating and expressing color differences. Developed by the Commission Internationale de l'Éclairage (CIE, International Commission on Illumination) in 1976, the CIELab color system includes L*, which represents lightness; a*, which represents the red-to-green spectrum; and b*, which represents the yellow-to-blue spectrum. The color difference, or ΔE, derived from the German word "Empfindung" for sensation, represents the differences between the L*, a*, and b* values of the standard or target color. ΔE is calculated using the equation: $\Delta E = (\Delta L2 + \Delta a2 + \Delta b2)1/2$. The magnitude of perceptible and/or acceptable color differences for human observers is still not well-defined, nor ideally measured in dental color research.[25–27] This study aims to evaluate the therapeutic impact of a herbal mouthwash prepared using two methods of extraction (ethanolic and decoction) obtained from the leaves of *Populus euphratica* and *Myrtus communis* compared to a mouthwash containing CHX, both clinically and photometrically.

Materials and Methods

Extract Preparation

Healthy leaves of *Populus euphratica* and *Myrtus communis* (30 kg, 15 kg of each plant) growing in Baghdad Province, Iraq, and identified by a specialized plant taxonomist, were collected in August 2023. The plant leaves were cleansed, desiccated for 7 days under room conditions, and then dried in an electric oven for 2 days at 40°C.[28] The dried leaves were then pulverized into a blended powder of equal weight from both plants using an electric blender. For the preparation of the ethanolic extract, 15 kg of the powdered dried leaves were immersed in approximately 75 L of 70% ethanol and frequently agitated for 15 days. The mixture was filtered through double-layered muslin cloth and then subjected to vacuum filtration to remove residues. The extract was then treated in a water bath and later in a rotary evaporator to remove the hydroalcoholic solvent at about 40°C. Aqueous extraction (decoction) was prepared by adding 100 L of sterilized boiling water to 15 kg of powdered dried leaves. After 24 hours, the mixture was filtered through double-layered muslin cloth and then vacuum filtered to remove residues. It was subsequently treated in a water bath and then in a rotary evaporator at 40°C. The gel-like material obtained from both methods of extraction was further freeze-dried with carbon dioxide (CO_2) dry ice to prepare a powdered form and to preserve the phytochemicals of the plant extracts. The powdered extracts were kept in a hermetically sealed container until use. The extract was diluted in distilled water to achieve an effective concentration of 120 mg/mL, as reported by previous studies.[29]

Study Population

A total of 80 patients aged between 8 and 11 years were selected for this randomized, double-blind, placebo-controlled study. Ethical approval was obtained from the College of Dentistry, Al-Mustansiriya University, Al-Rusafa, Baghdad, Iraq, with code number MUPEDO2. Parents of the children were provided with both written and verbal details regarding the study and signed their agreement to participate. Exclusion criteria included long-term medication, recent antibiotic use, systemic illness, allergies, prior use of mouthwashes in the last 1 month, and pathological alterations of the oral mucosa. The inclusion criteria were mild-to-moderate gingivitis and plaque accumulation according to the Loe and Silness indices of gingivitis and plaque.[30] Participants were randomly drawn from the patient pool of those treated in the clinic of the Department of Pediatric Dentistry, College of Dentistry, Al-Mustansiriya University, Al-Rusafa, Baghdad, Iraq, and assigned to one of three treatment groups. Eleven patients refused to undergo the initial clinical examination before treatment, two patients declined to have digital photographs

