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EFFECT OF INCORPORATING THREE NATURAL ANTIMICROBIAL AGENTS INTO TISSUE CONDITIONER IN INHIBITING THE GROWTH OF C. ALBICANS AND S. MUTANS: AN IN-VITRO STUDY

Mostafa A. Abdellatif* *and* Dalia A. Saba **

ABSTRACT

Background: Incorporation of natural antimicrobial agents into tissue conditioners might inhibit microbial colonization and prevent complications such as denture stomatitis.

Aim of the study: The present study compared antimicrobial properties of tissue conditioner (TC) incorporated with two (5% and 10%) concentrations of pumpkin (PK), rosemary (Rm), or propolis (Pp) extract powders at different storage times (3 and 7 days) against Candida albicans (C. albicans) and Streptococcus mutans (S. mutans).

Materials and Methods: Pilot study was performed to determine minimum inhibitory concentration (MIC) of Pk and Rm against C. albicans and S. mutans using broth-microdilution method. TC disc-shaped samples (n=3/group) mixed with (5% and 10%) concentrations of either Pk, Rm or Pp powders, positive and negative control groups were prepared. Agar-diffusion test was performed and zones of inhibition were measured in millimeters after 3 and 7-days. Data were statistically analyzed.

Results: Maximum inhibition zone against C. albicans was seen in 5% and 10% Pp after 3 & 7 days, which was greater than effect of +ve control group at same periods. For S. mutans, maximum inhibition zones were seen with +ve control group at both time intervals, followed by 10 Rm and 10 Pk extracts after only 3 days. Propolis extract produced larger inhibition zones than Rm and Pk after 7 days at both tested concentrations.

Conclusion: 5% and 10% Propolis extract powder incorporated into TC exhibited considerable antimicrobial efficiency against both *C. albicans and S. mutans*. Incorporation of natural antimicrobial agents in TC can be used as effective alternative to topical synthetic agents.

KEY WORDS: Natural antimicrobial agents, Tissue conditioner, Agar-diffusion test

* Associate Professor of Dental Materials Science, Biomaterials Department, Faculty of Oral and Dental Medicine, Egyptian Russian University; ERU.
** Associate Professor of Dental Materials Science, Biomaterials Department, Faculty of Dentistry, Cairo University.

INTRODUCTION

Tissue conditioners (TC) are temporary soft-lining materials utilized to uniformly distribute stress or force on supporting tissues, reducing masticatory pressures and therefore, conditioning and cushioning inflamed mucosal tissue. However, TCs, are prone to microbial colonization because of their soft-surface texture and lack of antimicrobial properties.[1–7]

Denture stomatitis is the most prevalent inflammatory condition of the oral mucosa, affecting around 15–65% of denture wearers. Denture stomatitis is caused by a variety of factors including; an ill-fitting denture, inadequate denture cleanliness, microbial colonization on the denture surface and oral mucosa, in addition to an impaired immune system.[1,2,8–10]

Denture stomatitis is primarily caused by the dentinophilic yeast, Candida albicans (C. albicans), whose mutualistic association with streptococcal species, including streptococcus mutans (S. mutans), results in a synergistic enhancement of an infectious disease's virulence.[1,8,11–13]

Denture stomatitis is often treated with topical application of medications, but their effectiveness is limited by being easily washed by saliva. Similarly, systemic drug administration may lead to undesirable side-effects. To address this, synthetic or natural antimicrobial chemicals have been integrated into TCs. This method helps minimize unwanted side-effects, improve patient compliance, and reduce treatment expenses. However, synthetic agents such as antibiotics can lead to microbial resistance and drug allergies. On the other hand, natural plant extracts and products are crucial sources of potent antimicrobials due to their abundance, safety, and efficacy without undesirable side effects.[1,14–16]

