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INTEGRATED BIOACTIVITY ANALYSIS OF BRANDED MOUTHWASH PREPARATIONS: ANTIMICROBIAL EFFECTIVENESS AND ANTIOXIDANT STRENGTH

Areeba Shafiq¹, Rizwan Ahmad¹, Jafir Hussain Shirazi^{1*} , Saba Tahir²

¹Department of Pharmaceutics, Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

²Direct Chemist Outlet, Springwood Place, Gawler East, Australia.

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ABSTRACT

Oral hygiene is essential for general health maintenance. Poor oral hygiene causes numerous mouth disorders including caries, gingivitis, periodontitis, and systemic illnesses. Recently, mouthwash usage has become a well-known tool for oral hygiene. The aim of this research was comparative analysis of different brands of mouthwashes for antimicrobial and antioxidant activities. Methods: Seven commercially available mouthwashes (i.e., Enziclor®, Protect®, Colgate Plax®, Listerine FreshBurst®, Closeup®, Touchme Minto®, and Oral-B® in Pakistan were selected. This research was carried out in vitro in a lab setting. The Agar well diffusion method was used to determine the antimicrobial activity of mouthwashes against *Staphylococcus aureus*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, and *Bordetella bronchiseptica*. Out of seven selected mouthwashes, (Protect® was the most effective mouthwash that showed the highest zone of Inhibition against the majority of tested bacterial strains [Inhibition zone diameter against *Bacillus subtilis* (20.5±2.121mm), Inhibition zone diameter against *Escherichia coli* (20±0.0 mm), Inhibition zone diameter against *Staphylococcus aureus* (18.5±0.7 mm) and Inhibition zone diameter against *Staphylococcus epidermidis* (18.00±0.000mm)]. On the other hand, (Listerine FreshBurst® showed no zone of Inhibition against any of the tested strains. The antioxidant activity of mouthwashes was evaluated by DPPH free radical scavenging assay. The (Protect® had the highest percentage of Inhibition (87.76%) of antioxidant activity. On the other hand, Enziclor® had the lowest % Inhibition (55.17±0.25) among all mouthwashes. Within the limitations of research, the current study demonstrated that mouthwashes had variable antimicrobial and antioxidant activities.

Keywords: Antibacterial activity, Agar well diffusion method, Zone of inhibition, Antioxidant activity, DPPH assay, Mouthwashes.

*Corresponding Author. E-mail: jafir.shirazi@iub.edu.pk

INTRODUCTION

Poor oral care causes the accumulation of microbes. In order to maintain proper oral hygiene removal of microbes from the oral cavity [1]. The indigenous microbiota in the oral cavity and host is in homeostasis. Under specific circumstances, changes in the oral microbiota might promote the development of non-oral pathogens that are difficult to eradicate due to their greater antimicrobial resistance, increasing the likelihood of therapeutic failure and recurrence. Numerous mouth disorders i.e., caries, gingivitis, periodontitis, and systemic illnesses with clinical relevance which include HIV,

rheumatoid arthritis, and fibrosis, have been linked to the occurrence of these bacteria in the mouth cavity [2]. Many methods have been developed to control dental diseases. Control of the production of dental plaque is the most efficient method of stopping dental diseases from occurring. Microbial action causes the formation of Plaque, a soft, thin film that builds up on teeth, gums, and all oral appliances. Plaque formation is aided by dietary sugars mainly sucrose, which also speeds up and thickens plaque development. Removing plaque from the teeth and surrounding areas is necessary to maintain a healthy mouth [3].

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The primary method for preventing bacterial biofilm formation in the oral cavity is the periodic removal of bacterial plaque with a toothbrush, toothpaste, and dental floss. However, bacterial plaque cannot be fully removed by toothbrushes which may not avoid dental diseases. Therefore, mouthwashes become a significant tool to overcome this problem. The use of mouthwash after brushing suppresses the development of microorganisms and subsequent tooth plaque because it contains antimicrobial components [4].