taken, and seven patients did not return for follow-up visits. The total number of patients available for evaluation was 60 (45 males and 15 females). Two pedodontists with extensive experience were chosen to conduct the clinical examination, document the clinical values, and transcribe the indices. Both pedodontists had undergone concentrated theoretical and brief clinical training sessions to standardize their assessment techniques and minimize interexaminer variability. The subjects were distributed into three groups—group I ($n = 15$)—treated with mouthwash prepared from ethanolic herbal extract, group II ($n = 15$)—treated with mouthwash prepared from herbal decoction, group III ($n = 15$)—using CHX mouthwash (0.12%) (positive control), and group IV ($n = 15$)—using placebo mouthwash (negative control) prepared from distilled water colored with beet peels giving solution red color.[31] Both the examiners and the patients were double-blinded to exclude bias. All solutions were prepared in matching bottles with the identical hue (red) to prevent bias. The bottles were then coded, and decoding was performed at the end of the study. Each patient was given one bottle (350 mL) according to their allocated group, along with a mm-calibrated cup. Children were instructed to continue brushing their teeth twice daily, in the morning and before bedtime, and to rinse their mouths with 10 mL of the supplied mouthwash from their respective group for 1 minute, twice daily. The interval between these two rinsing was approximately 12 hours. This schedule was consistent with the standardized protocol for CHX mouthwash, which must be used at 12-hour intervals as it has been found to reduce salivary bacterial counts for >12 hours.[32] The children were advised not to eat or rinse there mouth for the next 30 minutes. to linger the retention of CHX in the oral cavity.[33] The gingival index (GI) and plaque index (PI) (assisted with plaque disclosing tablets) were calculated before the treatment (0 reading), after 1 week (7 days), and after 2 weeks of treatment (14 days)[34] any adverse outcomes associated with treatments evaluated in this study were recorded.

Digital Images Acquisition and Analysis

A Nikon D610 full-frame digital single-lens reflex (DSLR) camera, along with a Tamron 90 mm macro lens, was used to image the patients' labial gingiva above the maxillary anterior teeth. The same dentist, room, and lighting conditions were used to ensure standardization. A tripod was employed, and the distance between the patient and the camera was set to 1.5 m for the facial images. The camera settings used in this study were as follows: manual mode, ISO 100, aperture of f/22, magnification ratio of 1:2, and shutter speed of 1/200 second. Two speed lights set to manual mode with 1/4 power served as the source of illumination. A gray card with 18% gray was used to calibrate white balance and was imaged with each photograph taken. The images were transferred to a personal computer monitor (Dell OptiPlex, United States of America) for evaluation and assessed using Adobe Photoshop CC 22 (Adobe Systems Inc., San Jose, United States of America) image software. The raw image profile was set to camera neutral to avoid software interference with the original colors. The image mode was set to CIELab. The "eyedropper tool" was used to pick color values in the CIELab from the specified area of the gingiva before and after treatment. Readings were repeated three times, and the average reading was used. ΔE was measured using Microsoft Excel software (Microsoft, United States of America) function: $((L1 - L2)^2 + (a1 - a2)^2 + (b1 - b2)^2)^{(1/2)}$ (Fig. 1).

Results

Data were charted in Excel sheets and analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics 21 (SPSS, United States of America). An intraclass correlation coefficient (ICC) reliability and consistency test was performed for the two pedodontists who conducted the clinical evaluation of the patients in this study, which showed high consistency (ICC = 0.981; $p < 0.05$) between the two examiners. Descriptive data of gingival and plaque scores at the start, on day 7, and at the end of the treatment on day 14 are tabulated in Tables 1 and 2. One-way analysis of variance (ANOVA) with Tukey *post hoc* test showed that both CHX and the *Populus euphratica* and *Myrtus communis* herbal mouthwashes, prepared by two different extraction techniques, resulted in comparably significant reductions in gingivitis and plaque accumulation ($p > 0.05$). Regarding CIELab parameters, there was a statistically significant increase in the L* value and a significant reduction in

Fig. 1: CIE lab values measurement using color picker tool of the photoshop software

Table 1: Gingivitis score at start of the treatment, after 7 days, and after 14 days

	Start				*Mid*				*End*			
Time\ treatment	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*
N	15	15	15	15	15	15	15	15	15	15	15	15
Mean	1.6	1.7	1.7	1.7	1.7	1.4	1.2	1.4	1.6	1.2	1.0	1.2
SD*	0.29	0.31	0.31	0.31	0.27	0.26	0.27	0.28	0.27	0.26	0.28	0.24
p-value**		1.000				0.000				0.000		
Tukey HSD		None significant				CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments				CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments		