Among these natural plant extracts, pumpkin (Cucurbita pepo L) is an annual vegetable from the Cucurbitaceae family. Pumpkins are commonly grown for commercial purposes in food and entertainment. Pumpkin is a good source of vitamin A because it contains beta-carotene, a precursor to the vitamin. Pumpkin has gained popularity due to its health benefits, which include antibacterial, antidiabetic, anti-inflammatory, and antioxidant properties. It has numerous medical applications, including wound healing, anticarcinogenic, and diuretic. It is also used to act as a preventive measure against hypertension and is claimed to lower cholesterol.[17,18]

Another medicinal plant is Rosmarinus officinalis L, also known as rosemary, a native Mediterranean plant species. Rosemary has long been used in cooking as a spice to enhance flavors of food. In addition, it is a highly valued medicinal plant in traditional medicine, where it is used to relieve muscle and joint pain, as well as to treat colds and rheumatism. Antibacterial, antioxidant, and antifungal qualities are present in the rosemary extract. It has several applications in dentistry, including disease prevention, dental hygiene, and anti-inflammatory medication. Its impact on microorganisms, however, has not been assessed.[19–23]

Propolis is a natural plant-derived resin, produced by bees by combining wax and saliva with resins collected from plants. Propolis and its extracts are used to treat a variety of diseases due to their antiseptic, antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antitumor, antiulcer, anticancer, scar-forming, tissue regeneration, local anesthetic, immunomodulatory, and cytostatic properties. It has long been recommended to treat oral ulcers and has been used in dentistry to treat surgical wounds, tooth hypersensitivity, pulp capping, and root canal therapy.[24–27]

However, no studies have been conducted to investigate the effect of pumpkin, rosemary extract powders or bee propolis powder incorporated into TC on C. albicans and S. mutans colonization.

Therefore, the purpose of the present study was to compare the antimicrobial effects of a tissue conditioner modified with either pumpkin, rosemary, or Bee propolis extract powders at two different concentrations against C. albicans and S. mutans. The null hypothesis was there would be no difference in the antimicrobial properties of the TCs containing different natural antimicrobial agents.

MATERIALS

Rosemary (Rm) (57% active ingredient) and pumpkin (Pk) (53% active ingredients) extract powders were purchased from MAKIN co., Egypt while Bee propolis (Pp) powder (100% active ingredient) was purchased from Imtenan Health Shop, Egypt. Nystatin oral suspension [100,000 International Unit (IU)/ml] (E.P.P.I.C.O, Egypt), and Ampicillin (E-MOX, 500mg, E.P.P.I.C.O, Egypt), tissue conditioner (Acrostone, Egypt) was used. C. albicans and S. mutans were provided by the Department of Microbiology, Faculty of Medicine, Cairo University.

METHODS:

Determination of the minimum inhibitory concentration (MIC)

A pilot study was performed to determine the minimum inhibitory concentration (MIC) of Pk and Rm extract powders against C. albicans and S. mutans using the broth microdilution method in a 96-well microtiter plate. According to the initial assessment findings for MIC, TC samples were prepared to evaluate the zone of inhibition (ZOI).

Broth microdilution method for MIC calculation

Strains of C. albicans and S. mutans were inoculated in Brain Heart Infusion (BHI) Broth and was incubated for 24 h at 37°C for cultures growth. Microbial suspension was adjusted to McFarland 0.5 standard solution (1.5x 10^8 CFU/mL) and its turbidity was adjusted to optical density OD= 0.13 using spectrophotometer at 625 nm. Serial dilution of the antimicrobial agents was started by dissolving 1g of each test powder in 1ml Mueller-Hinton broth resulting in 100% concentration. This results in 53% & 57% active ingredients concentrations of Pk & Rm, respectively. Accordingly, another 4 successive half dilutions were prepared down to concentration of 6.25% which resulted in active ingredient concentration of 3.3125% & 3.6525% for Pk & Rm, respectively. The dilutions were transferred to 96-well microtiter plate, then 5 μl of the prepared microbial cultures were added to each antimicrobial dilution. After incubation for 24 h at 37°C, using the same spectrophotometer under same previously mentioned parameters, the wells were optically checked for turbidity as an indication for bacterial growth. The lowest concentration of the antimicrobial agent needed to inhibit microbial growth compared to the negative control culture was defined as MIC. Because all antimicrobial dilutions resulted in turbid suspensions with high OD, so the real MIC was calculated based on previous research by Quan et al, 2021 [28] through the following equation:

$$Real\ OD = OD_t - OD_{am} \qquad (Equation\ 1)$$

Equation 1: Where OD_t represents the total optical density of the final mix of the microbial standard & antimicrobial agent, while OD_{am} is the optical density of the antimicrobial agent alone

Fungal or bacterial suspension added to Meuller-Hinton broth without the addition of the antimicrobial agents served as positive control, while the broth only was taken as the negative control.

TC samples' preparation:

The commercially available TC (Acrostone, Egypt) was specifically selected because it is supplied is a powder and liquid form, to facilitate the manipulation after addition of natural antimicrobial powders. According to MIC test results, 5 to 10% by weight of either Pk or Rm whole powders were

used. For Pp powder, and according to previous study by Hejazi et al (2014)[29], a concentration lower than 5% would be effective against tested micro- organisms, however, it was added to the tissue conditioner powder in the same 5 & 10% concentrations as other antimicrobial powders for standardization. Subsequently, the TC liquid was added and mixed on a glass slab using a stainless-steel spatula following the manufacturer's instruction. After mixing, disk-shaped TC samples (6 mm in diameter and 5 mm in thickness) were fabricated using a ring-shaped Teflon mold between double glass plates. Five minutes after mixing, the samples were removed from the mold, and the absence of air bubbles was checked visually before further testing. TC samples without any additives were designated as the negative control, while those mixed with Ampicillin powder or Nystatin suspension were used as positive controls for Gram-positive bacterial (S. mutans) and fungal strains (C. albicans), respectively.[6,16,30]

Triplicates were done for each group to check the repeatability of the antimicrobial effect.

The groups were classified as follows:

- Group (5 Pk): TC mixed with 5% Pumpkin extract powder
- Group (10 Pk): TC mixed with 10% Pumpkin extract powder
- Group (5 Rm): TC mixed with 5% Rosemary extract powder
- Group (10 Rm): TC mixed with 10% Rosemary extract powder
- Group (5 Pp): TC mixed with 5% Propolis extract powder
- Group (10 Pp): TC mixed with 10% Bee Propolis extract powder
- Group (+ve cont.): TC mixed with either Ampicillin or Nystatin suspension
- Group (-ve cont.): TC without any additive.

Candida albicans and Streptococcus mutans inoculation preparation

The antimicrobial activity of the tested compounds was determined using the agar diffusion method. All the samples were tested *in vitro* for their antibacterial activity against Gram-positive bacteria, *(ATCC:13565)* using nutrient-blood agar culture medium. The antifungal activity of the prepared samples was tested against *C. albicans (ATCC:10231)* using Sabouraud dextrose agar culture medium.

Agar diffusion testing method

The assigned sterilized media was poured onto sterilized Petri dishes 25 ml in diameter, and allowed to solidify at room temperature. Microbial suspension was prepared equivalent to McFarland 0.5 standard solution (1.5x 10^8 CFU/mL) and its turbidity was adjusted to OD= 0.13 using spectrophotometer at 625 nm. Optimally, after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension and was flooded on the dried sterilized media surface then allowed to dry for 15 minutes with the lid in place. Four - Six TC/antimicrobial discs were planted by sterile forceps carefully onto the medium inside the plate and gently pressed on the agar surface until the whole disc was in contact with the agar. The plates were incubated at 37°C for 7 days. After 3- and 7-days, plates were vertically photographed and images of plates were then analyzed to measure zones of inhibition for each test group in millimeters using ImageJ software (ImageJ 1.54f (64-bit), National Institutes of Health, USA). Two diametric readings were taken for each sample and their mean was taken as the final reading for that sample.[1,3,4,11,31,32]

Statistical Analysis

Mean distances of zones of inhibition for each group were calculated and statistically analyzed using SPSS (IBM® SPSS® Statistics for Windows, Version 26.0., IBM Corp.). After proofed means

normality, differences between means were analyzed by Analysis of Variance (ANOVA) test at level of significance p-value = 0.05. For significantly different results, Scheffe post-hoc test was used for pairwise comparisons.

