

Oral Health Care Improvement using Combined Toothpaste with Traditional Medicinal Plants

^{1,2}Muna Jalal Ali, ^{1,3}Essam A. Makky, ¹Mashitah M. Yusoff

¹Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

²Department of pathological analyses, Al-Haweeja Technical Institute, Northern Technical University, Kirkuk, Iraq

³Center of Excellence for Advanced Research in Fluid Flow (CARIFF), University Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

Address For Correspondence:

Essam A. Makky, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

E-mail: essam22001@gmail.com; Phone: +60139553169; Fax: +609-5492766

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ABSTRACT

The evolution of microbes, including the development of resistant strains and adaptation, depend on diverse members of the microbial population that can thrive in new condition. Therefore, microbes present remarkable abilities to evolve faster than their hosts do. Oral isolates of different patients aged 3 years to 60 years were obtained, purified, and tested against four different commercial medicinal plants extracts for antimicrobial activity. A total of 10 different commercial toothpastes (different brands and prices) were collected, and the combined action of the medicinal plants combination and toothpaste was studied by using disc diffusion method to determine the antimicrobial activity of medicinal plants with toothpaste. We found a higher bacterial population in the age group of 3–40 years than the group of 40–60 years, with approximately 44% and 32%, respectively. The combined action of ethanol medicinal plant extracts (alone) against oral isolates showed a synergistic effect, with 32.20%, for combinations A (Ci/Ca). By contrast, the combined action of medicinal plants with 10 different toothpastes improved the antimicrobial sensitivity by 60% for combinations A. In addition, the effectiveness of toothpaste is not related to the price. The ethanol extract of combinations medicinal plants Ci/Ca with commercial toothpaste showed higher antimicrobial activity against oral isolates than other combination groups and the effectiveness of toothpaste is not related to the price.

KEYWORDS: microbial evolution, bacteria, medicinal plants, toothpaste, antimicrobial activity.

INTRODUCTION

The ecological and evolutionary responses to new environments could alter productivity and diversity–productivity relationships. In turn, diversity may affect the adaptation of component species to new conditions [9]. These ideas were tested in this study. As soon as a baby is born, bacterial colonization of the mouth begins. In addition to frequent contact and sufficient inoculation size, colonization by oral bacteria requires a suitable attachment site and nutrients for growth. For example, children whose mouths are colonized by *Streptococcus mutans* are more likely to have been exposed to behavior that allows for contact with adults' saliva and frequent sugar exposure than children without *S. mutans* [24]. Since precedent days, toothpastes are widely used as cleaning agents. Fluoride principally results in vital enhancements to the oral health of populations worldwide and thus became essential for oral health maintenance in comparison to plain toothpastes (i.e., without fluoride). The main function of toothpaste is to help remove dental plaque. Tooth brushing compliance is reduced below the use of associated abrasion-free formulas as a result of the simple improvement capability of toothbrush alone in removing plaque, leading to exaggerated tooth staining and fast dental biofilm regrowth. This practice should be performed with a toothbrush and a toothpaste for a minimum of two times each day and preferably after every meal or snack [6]. However, for most people, brushing alone is inadequate to remove the oral biofilm to prevent the development of periodontal diseases [8]. Therefore, a variety of toothpastes with antibacterial

properties have been evaluated. Numerous toothpaste formulations with antibacterial activity have been proven effective [11].

Medicinal plants have attracted increasing interest because of their antimicrobial activity against pathogenic oral microorganisms. Plants that are used for traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases [12]. The World Health Organization (WHO) estimates that 80% of the population of certain Asian and African countries presently uses medicinal plants in several aspects of primary health care. Studies in the United States and Europe have shown that the use of these plants is less common in clinical settings but has increased in recent years, as scientific evidence regarding the effectiveness of herbal medicine has become more widely available [19]. Medicinal plants are important sources for pharmaceutical manufacturing and account for a significant percentage of the pharmaceutical market. For example, in Malaysia, the market for traditional medicine is estimated to be at 1 billion Malaysian ringgit annually [5].