*SD, standard deviation; **$p < 0.05$ indicates significant

the a* value between baseline and posttreatment ($p < 0.05$), while the b* value showed a nonsignificant decrease ($p > 0.05$). There were obvious changes in ΔE values between groups treated with herbal and CHX mouthwashes, while the water placebo showed none when compared with the critical ΔE threshold of 3.7 for intraoral color distinction as perceived by the naked eye. Significant differences were observed between CHX, herbal mouthwashes, and the placebo mouthwash ($p < 0.05$). No significant differences in ΔE were observed between patients treated with herbal and CHX mouthwashes ($p > 0.05$) (Tables 3 and 4). The Pearson correlation test showed a high positive correlation between the a* value and GI ($r = 0.785$; $p < 0.05$), and a low positive correlation between the b* value and GI ($r = 0.358$; $p < 0.05$). The L* value showed a high negative correlation with GI ($r = –0.607$; $p < 0.05$).

Discussion

Several studies have found a significant prevalence of caries and periodontal disease in Iraq due to the fact that the majority of the population places little value on basic oral health, particularly in rural and impoverished areas.[35] Generally, mechanical plaque removal has been the main method for treating orodental diseases in all regions of the world. However, data reveal that mechanical cleaning procedures are often insufficient.[36] *Populus euphratica* and *Myrtus communis* are naturally growing trees and shrubs found throughout much of Iraq and are used locally in many traditional herbal remedies with minimal effort and cost to cultivate and make extracts from these plants. In an effort to preserve their traditional use, we studied the medicinal properties of their leaves to determine whether a mouthwash made with herbal extracts from these plants can effectively reduce gingivitis in children.[20,37,38] The present study shows that the herbal mouthwash prepared from *Populus euphratica* and *Myrtus communis* is equally effective in controlling oral health compared to the standard control of CHX mouthwash. This effectiveness may be related to the antimicrobial, anti-inflammatory, and antioxidative activities of the herbal extracts, as reported by previous studies. Several studies have reported the isolation of diterpenoids with cytotoxic and potent wound-healing promotion properties from the leaves of *Populus euphratica*.[20,39–41] Other research has shown that the leaves of *Myrtus communis* contain many secondary metabolite compounds that are effective against bacteria, fungi, and viruses.[42] Aqueous herbal extracts of *Myrtus communis* have been reported to have antibacterial activity against several oral pathogenic bacteria involved in periodontitis, such as *S. aureus*, *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia*.[42–44] *Myrtus communis* may have an anti-inflammatory effect on disorders associated with inflammation and can help reduce edema.[45] The present study evaluated a mouthwash made from a combination of two types of herbal extracts. Herbal products, in the form of dentifrices and mouth rinses, can be based on a single natural component or a mixture of several medicinal plants. Additionally, some studies have assessed the effect of combining herbal treatments with conventional mechanical dental practices, such as scaling, and have shown a synergistic effect. This suggests

Table 4: Color change (ΔE) measured at the end of treatment

	ΔE			
Treatment	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*
Mean	0	6.61	6.56	6.74
SD*	0	0.218157	0.227851	0.344344
p-value**	0.000			
Tukey HSD	CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments			

*SD, standard deviation; **$p < 0.05$ indicates significant

Table 2: Plaque score at start of the treatment, after 7 days, and after 14 days

	Start				*Mid*				*End*			
Time\treatment	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*
N	15	15	15	15	15	15	15	15	15	15	15	15
Mean	1.75	1.77	1.77	1.76	1.71	0.99	0.85	0.99	1.84	0.49	0.36	0.49
SD*	0.26	0.25	0.25	0.25	0.26	0.28	0.27	0.29	0.21	0.28	0.25	0.29
p-value**	0.997				0.0000				0.0000			
Tukey HSD	None significant				CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments				CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments			

*SD, standard deviation; **$p < 0.05$ indicates significant

Table 3: CIE L*a*b* values at the start and at the end of treatment

	L value*				*a* value*				*b* value*			
Time\treatment	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*	*Water*	*Herbal ethanolic extract*	*CHX*	*Herbal decoction*
Mean before	54.53	54.33	54.13	54.87	36.47	36.13	36.00	36.07	25.60	26.00	26.07	25.53
Mean after	54.53	50.33	50.13	50.87	36.47	31.13	31.00	31.07	25.60	27.13	27.00	27.07
p-value*	0.000				0.000				0.382			
Tukey HSD	CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments				CHX = Herbal mouthwashes (decoction, ethanolic) Water < other treatments				None significant			

