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COMPARATIVE EVALUATION OF ANTIMICROBIAL AND ANALGESIC ACTIVITIES OF SYNTHETIC AND HERBAL ORAL HYGIENE PRODUCTS IN PAKISTAN

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Abstract

Objective: *The aim of this study was to evaluate and compare the antimicrobial and analgesic properties of commonly available oral hygiene products in Pakistan, including both synthetic and herbal toothpastes and mouthwashes. With the increasing popularity of herbal formulations, it has become essential to assess their actual biological effectiveness beyond marketing claims.*

Methodology: *Nine commercially available dentifrices including, six toothpastes and three mouthwashes were selected and categorized into synthetic herbal and mouth wash groups. Their antimicrobial activity was tested in vitro against ten bacterial and fungal strains using the agar well diffusion method. Analgesic potential was assessed in vivo using the acetic acid-induced writhing test in NMRI strain mice. All data were analyzed statistically using two-way ANOVA, with $p<0.05$ considered significant.*

Results: *Synthetic products, toothpastes, showed strong antimicrobial activity, especially against Candida albicans and Streptococcus pyogenes. Herbal formulations generally demonstrated limited antimicrobial effects. In terms of analgesic activity, among synthetic mouthwashes MW-1 stood out by completely suppressing pain responses in mice, despite showing no antimicrobial action. Other products had either moderate or negligible analgesic effects, with no consistent overlap between antimicrobial and pain-relieving capabilities.*

Conclusion: *The findings reveal that antimicrobial and analgesic activities do not always go hand in hand in oral care products. While some synthetic formulations excelled in microbial inhibition, only MW-1 showed notable analgesic benefit. These results emphasize the need for dual-action products and more rigorous scientific testing, especially for herbal dentifrices, to ensure safety, consistency, and therapeutic value.*

INTRODUCTION

Oral hygiene has been an essential aspect of human health and culture since ancient times. Archaeological and historical records reveal that humans have practiced dental care since 7000 B.C, signifying its

importance in societal and cultural evolution [1]. Ancient civilizations such as the Egyptians, Greeks, Romans, Hebrews, and Buddhists documented oral care, using tools like chew sticks, sponges, and bone picks to remove debris and clean the mouth [2]. Archaeological findings and early texts, such as the *Ebers Papyrus* of ancient Egypt, illustrate the formulation of early dentifrices using ingredients like green lead, verdigris, honey, incense, and powdered flint stone [3]. These early practices, though rudimentary and sometimes harmful, reflect an enduring recognition of oral cleanliness as a symbol of personal hygiene and social civility.

With the advent of modern science, these primitive formulations evolved into scientifically developed products, paving the way for present dentifrices. Today, toothpastes and mouthwashes are not only used to enhance cosmetic appearance and reduce odour but also serve as preventive agents against dental caries, plaque accumulation, gingival inflammation, and oral microbial infections [4]. Dentifrices are now available in various forms like paste, gel, powder, and mouthwashes. Mouthwashes are usually formulated with antiseptics, antimicrobials, fluorides, and herbal extracts [5].

The progression from abrasive, sometimes even toxic substances like lead compounds to sophisticated, evidence based formulations marks a significant achievement in pharmaceutical and dental sciences. Since the mid-20th century, attention has shifted toward therapeutic dentifrices products designed not only for mechanical cleaning but also for delivering pharmacologically active agents [6]. In 1960, the American Dental Association officially recognized stannous fluoride-based toothpaste as a therapeutic agent, capable of significantly reducing dental caries [7]. Subsequently, formulations with antimicrobial agents such as triclosan, chlorhexidine, cetylpyridinium chloride, and essential oils have been developed to broaden the functional scope of oral care products.

Despite these advancements, a new trend has emerged with increased consumer interest in herbal and plant-based oral hygiene products. Public perception of synthetic chemicals as potentially harmful, combined with growing awareness of natural remedies, has led to a surge in the use of herbal toothpastes and mouthwashes [8]. These products often include extracts of *Azadirachta indica* (Neem), *Salvadora persica* (Miswak), *Mentha* species, and *Camellia sinensis (tea)*, among others [9, 10, 11]. Manufacturers claim antimicrobial, anti-inflammatory, analgesic, and anti-cariogenic benefits, often referencing traditional medicine systems such as Ayurveda and Unani. This has initiated a debate about the comparative efficacy and safety of herbal versus synthetic formulations. While synthetic oral hygiene products are well regulated and supported by clinical evidence, herbal alternatives are often under-researched, lacking robust pharmacological and toxicological data [12]. Furthermore, differences in standardization, formulation practices, and quality control of herbal products raise additional concerns regarding their consistency and therapeutic outcomes [13].