Toothpaste contains ingredients based on medicinal plants that provide high antibacterial activity, with the right balance of all essential elements to ensure proper oral health care [16]. Several recent studies show that plant extracts exhibit significant antibacterial activity against oral bacterial isolates. As a result, some of these agents have been incorporated in different products, including toothpaste [13,20]. However, an effective combination of medicinal plants and toothpaste against initial oral bacterial isolates has not been reported. The present study aims to obtain extracts of different medicinal plants and screen its antibacterial activity against oral bacterial isolates from different patients aged 3 years to 60 years. In addition, the antimicrobial activity of different commercial toothpastes was also evaluated, and the combined action of medicinal plants and toothpaste against oral isolates was studied.

MATERIALS AND METHODS

2.1 Isolation of oral isolates:

All oral isolates were collected from 50 patients at dental clinics in Gambang, Pahang, Malaysia. Sterile swabs were used for patients of both genders with ages ranging from 3 years to 60 years. Collected samples were transferred to a laboratory in University Malaysia Pahang. The patients' oral samples were cultured on nutrient agar plates and incubated at 37 °C for 24 h, and then purified, cultured on agar slants, and kept in a chiller until use.

2.2 Medicinal plants collection and extraction:

The commercial plants parts used in the study, namely, *Cinnamomum zeylanicum* (Ci) (park), *Elettaria cardamomum* (Ca) (fruits), *Piper nigrum* (P) (seeds), and *Nigella sativa* (N) (seeds), were purchased from Kuantan Market, Malaysia. The four plants were crushed using a blender and then sieved to obtain fine powders. Approximately 25 g of the powdered plants were soaked in 200 ml of three different solvents (95% ethanol, hot water, and cold water), kept at room temperature for 72 h, and then filtered using Whatman No.

1 filter paper. The filtrate was evaporated using a rotary evaporator (BUCHI, Rotavapor R-3 Vacuum pump V-700), according to a previous study [10], but with certain modifications. The extracts were stored in sterile bottles and stored at 4 °C until further use. Filter paper discs of 6 mm diameter were prepared using Whatman No. 1, sterilized using UV, and loaded with 25 µl plant extracts, left to dry, and then used for antibacterial screening.

2.3 Antibacterial sensitivity test using disc diffusion method:

Muller–Hinton agar medium was used for antibacterial activity. The plants extracts discs from previously prepared solvents were allowed to set onto the inoculated agar surface. Inoculums from primary culture plates were prepared by touching 3 to 5 colonies with a swab, inoculating into a replicated plate, and incubation at

37 °C for 24 h. After incubation period, each plate was observed, and the inhibition zone of all isolates were recorded in millimeter (mm) [18]. Control discs for different solvents were used.

2.4 Combination of medicinal plants:

The combination of plants extracts obtained by using different solvents was studied at a ratio of (1:1) and prepared by using mixture of two plants extracts viz : (P/Ci, P/N, P/Ca, Ci/N, Ci/Ca, N/Ca). The combinations were tested for antimicrobial activity against oral isolates as mentioned previously.

2.5 Plant–toothpaste combination:

Ten different commercial toothpastes (different brands and prices), which were denoted by 1 to 10, were collected from Kuantan, Malaysia and mixed with the ethanolic plant extracts of combinations A (Ci/Ca) at a ratio of 1:1 (v/v). Filter paper discs were prepared and loaded with a plant–toothpaste combination. The antimicrobial sensitivity test was performed as previously mentioned, and a plant-free toothpaste disc was used as a control.

RESULTS AND DISCUSSION

3.1 Oral Isolates:

Oral isolates were obtained from 50 individuals of different ages and of both genders (54% males and 46% females), and a total of 59 bacterial and yeast isolates were collected. As shown in (Fig.1) the impact of age group on the infection rates of tooth caries showed that age groups of (3–20 years) and (20–40 years) were more susceptible to infection than the older group (40–60 years), with incidence of 32% and 44%, respectively.