RESULTS

Results of Micro-dilution broth test:

It was found that MIC of Pk extract powder against C. albicans and S. mutans was 26.5% and 3.3125%, respectively. Also, MIC of Rosemary extract powder was 3.56% against both C. albicans and S. mutans [Figs. 1& 2, black arrows]. Negative values in Fig 2 are due to real OD calculations through Equation *1*.

Results of Agar-diffusion test:

Results of antifungal activity against C. albicans

The results of the antifungal activity of the TC mixed with any of the three natural antimicrobial agents, or mixed with Nystatin (+ve control) and -ve control against C. albicans were determined by measuring the zones of inhibition after three and seven days at two different concentrations, are summarized in [Table 1/Figs-3&5].

The results showed that the maximum inhibition zone against C. albicans was shown only around TC/Pp at both concentrations of 5 & 10% (10.32 and 9.97 mm) after 3 days and (10.71and 10.80 mm) after 7 days, respectively, and around TC/Nystatin +ve control (9.04 and 8.97 mm) at 3 and 7 days. Neither concentration of Pp nor time resulted in significant difference of Pp effect on C. albicans. The inhibitory effect of Pp at its 2 concentrations was significantly higher than that of Nystatin at 7 days evaluation. No inhibition was seen with -ve control gp or with the rest tested gps at both time intervals.

TABLE (1) Statistical analysis of inhibition zones of different gps against C. albicans using ANOVA and Scheffe post hoc test.

Different Groups concentrations	3 Days	7 Days	p-value
5 Pk	0.00±0.00 ^{c}	0.00±0.00 ^{c}	1.000
10 Pk	0.00±0.00 ^{c}	0.00±0.00 ^{c}	1.000
5 Rm	0.00±0.00 ^{c}	0.00±0.00 ^{c}	1.000
10 Rm	0.00±0.00 ^{c}	0.00±0.00 ^{c}	1.000
5 Pp	10.32±0.70 ^{ab}	10.71±0.83 a	1.000
10 Pp	9.97±0.70 ^{a}b	10.80 ±1.03^{a}	0.903
+ve Cont	9.04±0.33 ^{b}	8.97±1.07 ^{b}	1.000
-ve Cont	0.00±0.00 ^{c}	0.00±0.00 ^{c}	1.000
p-value	**0.0**	**0.0**	

p<0.05 statistically significant p>0.05 non-significant, NS

Values sharing same letters have statistically insignificant difference within same column

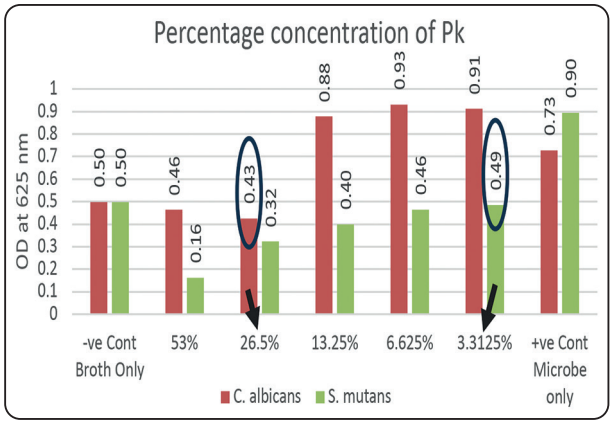

Fig. (1) MIC observed with 26.5% Pumpkin extract against C. albicans and 3.3125% against S. mutans