In this perspective, mouth rinse also known as mouthwash received extraordinary attention and its consumption/use is potentially increasing to maintain oral hygiene [5]. Doctors frequently suggest mouthwashes to their patients to lower dental plaque and prevent gingivitis [6]. Thus, it became necessary to study the side effects of mouthwash and to test their ability to control the microorganisms and affect plaque formation. Recently, many studies on mouthwash have been performed to test their efficacy and to study the side effects associated with their use. Mouthwash containing chlorhexidine had excellent antibacterial action against *S. mutans*. The study also reveals that the mouthwash containing triclosan and tea tree leaves showed a moderate level of effectiveness. The mouthwash made with aloe vera has the least antibacterial action [7]. Mouthwashes contain fluoride, benzethonium chloride, benzydamine hydrochloride, Chlorhexidine (CHX), and Cetylpyridinium chloride. In particular, Chlorhexidine is renowned as a Plaque-control agent that prevents the sticking of microorganisms on the surface of teeth and in the cavity of the mouth [8]. Fluoride is widely employed in dental caries prevention due to its remineralization and anti-cariogenic properties. It is not advised for small kids because of the possibility of ingesting it and fluoride toxicity [9]. Mouthwashes typically include Chlorhexidine, Cetylpyridinium chloride, and triclosan as antimicrobial agents. The most common fluorine compounds found in chewing gum, mouth rinses, dental flosses, and toothpaste are sodium monofluorophosphate, stannous fluoride, sodium fluoride, and amino fluorides [10].

Chlorhexidine gluconate is recognized as the "gold standard" among the chemotherapeutic ingredients found in mouthwashes. The Chlorhexidine has been shown to reduce *S. mutans* colonization by 30-50% when used daily [11]. The prolonged use of chlorhexidine has been associated with several adverse effects including genotoxicity of buccal epithelial cells, tongue and tooth discoloration, a transient alteration in sense of taste, a rise in calculus deposits, and burning sensations [12].

The effects of free -radicals and ROS can be reduced by antioxidants in humans. They slow the progression of several chronic illnesses and lipid peroxidation [13]. Free radicals are produced more frequently when an antioxidant shortage or oxidative stress is linked to periodontal disorders. In the initial phases of the development of periodontal disorders, reactive oxygen, and nitrogen species increased. This process can cause an imbalance in the body's reaction, biomolecule changes, and periodontal tissue damage. The antioxidant defense system can potentially reduce the damage caused by reactive nitrogen and oxygen species. Antioxidant mouthwashes help to reduce plaque formation and subgingival periodontopathic microorganisms and have been proven to have a beneficial effect on gingival inflammation [14]. Therefore, the use of antioxidant mouthwashes has become necessary for oral health. In a study, a particular spectrophotometric technique was used to evaluate the antioxidant properties of 11 mouthwashes and their active ingredients. Several mouthwashes have antioxidant activity which is due to their active ingredient or vehicle or both of them and the mouthwashes containing methyl-salicylate have the best antioxidant activity [15].

According to our knowledge, little work is done on the antioxidant activity of commercially available mouthwashes. Therefore, current study aims not only to assess which mouthwash has the remarkable efficacy among the selected commercially available mouthwashes but also to determine whether these mouthwashes contain any antioxidant activity besides their antimicrobial efficacy because many of the ingredients present in these washes have reported antioxidant activity in literature. In this study, we determine the antimicrobial activity of seven commercially available mouthwashes by the Agar well diffusion method. Out of seven selected mouthwashes, Protect mouthwash was the most effective mouthwash and showed the highest zone of inhibition against many tested bacterial strains. The antioxidant activity of mouthwashes was evaluated by DPPH free radical scavenging assay. The Protect mouthwash had the highest percentage of inhibition (87.76%) of antioxidant activity.

MATERIALS AND METHODS

Collection of Mouthwashes

Seven commonly available mouthwashes: Enziclor®, Protect®, Colgate Plax®, Listerine FreshBurst®, Closeup®, Touchme Minto®, and Oral-B® were purchased from the local market of Bahawalpur, Pakistan. Manufacturing and expiry date of mouthwashes were checked at the time of purchase and stored at room temperature as per the manufacturer's recommendation.

Table 1: Mouthwashes and their composition.