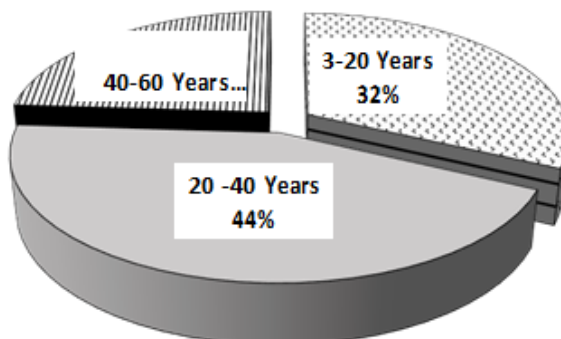


Fig. 1: Percentage of isolates according to age group

This finding may be related primarily to immune shortages of infected people, as well as health-consciousness or other factors related to nutrition and public health that increase the rates of infection in children and younger adults. Our results stated that children are more susceptible to the bacteria that cause decay, in which frequent consumption of sugar is a major factor in infection. Moreover, emphasis has been placed on the function of the mother as a source of disease transmission from her infected teeth to the baby, as shown by the similar levels of bacteria in mothers to those found at their children [21].

3.2 Antimicrobial activity of medicinal plants against oral isolates:

The ethanolic extracts of Ci and Ca showed higher percentages of antimicrobial activity against oral isolates, reaching up to 88.13%, that the hot and cold-water extract. By comparison, only 22.03% and 3.38% antimicrobial activity was exhibited by the ethanolic extracts of P and N (Fig.2).

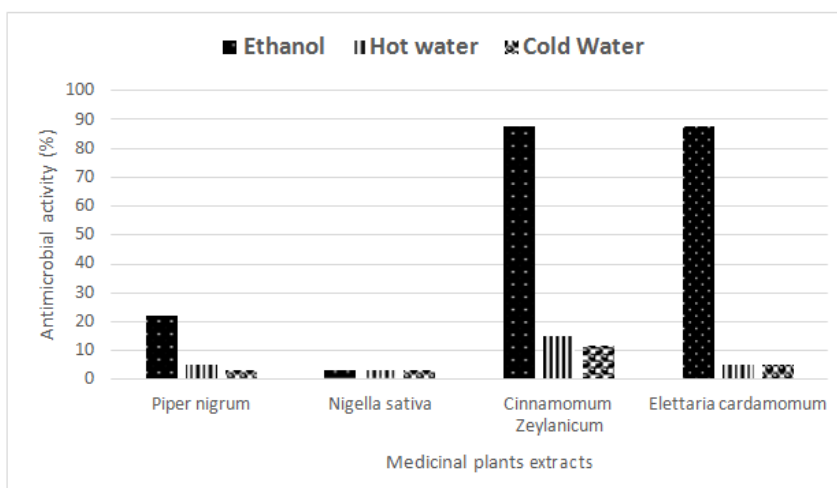


Fig. 2: Antimicrobial activity percentage of medicinal plants using different solvents against oral isolates

The ethanolic extracts of *C. zeylanicum* and *E. cardamomum* were found to be effective antibacterial agents against oral isolates. These extracts were not harmful when consumed in products and used as agents that inhibit the growth of bacteria, and yeast, in accordance with the results obtained in [15]. The hot and cold-water extracts of four medicinal plants showed the least antibacterial activity, which may be due to the polarity of compounds; most antibacterial agents are extracted more effectively by organic solvents than by aqueous extraction [3]. The results of our study were contrary to those in the study of Arora and Kaur [4], who reported that the aqueous extracts of *E. cardamomum* were effective against pathogenic microorganisms. By contrast, Ahmad *et al.* [1] found no antibacterial activity of aqueous extracts. The variations may be due to the method of extraction or strain differences.

3.3 Combination between medicinal plants using different solvents:

The results of antimicrobial activity testing of 6 combinations, with the use of different solvents to obtain medicinal plants extracts, are shown in (Table 1).