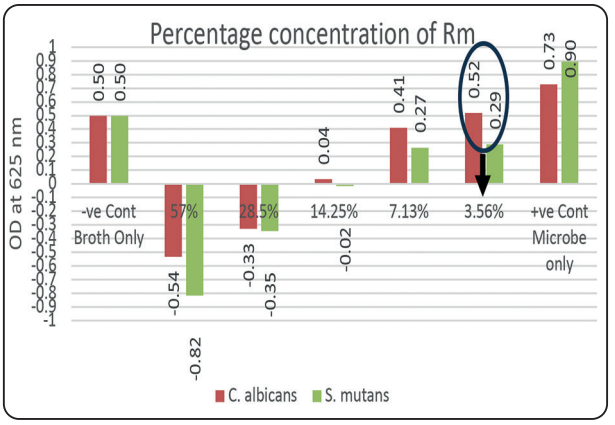

Fig. (2) MIC observed with 3.56% Rosemary extract against both C. albicans and S. mutans

Results of antibacterial properties against S. mutans

The results of the antibacterial efficacy of the TC mixed with any of the three natural antimicrobial agents or mixed with either ampicillin (+ve control) and (-ve control) against S. mutans was determined by measuring the zones of inhibition after three and seven days at two different concentrations, are summarized in [Table 2/Figs 4&5].

The results showed that the maximum inhibitory zone against S. mutans (41.98 and 38.08 mm) was seen with positive control gp after both three and seven days, respectively with no significant difference between the time intervals (p-value =0.799). This was followed by both 10 Rm and 10 Pk extracts after three days (27.44 and 23.44mm), respectively. The least inhibition zone after three days was shown with 5 Pp gp (14.23mm). However, only Pp showed increase in inhibition zones at 7 days, and this increase was significant at 5% concentration.

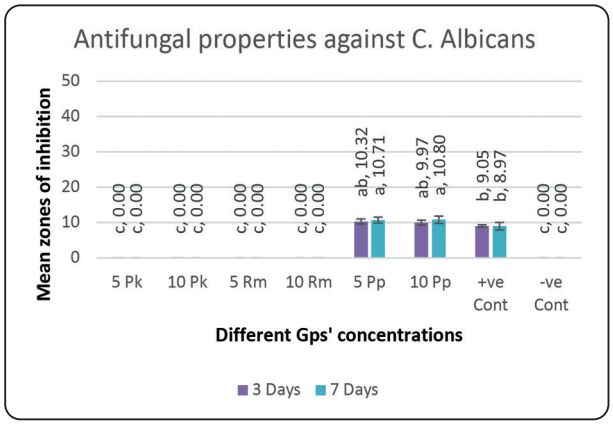

Fig. (3) Mean inhibition diameter (in mm) of different tested groups against *C. albicans*

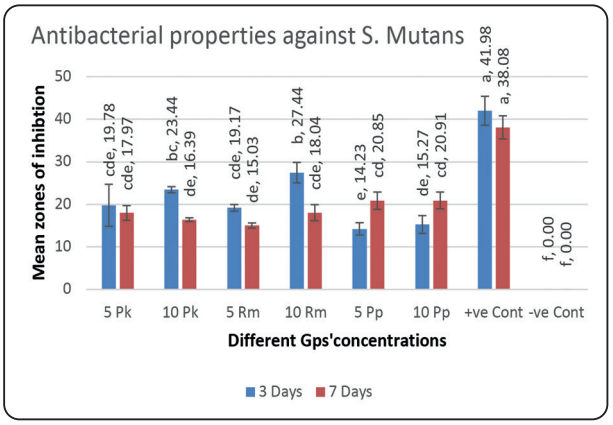

Fig. (4) Mean inhibition diameter (in mm) of different tested groups against *S. mutans*

TABLE (2) Statistical analysis of inhibition zones of different Gps against Streptococcus mutans using ANOVA and Scheffe post hoc test.