Formulation Code	Name of Mouthwash	Ingredients listed on packages
E1	Enziclor	0.2 % w/v Chlorhexidine Gluconate, Benzydamine Hydrochloride 0.15% w/v. Product Complies Platinum Specs.
CO2	Colgate Plax	Glycerin, Sorbitol, Polaxamer407, Flavor, Cetylpyridinium Chloride, Water, Sorbate, Sodium Fluoride, Potassium, Sodium Saccharin, Propylene Glycol, Menthol, CI 42051
O3	Oral-B	Alcohol 11.5%, Cetyl pyridinium Chloride, Polysorbate 80, Aroma, Benzoic acid, Aqua, Sodium Saccharin, Glycerin, Sodium Benzoate, CI 42090.
L4	Listerine Freshburst	Sorbitol, Eucalyptol, Poloxamer 407, Sodium Saccharin, Alcohol, Flavor, Benzoic Acid, Methyl Salicylate, CI 47005, Water, Sodium Benzoate, Thymol, Menthol, CI 42053.
P5	Protect	Chlorhexidine Gluconate 0.12%, Sodium Fluoride 0.05% with a fresh mint taste.
Cl6	Closeup	Sorbitol, Sodium Saccharin, Basil Extract, PEG-40 Hydrogenated Castor Oil, Glycine, Benzyl Alcohol, Elettaria Cardamom Oil, Phenoxyethanol, Sodium Lauryl Sulphate, Flavor, Water, Mentha Piperita Extract, Sodium Fluoride, Zinc Sulphate, Potassium Citrate, CI 47005, CI 42051. Contains 225 ppm of available fluoride when packed. Contain Sodium Fluoride.
M7	Touchme Minto	Sodium Mono Floro Phosphate, Propylene Glycol, Menthol, Benzoic Acid, Cremophore, Ice cool Flavor, Eucalyptus Oil, Sorbitol, Certified Color, Glycerin, De-Ionized Aqua.

Antimicrobial Assay**Bacterial Strain**

Drug Testing Laboratory (DTL) of Bahawalpur provided the bacterial strain in the form of Stock Culture Agar. American Type Culture Collection (ATCC) Bacterial Strains used for antimicrobial activity including five gram-positive bacterial strains: *Bacillus pumilus* (ATCC 14884), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), two gram-negative bacterial strains:

Bordetella bronchiseptica (ATCC 4617) and *Escherichia coli* (ATCC 10536). The fresh culture was prepared from the stock culture in nutrient agar media and incubated for 24 hours at 37°C.

Agar Well Diffusion Method

The antimicrobial activity of mouthwashes was assessed by the Agar well diffusion method [16] with minor modification. In this method, Inoculum suspension was prepared by directly taking 3-4 isolated colonies from 24 hours freshly prepared bacterial culture plate by sterile loop into 0.9% normal saline and adjusted its turbidity to 0.5 McFarland turbidity standard by visually comparing the test and standard against a white background with thick black lines. Muller Hinton agar plates were inoculated within 15 minutes of preparation of Inoculum suspension under an aseptic condition in a

laminar flow hood. Wells having a diameter of 7 mm were made in Muller Hinton agar plates aseptically using a sterile blue micropipette tip .100 µl Sample of each mouthwash concentration at full strength was poured into respective wells of muller Hinton agar plates. 100 ul Sterile distilled water was used as a negative control. The Muller Hinton agar plates were incubated for 18-24 hours at 37°C. The method was performed two times, and the antimicrobial activity of each mouthwash sample was assessed by measuring the zone of Inhibition in millimeters. The mean value of the diameter of the zone of Inhibition of the duplicate sets was taken as the final value.

Antioxidant Assay of Mouthwashes**Mouthwashes Antioxidant Activity was Assessed by the DPPH Method.****Chemicals**

The chemicals used in DPPH Free radical Scavenging Assay are the following: Mouthwash samples, Ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant Assay

The antioxidant assay of mouthwashes was assessed through DPPH free radical method [17, 18]. In this method, 1 ml of each mouthwash concentration at full strength was mixed with 1 ml of 0.2 mM DPPH solution and stored in a dark environment for thirty minutes. Using a UV-Visible Spectrophotometer, the absorbance was measured at 517 nm. The

Standard used was Ascorbic acid, and DPPH with methanol was used as a Control.

The % radical scavenging activity of mouthwash samples was determined by the formula given below:

% RSA = [(Absorbance of control – Absorbance of the sample) ÷ (Absorbance of Control)] × 100

Where, “RSA” is a Radical Scavenging Activity. The “absorbance of control” is the absorbance of DPPH radical+methanol; “the absorbance of the sample” is the absorbance of the DPPH radical +Sample.

Statistical Analysis

All results of the antimicrobial and antioxidant assay were expressed in terms of mean ± standard deviation. Statistical analysis was performed by GraphPad Prism 8 XML. One-way analysis of variance (ANOVA) was used to determine the statistically significant difference among the mean.