Table 1: Summary of antimicrobial activity percentage of medicinal plants combinations using different solvents

Combined groups	Combination effect of medicinal plants using different solvents (%)								
	Ethanol			Hot water			Cold water		
	¹ S	² A	³ NE	S	A	NE	S	A	NE
P/Ci	22.03	42.37	35.59	3.38	1.69	94.91	1.69	1.69	96.61
P/N	15.25	1.69	83.05	3.38	0.00	96.61	1.69	1.69	96.61
P/Ca	15.25	30.50	54.23	1.69	1.69	96.61	1.69	1.69	96.61
Ci/N	23.72	40.67	35.59	3.38	1.69	94.91	3.38	1.69	94.91
Ci/Ca	32.20	59.32	8.47	6.77	0.00	93.22	5.08	0.00	94.91
N/Ca	11.86	23.72	64.40	3.38	0.00	96.61	3.38	0.00	96.61

¹S: Synergism ; ²A:Antagonism ; ³NE: No Effect

Among ethanol, hot-water, and cold-water extracts, the ethanol extract for (Ci/Ca) combination showed antimicrobial activity against 59 oral isolates with a higher synergistic effect of up to 32.20% than other combinations, as shown in (Fig.3).

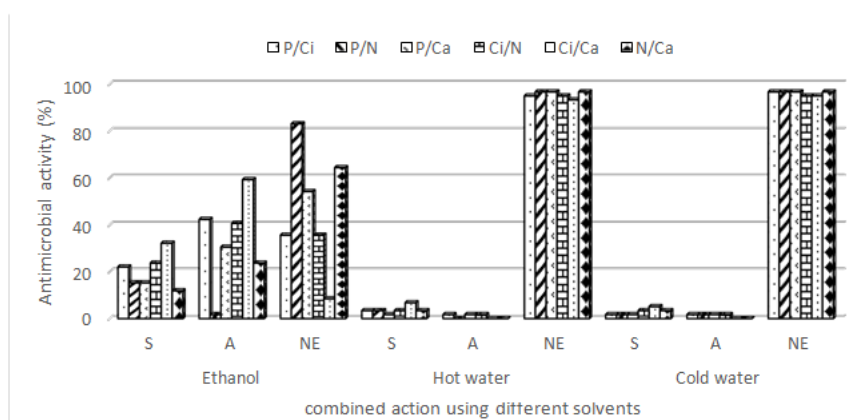


Fig. 3: Antimicrobial activity of medicinal plants combinations by using different solvents

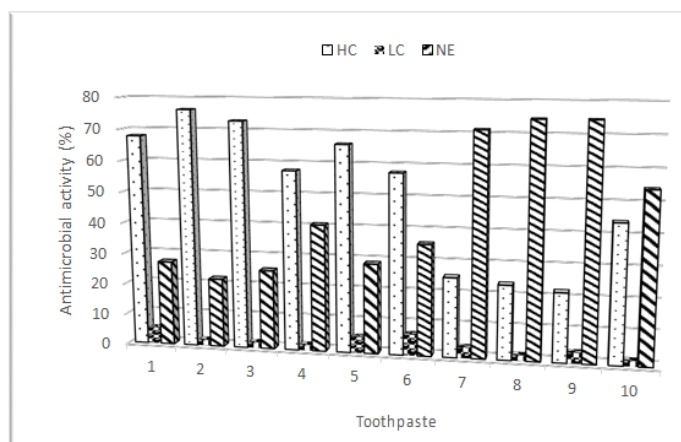
The combination of aqueous extracts of medicinal plants did not exhibit an inhibitory effect against the tested oral microorganisms. In this study, the ethanolic extract preparation shows better antimicrobial activity, in accordance with the results obtained by (K. Aneja and Joshi, 2009). Our results agree with those in (Manurung et al., 2008) which reported that ethanol is capable of extracting tannin, flavonoid, polyphenols, terpenoids, alkaloids, and essential oils in low amounts. Flavonoids, alkaloids, and phenolics inhibit the synthesis of bacterial cell walls. Phenolics and tannin coagulate protein in the cell wall and cut cross-linked peptide bonds within peptidoglycans, resulting in the weakening of the bacterial cell wall structure in bacteria that may affect cell permeability. Alkaloids disrupt the formation of cross-linked peptide bonds in the peptidoglycan structure and compromise the cell's ability to defend itself from external attacks.