Different Groups' concentrations	3 Days	7 Days	p-value
5 Pk	19.78±4.93^{cde}	17.97±1.73 ^{cde}	1.000
10 Pk	23.44±0.7$^{1\ b}$c	16.39±0.44 ^{de}	0.012
5 Rm	19.17±0.78 ^{cde}	15.02± 0.60^{de}	0.710
10 Rm	27.44±2.40 b	18.03±1.88 ^{cde}	0.000
5 Pp	14.23±1.44 ^{e}	20.84±2.04 ^{cd}	0.030
10 Pp	15.27±2.08 ^{de}	20.91±1.97 ^{cd}	0.159
+ve Cont	41.98±3.39 a	38.08±2.71 a	0.799
-ve Cont	0 ^{f}	0 ^{f}	1.000
p-value	**0**	**0**	

p<0.05 statistically significant** ***p>0.05 non-significant, NS

Values sharing same letters have statistically insignificant difference within same column

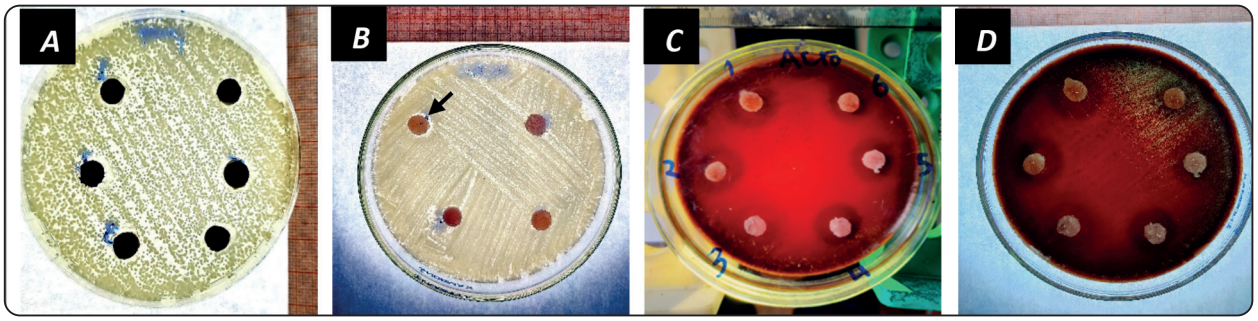

Fig. (5) The inhibition zones of different groups against C. albicans or S. mutans after either 3- or 7-days; ***A***:TC/Pp discs against C. albicans after 3 days. ***B***: TC/Nystatin positive control discs against C. albicans after 7 days. ***C***: TC/Rm and TC/ Pk discs against S. mutans after 3 days. ***D***: TC/Rm and TC/ Pk discs against S. mutans after 7 days.

DISCUSSION

Natural products have been shown to possess antimicrobial properties in addition to anti-inflammatory and antioxidant effects. Moreover, they have been highly recommended as effective substitutes having minimal side effects compared to conventional chemical drugs. The main advantages of using natural products are their accessibility, affordability and low toxicity documented microbial resistance.[1,26,33]

Because these extracts are derived from natural sources; pumpkin, rosemary, and propolis which are well known for their usual use in traditional medicine and herbal remedies. These natural ingredients have been researched and used in a variety of application forms, including food and herbal treatments, with no substantial reports of toxicity. For example, pumpkin has been widely investigated for its nutritional and therapeutic characteristics, with no considerable reports of toxicity [34]. Rosemary is widely used in food and medicine due to its antioxidant and antimicrobial properties, and their biocompatibility and safety are extensively investigated [35]. Propolis has been long used in traditional medicine and is known for its antimicrobial and anti-inflammatory effects without recorded toxicity. Hence, and due to reported safety and biocompatibility of these natural antimicrobial agents, this study focused on their antimicrobial efficacy as the primary outcome of interest.