*$p < 0.05$ indicates significant

that herbal products can be used as a complementary therapy to improve the effectiveness of conventional treatments.[46] Results also show that both extraction techniques (decoction and ethanolic extraction) are equally effective in treating gingivitis and reducing plaque. This finding limits the need for the costly and time-consuming ethanolic extraction process, as decoction is faster and cheaper.[39,42] It is worth mentioning that none of the patients who participated in the study suffered from adverse effects from using herbal mouthwashes, whether prepared by decoction or ethanolic extraction. In contrast, eight patients in the CHX mouthwash group reported adverse effects, such as sloughing, ulceration, and teeth staining.[47] Further investigation is required to evaluate the full potential of these herbs on a larger population sample and to develop more convenient methods of delivery. Currently, clinical examination using specific criteria to determine the presence of inflammation, which may be subjective is the gold standard for assessing the efficacy of periodontal treatment. This study assessed the potential application of a convenient photometric analysis method, which has been suggested by several other studies, for the quantitative assessment of gingivitis and monitoring the outcomes of periodontal therapy for children. Gingival color variations before and after treatment can be distinguished using the CIELab color space values derived from digital photos.[26,48] Comparisons of color analysis methods for tooth enamel revealed that photography and colorimeters produced E values lower than those recognized by the human eye.[49] Red is represented by the a* value in the CIELab color space; a higher a* value indicates a more reddish color. L* indicates the lightness of color. In this study, the a* value was found to have significantly decreased ($\Delta a^* = 5$) following treatment, while the L* value significantly increased ($\Delta L^* = 4$). No significant differences were found in the amount of a* value reduction and L* increase between herbal and CHX mouthwashes ($p > 0.05$). The decrease in a* is positively correlated with a decrease in the GI, while the L* value is negatively correlated. This could be interpreted as periodontal treatment and the subsequent mouthwash regimen, whether chemical or herbal, effectively reduce inflammation, with a reduction in redness and a restoration of a healthier (rose-pink) color of the gingiva. The ΔE value denotes the whole change of color symbolized by the L*, a*, and b* parameters. It is expected that naked eye is the typically capable to sense any color variation of a Δ E = 1:2.[50,51] ΔE acceptable threshold was determined to be 3.1 using a photographic approach to evaluate gingival color.[52] While in another study, the ΔE threshold was 3.7 which was used in this study.[26] In this study, there was a significant difference in ΔE before and after treatment ($p < 0.05$). Herbal and chemical mouthwashes had a similar impact on ΔE ($p > 0.05$). The color change ($\Delta E = 6.6$) is very suggestive of the effectiveness of the therapies used. It also demonstrates the photometric analysis capabilities to monitor color changes; and thus the healing process for pediatric patients suffering from periodontal disorders such as gingivitis. The results of this study are consistent with previous studies regarding the ΔE and a* parameters, while the L* parameter results showed some disagreement. This discrepancy could be related to differences in study groups, the use of a single-site evaluation rather than a comprehensive mouth assessment, and the influence of tissue biotype (thin vs thick) on the extent of color shift. Additionally, some ethnic groups with gingival pigmentation may require special adjustments to recognize such photometric shifts.[25,26,53,54]

Finally, the type of camera and illumination used (in this study, a full-frame camera with a polarized filter) may also influence the results. Further investigations are required to corroborate the current study's findings and to design an adequate gingival color scale for pediatric patients.[53–58] Because color is a key indicator of gingival inflammation, digital photos can be an excellent tool for diagnosing gingival inflammation, both pre- and postoperatively in pediatric patients undergoing periodontal therapies. Digital photography is widely used in modern dentistry, making it a plausible option for diagnosing and monitoring the outcomes of periodontal therapy, as this study has shown. Additionally, it encounters less objection and fear from pediatric patients compared to conventional clinical examination methods; for instance, 11 patients refused clinical examination, whereas only two patients refused to have photographs taken.[59,60]

Conclusion

Herbal mouthwashes offer significant promise for treating and preventing periodontal diseases in young children and have fewer adverse effects compared to traditional chemical mouthwashes.

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