In the global and regional market including Pakistan, a wide variety of oral hygiene products are available to consumers, many of which advertise similar benefits despite having vastly different compositions. Yet, comprehensive comparative studies that assess both the pharmacognostic characteristics and pharmacological effects of these products are scarce. Most available research is either limited to single parameter evaluation or focuses narrowly on one product category. There is a distinct lack of integrative studies that assess multiple biological activities across a spectrum of products used in everyday oral hygiene. Hence the aim of our study was to evaluate the antimicrobial as well as analgesic activity of wide range of dentifrices available in Pakistan by using multiple in-vitro assays. We selected nine commercially available oral hygiene products, six toothpastes and three mouthwashes and compared their activity over a broad range of assays. The results are expected to aid clinicians, researchers, and consumers in making informed choices based on scientific merit rather than marketing claims, and promote evidence based practice.

Material and Method

In-vitro

Tested products

Nine different oral hygiene products were selected and divided into three groups, Group 1 included three synthetic toothpastes TPS-1, TPS-2 and TPS-3, Group 2 included three herbal toothpastes TPH-1, TPH-2 and TPH-3, and Group 3 included synthetic

mouthwashes MW-1, MW-2 and MW-3, they were procured from different pharmacies located in Karachi. Each product was authenticated and verified for quality and labeling accuracy by Prof. Dr. Mansoor Ahmed, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Additionally, a standard drug vibramycin (doxycycline hyclate) was used as a standard/positive control for antibacterial assays.

Culture media

For bacterial growth, Brain Heart Infusion (BHI) media (dehydrated; Merck, Germany), Sodium Chloride (NaCl; Merck, Germany), and Nutrient Agar (Biolab, USA) were used. For fungal growth Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were used.

Antimicrobial assay

Anti-fungal and antibacterial activity was carried out against *Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri, Staphylococcus epidermidise, Salmonella paratyphi B, Shigella dysentery, Streptococcus faecalis,* and *Streptococcus pyogenes*. Nutrient agar was used as the assay medium and brain heart infusion served for culture maintenance for bacteria, while SDA was used as an assay medium and SDB acted as a culture maintenance media for fungi. Cultures were incubated at 37 ± 1°C for 24 hours and diluted to a 1:1000 concentration using sterile saline. Agar plates were seeded with 0.1 mL of the diluted culture and allowed to dry. Wells of approximately 6 mmin diameter were bored into the agar and filled with test samples (10% toothpaste solution or undiluted mouthwash), distilled water (negative control), and doxycycline (positive control). Plates were incubated at 37°C for 48 hours, after which inhibition zones were measured to determine antimicrobial efficacy [14]. All test samples were evaluated in triplicate against each microbial strain.

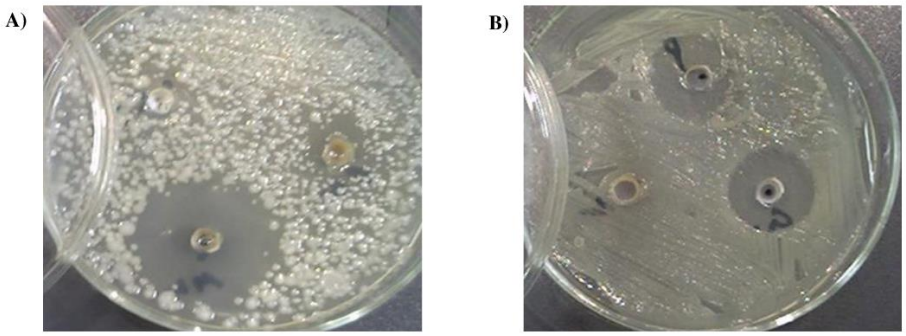


Fig. 1. Zone of Inhibition of different bacterial strain using test samples.

In-vivo

Animals

Nine groups of albino mice of NMRI strain, either sex, weighing18-23 g were used for analgesic studies. Each group included three animals plus a control (n=4), meaning a total of 36 mice were used. The mice were obtained from Salim Habib University, Karachi, and acclimatized for at least five days under standard laboratory conditions (12-hour light/dark cycle) with free access to food and water.

Dosing

To prepare test solution for evaluating analgesic activity, a dose of 0.071mg/g was used for tooth pastes, while for mouthwashes a dose of 0.041ml/g of undiluted mouth wash was used.

Analgesic activity

In order to check the analgesic activity of tested products, acetic acid induced writhing response test was used [15]. Thirty-five minutes after oral administration of the respective toothpaste solutions to treated mice and normal saline to the control, each mouse received an intraperitoneal injection of 0.06%

acetic acid. The number of abdominal writhes was observed over a thirty-minute period, recorded in three consecutive ten minute intervals to assess analgesic activity.