In the present study, the natural antimicrobial products incorporated in TC aimed to inhibit the growth of C. albicans and S. mutans as they are the cause of most oral diseases. C. albicans in association with S. mutans, promotes yeast colonization on acrylic surfaces and oral epithelium.[1,8,11,12]

Although our results of the broth microdilution test showed that the MIC of Pk extract powder against C. albicans was 26.5%, so that each 1g TC would require a whole of 1g of Pk powder in a ratio of 1/1 to achieve 26.5% of active ingredient in the whole TC/Pk mix. However, mixing at 1/1 ratio with TC powder resulted in a very friable mix and could not be used practically.

On the other hand, to achieve the desired MIC in each TC/Antimicrobial whole powder mix, about 1/0.075 ratio was the optimum, which represents 7% of antimicrobial whole powder in the TC/ Antimicrobial mix. This 7% results in more than 4% active ingredient in the whole TC/Antimicrobial mix, which exceeds the required MIC of RM against both C. albicans and S. mutans, and it only exceeds the MIC of Pk against S. mutans. Hence, slightly lower and higher percentages were selected which were 5 and 10%. To be more specific, mix of TC powder with 5% & 10% Rm powder resulted in 2.85 & 5.7 % active ingredient in the final mix, respectively, and 5% & 10% Pk powder resulted in concentrations of 2.65 & 5.3 % active ingredient,

respectively, in the final TC powder / antimicrobial mix.

Regarding Pp powder and while being claimed to be pure by the manufacturer, and according to Hejazi et al (2014)[29], the MIC of Egyptian Bee Propolis for Gram +ve bacteria and C. albicans were 1.6 and 1.048%, respectively. Therefore, and for standardizing the concentrations, 5 and 10 % concentrations of Pp which results in 5 & 10% active ingredient, were also selected.

Antimicrobial efficacy can be evaluated by a number of methods. Agar diffusion, similarly known as disc diffusion test, was used in this study as it is a widely used method owing to its ease of use, affordability, and the findings that are simple to comprehend.[2,36]

In the current invitro study, the results showed that the TC, Acrostone alone (-ve control group) has no antimicrobial effect against both C. albicans and S. mutans. Moreover, it was observed that TC mixed with both concentrations of local Pp powder at both concentrations have the highest antifungal activity against C. albicans, which was significantly higher than Nystatin (positive control) (p-value <0.05) especially after 7 days indicating powerful antifungal activity against C. albicans even over longer period of this test time.

According to several studies, Propolis' antifungal activity against C. albicans is attributed to its high concentration of flavonoids, phenolic acids, ketones, and esters. Other substances that are known to damage the membrane or cell wall of microorganisms structurally and functionally include cinnamic acid, benzoic acid, and caffeic acid. Furthermore, Pp has been shown to prevent the development of germ tubes, which aids in C. albicans adhesion. Other studies have shown that Propolis, like some antibiotics, breaks down the cytoplasm and fungal cell wall in addition to preventing fungal cell division. [24,25,37]

In addition, and according to Stähli et al[33], the TEM images of C. albicans reveal enlarged cells and indicate a loss of cell wall integrity following exposure to propolis.

In case of S. mutans, our results showed that TC mixed with Ampicillin (positive-control) had the highest antibacterial efficacy. This goes in concurrence with the study by Liu J. et al. which found that Ampicillin at all concentrations tested inhibited S. mutans biofilm attachment and reduced the percentage of viable detached cells. [38]

This antibacterial activity was followed by TC mixed with 10 Rm and 10 Pk extract powders. The antibacterial activity of Rm extract powder is attributed to its active ingredients including phenolic acids, such as ferulic acid, cafeic acid, and rosmarinic acid; flavonoids, such as apigenin and rutin; and phenolic diterpenoids, such as carnosic acid and carnosol. The antibacterial activity of the Rm extract against S. mutans is most likely attributed to the nonpolar phenolic diterpenoid compounds like carnosic acid and carnosol.[19] According to the study by DEL CAMPO et al.[39], gram-positive bacteria were the most sensitive to the Rm extract because they are typically more sensitive to nonpolar phenolic compounds than gram-negative bacteria.