Despite the development of antimicrobial activity in products, oral bacterial infections remain a major health problem because of environmental changes, as well as microorganism behaviors. Therefore, developing new antimicrobial compounds with high activity and low toxicity and side effects is urgently needed. Our study showed that all of the studied medicinal plant combinations exhibited high antimicrobial activity against oral isolates, with different synergistic or antagonistic effects.

3.4 Toothpaste combination test with medicinal plants:

Fig.4 Show the antimicrobial activity percentages against 59 oral isolates of 10 commercial toothpastes combined with the highest medicinal plant combinations that were previously obtained. Approximately 60% total toothpaste combined with medicinal plant combination of (Ci/Ca) revealed higher antimicrobial activity against

oral isolates.



*HC: Higher than control **LC: Lower than control ***NE: Not effect compared with control

Fig. 4: Shows antimicrobial activity of combined action between toothpaste and medicinal plant

Numerous types of toothpastes that have been used in the past years contained antibacterial activity, with the aim of preventing dental caries. Different toothpastes contain their own composition and concentration of ingredients for this activity. The findings suggest that the antimicrobial activity of ten different commercial toothpastes combined with the highest-activity ethanol extracts of medicinal plant combination exert an inhibitory effect on pathogenic bacteria that cause dental caries in comparison with the action of toothpaste alone against oral isolates. This study agreed with [25], which reported that herbal toothpastes exhibit high antifungal activity against *Candida*. Interest in alternative toothpastes based on plant extracts has increased recently. These toothpastes have been classified as drugs and not cosmetic toothpastes, which are daily oral care products. The effect of toothpastes mixed with medicinal plants is better than toothpaste alone on oral isolates, because medicinal plants contain different components at sufficient concentrations that exhibit better antibacterial activity than toothpaste alone. Thus, recommending and selecting the best toothpaste has become increasingly difficult for dentists and patients, respectively. The mixture of toothpaste with a combination of medicinal plants presented high antimicrobial activity in comparison with the control. These results were in agreement with the study of Mazumdar et al. [16], who found that complete care herbal toothpaste showed significant reduction in gingival problems, plaque, and dental caries.

The results of our study are in contrast with the study of Mohammed [17], who reported that the antibacterial activity of toothpastes alone was more than plants extracts on tested isolates. Shetty et al. 2015 carried out a comparative evaluation of the effect of herbal and a regular toothpaste on beneficial oral microflora they found that herbal toothpaste may be slightly more effective in preservation of beneficial oral microflora when compared to regular toothpaste, although both are equally effective in inhibiting the pathogenic microorganisms [22]. Bhat et al [7] formulated the efficacy of herbal toothpaste containing Propolis with Miswak and Colgate total toothpastes in controlling plaque formation, they reported that Propolis was to be safe and effective in reducing plaque accumulation when compared to Miswak and Colgate total toothpaste Vohra et al [23] extended the previous work in evaluation and comparison of herbal toothpowder with various marketed toothpaste brands they were found that the herbal toothpowder was effective as comparable to the other synthetic toothpastes available in the market. Finally, the combinations of medicinal plants (Ci/Ca) can be added to the toothpastes since it is inexpensive and available. Thus it appears to be a potent antimicrobial agent that could be considered as a medicinal plant.

3.5 Antimicrobial activity in relation to patient age:

The test aims to find the antimicrobial activity relationship between the combination of toothpaste and medicinal plants, and patient age. Data presented in (Fig.5a) show the impact of patient age on dental caries infection in relation with the antimicrobial activity of commercial toothpaste combined with medicinal plants. We found that combination (Ci/Ca) mixed with toothpaste exhibited antimicrobial activity of up to 70% on all age groups, 20% effective on two different age groups, and only 10% on only one age group. On the other hand, antimicrobial activity of only toothpaste as a control in relation to patient age was studied and represented in (Fig.5b).