Statistical analysis

To visualize and interpret the data, GraphPad Prism v8.0.1 was used to make graphs and carry out statistical tests. Two-way ANOVA test was conducted in which the p-value <0.05 was considered to be statistically significant.

Ethical approval

Standard guidelines were followed for this research study and it was approved from the Ethical Review Committee of Salim Habib University, Reference no. SHU-ERC/Pharmacy/Pharmacognosy/Study-001/04-2025

Results

Antimicrobial activity

The antimicrobial activity average value of zone of inhibition (ZOI) in millimeter (mm) against all bacterial strains for each dentifrice is presented in **Table 1**. MW-1 consistently showed no antimicrobial activity (0 mm ZOI) against all tested strains while TPS-1 only showed minimal activity only against *Candida albicans* (5.2mm). In contrast, TPS-2 demonstrated the highest overall efficacy, exhibiting the largest ZOI against *Candida albicans* (37.1 mm), *Shigella dysenteriae* (31.1 mm), and *Shigella flexneri* (28.4 mm). TPS-3 also showed substantial inhibition against *Streptococcal pyogenes* (41.1 mm) and *C. albicans* (23.5 mm). MW-2 and MW-3 exhibited moderate antimicrobial activity, particularly against *C. albicans*, *S. paratyphi B*, and *S. typhimurium*. However, herbal products demonstrated relatively limited or selective activity, with TPH-1 being effective primarily against *S. pyogenes* (23.2 mm).

Table 1: Antimicrobial activity of test samples

Name of Micro organism	TPS-1 (ZOI)	TPS-2 (ZOI)	TPS-3 (ZOI)	TPH-1 (ZOI)	TPH-2 (ZOI)	TPH-3 (ZOI)	MW-1 (ZOI)	MW-2 (ZOI)	MW-3 (ZOI)
Salmonella typhimurium	0	24.93	22.5	0	0	0	0	12.8	14.5
Klebsiella Pneumoniae	0	21.7	20.1	0	4.4	4.4	0	11.8	13.1
Pseudomonas aerogenousa	0	0	0	0	8.6	8.6	0	8.6	10.7
Shigella flexneri	0	28.4	20.7	3.9	3.7	4	0	10.2	11.6
Staphylococc-us epidermidise	0	15	14.4	7.7	7.6	8.1	0	13.5	14.4
Candida albicans	5.2	37.1	23.5	0	0	0	0	21.0	23.9
Streptococcus fecalis	0	19.5	13.4	8.3	7.2	8.5	0	12.2	12.2
Streptococcal Pyogenes	0	25.2	41.1	23.2	17.9	17	0	15.8	17.3
Salmonella paratyphi B	0	25.3	22.3	13.5	7.1	0	0	21.0	23.7
Shigella dysentery	0	31.1	23.2	0	0	0	0	9.9	12.1

Anti-microbial activity is shown in terms of zone of inhibition (ZOI) in millimeters (mm) of each dentrifice against particular micro-organisms.

Analgesic activity

Among the tested samples, only MW-1 demonstrated the highly significant analgesic effect compared to control, with twitch counts dropping to 1.3 in the first 10 minutes and completely absent in the subsequent intervals. Meanwhile, TPS-2, TPH-1, and MW-2 also showed notable reductions in writhing numbers over time, indicating moderate analgesic activity though no significance was found with control. In comparison, TPS-1, TPH-2, and TPH-3 exhibited nearly similar response compared to control **(Fig. 2)**.