There are several mechanisms for the antibacterial action of Rm extract. The active compounds can pass through the cell wall and cytoplasmic membrane, disrupting the various layers of fatty acids, phospholipids, and polysaccharides in bacterial cell walls. This causes cell permeability and ions and other cell contents to leak, resulting in bacterial cell death. Another theory is that certain phytocompounds derived from Rm extracts, including rosmarinic acid, carnosol, and carnosic acid, have been demonstrated to target S. mutans surface molecules, thereby impeding their adhesion and hindering the formation of biofilms.[20,40] This goes in concurrence with Silva et al. [41] who stated that oral plankton

bacteria (S. mitis, S. sanguinis, S. mutans, S. sobrinus, and L. casei) that contribute to the formation of oral biofilm were successfully inhibited by the hydroalcoholic extract of Rosmarinus officinalis.

On the other hand, the antibacterial activity of Pk is primarily attributed to its composition as well. In particular, the presence of bioactive compounds such as phenolic compounds, carotenoids, and cucurbitacins. [17] Pk contains a variety of phenolic compounds, such as flavonoids and phenolic acids, which have been demonstrated to disrupt bacterial cell membranes, interfere with enzyme activity, and inhibit bacterial growth.[42] Carotenoids, particularly beta-carotene have been found to inhibit the growth of certain bacterial species. Pk also contains cucurbitacins, which are a group of triterpenoid compounds. Cucurbitacins have been reported to exhibit antibacterial effects against both Gram-positive and Gram-negative bacteria.[43] In addition, Pk extract contains bioactive compounds like saponins. the antibacterial mechanism of saponins was related to damage to the cell wall and membrane. in addition to saponins, Pk extract also contains tannins, flavonoids, terpentoids, phenols, sterols, alkaloids, komarins, etc., which directly or indirectly in a synergistic relationship linked to each other affect microbial cells, causing their death.[18,44]

In addition, the fatty acids composition of Pk extract is essential for the antibacterial properties. It contains mainly unsaturated fatty acid as linolenic acid and oleic acid, in addition to saturated fatty acids as palmitic and stearic acid. In their review report, Yoo et al. [45] highlighted the broad spectrum of antibacterial activity of lipids with emphasis on fatty acids with 18 carbons such as α-linolenic, linoleic, and oleic acid, which were the most prevalent fatty acids in the Pk extract.[46,47]

Moreover, our results showed that the higher the concentration of both Pk and Rm extract powders mixed with TC, the greater the antibacterial effect which was statistically significant ($p<0.05$). This might be attributed to the increase in the percentage of active compounds in the extract powders.

In addition, the decrease in the S. mutans inhibition zones over time which was found statistically significant in 10% Pk and Rm groups might be attributed to the decrease in the diffusion of the antimicrobial agents with time, with subsequent regaining microorganisms their capabilities to proliferate.

Since the results of the present study showed that there were differences in the antimicrobial properties of the TC containing different antimicrobial agents, therefore, null hypothesis was rejected.

The use of only one type of TC was used in the current study, which is considered a limitation. Moreover, the evaluation of only the antimicrobial properties of the natural products is a further limitation. Therefore, further research should investigate the mechanical properties after the incorporation of the natural antimicrobial agents into the TCs. Further researches are still required to investigate the actual mechanism of action and pharmacokinetics of these natural antimicrobial agents.

CONCLUSION

Within the limitation of the current study, the following can be concluded:

- 5% and 10% Propolis extract powder incorporated into TC exhibited considerable antimicrobial efficiency against both C. albicans and S. mutans.
- Incorporation of natural antimicrobial agents in TC can be used as effective alternative to topical synthetic agents.

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