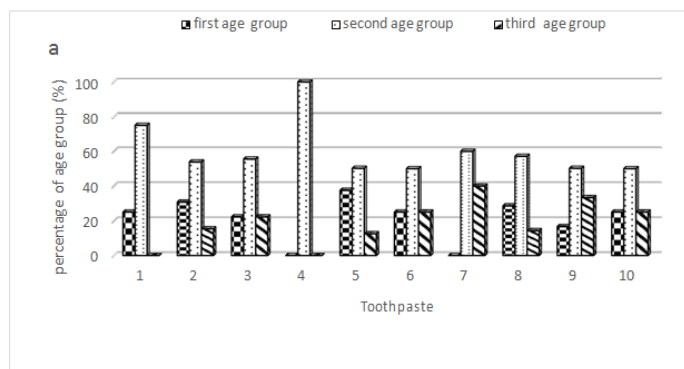


Fig. 5a: Shows the relationship between patient age group percentage and antimicrobial activity of combined action between toothpaste and medicinal plants

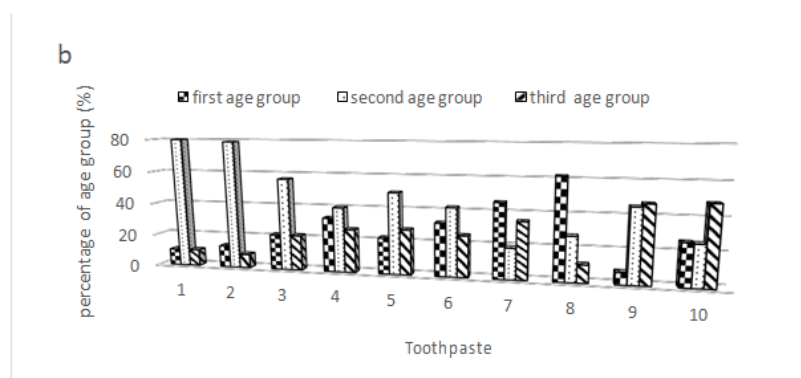


Fig. 5b: Shows antimicrobial activity of toothpaste control in relation to patient age

3.6 Antimicrobial activity in relation to toothpaste price:

The image in (Fig.6) explains the total relationship of antimicrobial activity of the toothpaste–medicinal plant combination and toothpaste price. We found a good relationship between the toothpaste–medicinal plants combinations against oral isolates, in which the antimicrobial activity had increased compared with the toothpastes alone. However, no relationship was found between toothpaste activity and price, and we found that cheaper toothpastes exhibited good antimicrobial activity.

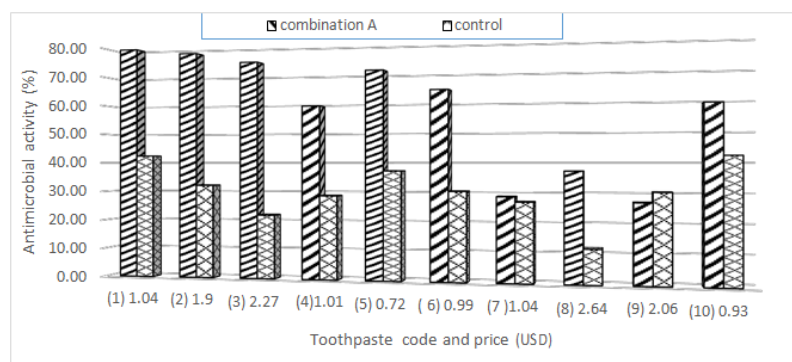


Fig. 6: Antimicrobial activity of combined toothpaste with medicinal plants combinations in relation to toothpaste price

Conclusions:

Although numerous studies on toothpastes have evaluated their effectiveness in preventing and controlling dental caries, no studies on the antimicrobial activity of toothpaste–medicinal plant combinations have been reported. The present study revealed that the ethanol extract of combinations Ci/Ca with commercial toothpaste showed high antibacterial activity against oral isolates compared with the control (i.e., toothpaste alone). Moreover, we found that the antimicrobial activity of toothpaste is unrelated to its price. The presented combinations can be used to develop novel herbal toothpastes.

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