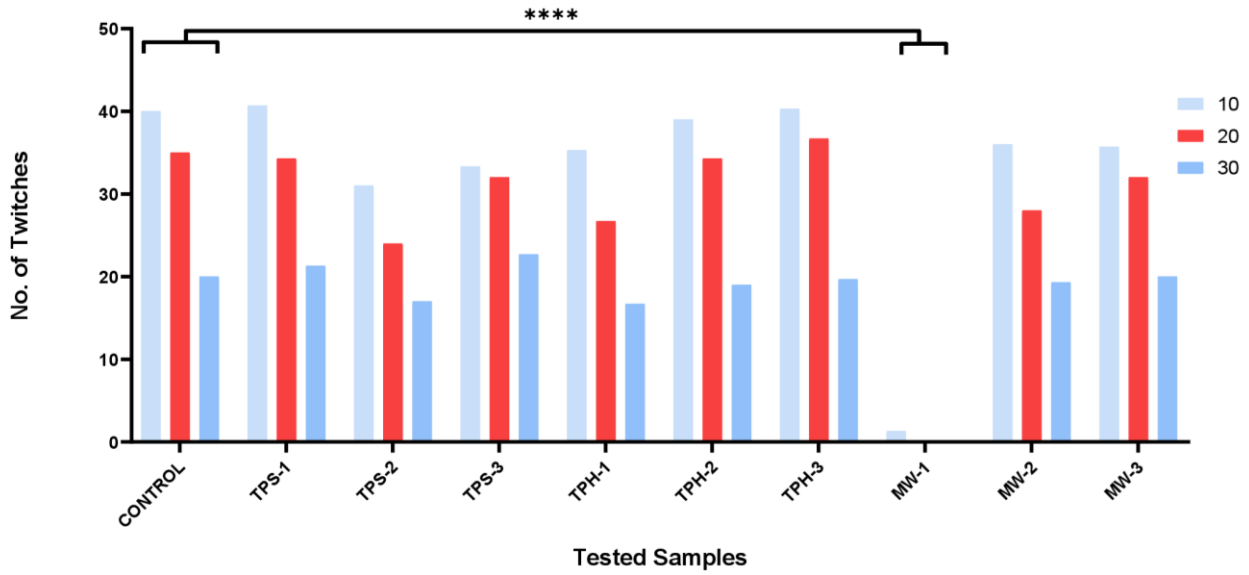


Fig. 2. Number of twitches were observed against all tested samples at 10, 20 and 30 minutes and it was compared with control. "**" shows degree of significance (p= <0.0001).**

Discussion

This study aimed to assess and compare the antimicrobial and analgesic properties of nine commonly used oral hygiene products in Pakistan, including synthetic and herbal toothpastes and medicated mouthwashes. The results revealed major differences in assessed activities, primarily based on formulation type and active constituents.

Among synthetic toothpastes, TPS-2 and TPS-3 showed the most significant antimicrobial activity. TPH-2 was particularly effective against Candida albicans and Shigella dysenteriae, which may be attributed to triclosan and bisabolol present in them, both known for disrupting bacterial membranes and inhibiting microbial metabolism [16, 17]. TPS-3, containing zinc citrate and enoxolone, also demonstrated strong efficacy against Streptococcus pyogenes, aligning with existing data on these agent's antimicrobial roles in dentifrices [18, 19].

In contrast, TPS-1, despite containing hexetidine and fluorides, exhibited limited activity. This may relate to subtherapeutic concentrations or microbial resistance, warranting further formulation review [20, 21].

Herbal products like TPH-1, TPH-2, and TPH-3 showed modest or no antifungal activity, although TPH-1 showed some effectiveness against S. pyogenes. This supports the traditional antimicrobial claims of Salvadora persica [22]. However, inconsistencies in herbal efficacy likely stem from variability in phytochemical content, extraction methods, and the absence of standardized dosages [23, 24].

Among the mouthwashes, MW-2 and MW-3, both chlorhexidine based, showed strong broad spectrum antimicrobial activity. MW-3 (0.2% chlorhexidine) and MW-2 (0.12%) performed similarly, consistent with chlorhexidine's proven efficacy as a dental antiseptic [25]. MW-1, surprisingly, showed no antimicrobial effect despite literature supporting its effectiveness against dental plaque and bacteria. The likely reason may be related to alcohol-free variants or batch variability or the absence of standardized dosage

form as discussed with TPS-1 as both of these products have similar constituents while having different dosage form [23, 24, 26].

Analgesic testing using the acetic acid-induced writhing model revealed that MW-1 showed the most significant pain-relieving activity, completely inhibiting abdominal writhes. This can be attributed to menthol, eucalyptol, and methyl salicylate, which modulate inflammatory mediators and act via TRP channel activation and prostaglandin synthesis inhibition [27, 28].

Other products, including TPS-2, TPH-1, and MW-2, exhibited moderate but statistically insignificant analgesic effects, likely due to mild anti-inflammatory constituents like flavonoids or isothiocyanates present in some herbal extracts [29]. Products like TPH-3 and TPS-1 had negligible effects, reinforcing that analgesic action is not a universal property across all oral care products.

A significant finding of this study is that, it helped us determine which dentrificial formulation is the best to use and which condition one is preferable over the other, and it also showed that there is no necessary direct relationship between analgesic and antimicrobial activity. Products having strong antimicrobial activity like TPS-2 and TPS-3 did not show significant analgesic property while MW-1 showed a complete opposite results. This study emphasizes the need of an oral formulation that can target both and it also underscores the importance of proper scientific validation and standardization of herbal products before they can be marketed as therapeutic agents.

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