RESULTS AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity of mouthwashes is determined by the zone of inhibition against seven bacterial strains given. The One-way analysis of variance (ANOVA) revealed significant differences among means ($P < 0.0001$). The most effective mouthwash was Protect because it showed the highest zone of Inhibition against the majority of tested bacterial strains followed by Enziclor, Oral-B and Colgate Plax have moderate antimicrobial activity while Minto and Close-up showed minimum activity among all mouthwashes and Listerine showed no antimicrobial activity against all tested bacterial strains. Protect showed the highest Inhibition zone (20.0 ± 0.0 mm) and Minto showed the lowest Inhibition zone (10.5 ± 0.707 mm) against *Escherichia coli*. Oral-B showed the highest Inhibition zone (24 ± 1.414 mm) and Closeup showed the lowest zone of inhibition (12.5 ± 0.70 mm) against *Micrococcus luteus*. Protect showed the highest Inhibition zone (20.5 ± 2.12 mm), and Colgate Plax and Minto both showed the lowest Inhibition zone (15 ± 1.41 mm) against *Bacillus subtilis*. Enziclor showed the highest Inhibition zone (18.00 ± 1.414 mm), and Minto showed the lowest Inhibition zone (10.5 ± 0.707 mm) against *Bordetella bronchiseptica*. Enziclor showed the highest Inhibition zone (19 ± 0.0 mm), and Minto showed the lowest inhibition zone (12 ± 0.0 mm) against *Bacillus pumilus*. Protect showed the highest inhibition zone (18.5 ± 0.707 mm) and Minto showed the lowest Inhibition zone (13.5 ± 0.707 mm) against *Staphylococcus aureus*. Protect and Enziclor showed the highest inhibition zone (18 ± 0.0 mm) and Oral-B showed the lowest inhibition zone (12.5 ± 0.707 mm) against *Staphylococcus epidermidis*.

Mouthwashes containing chlorhexidine gluconate have the greatest antimicrobial activity. Protect and Enziclor exhibited a higher zone of Inhibition against

most of the tested strain. Protect is most effective against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. Enziclor has potent antimicrobial activity against *Bordetella bronchiseptica* and *Bacillus pumilus*. Enziclor has the same antimicrobial activity against *Staphylococcus epidermidis* as Protect. These mouthwashes contain chlorhexidine gluconate as an active ingredient which is an effective antimicrobial agent. reported that Chlorhexidine gluconate is regarded as a “gold standard”. It has an antiplaque and antigingivitis effect. The most effective antimicrobial agent was (0.12%) chlorhexidine. Because of its reported side effect, it has not been advised for long-term use [21].

A study compared the antimicrobial ingredients of commercially available mouthwashes. It reported that mouthwashes that contain triclosan have the highest zone of Inhibition, followed by chlorhexidine gluconate. Most researchers suggested Chlorhexidine as a potent antimicrobial agent for preventing oral diseases [22].

Oral-B showed the highest zone of Inhibition only against *Micrococcus luteus*; otherwise, Oral- B showed a moderate Inhibition zone against most strains. Colgate Plax also showed a moderate Inhibition zone against most strains. These mouthwashes contain Cetylpyridinium chloride as an active ingredient, a well-known antimicrobial agent. The outcomes are supported by Miranda et.al., 2020 [23]. Cetylpyridinium chloride is a broad-spectrum antimicrobial agent and a common ingredient in most mouthwashes. Almas et.al., 2005 [24] reported that Cetylpyridinium chloride has moderate antimicrobial activity, which supported this study but the method used in both studies are different. Witt et.al., 2006 [25] reported that mouthwash containing cetylpyridinium chloride when used alone or in combination with brushing, has antiplaque activity.

The Closeup contains Sodium fluoride as an active ingredient. Mentha piperita extract, essential oils like PEG-40 Hydrogenated Castor Oil, Elettaria cardamom oil, and many other components are also present in Closeup. Closeup was most effective against *Bordetella bronchiseptica*, *Staphylococcus aureus*, and *Bacillus pumillus* while less effective against other tested microbial strains and had no antimicrobial activity against *Bacillus subtilis*. The results are supported by Araújo et.al., 2019 [26] who reported that sodium fluoride has little antimicrobial activity when used alone in mouthwashes; however, when used in combination with essential oils and Cetyl pyridinium chloride has effective antimicrobial activity. Singh et.al., 2015 [27] reported that mentha Piperita extract has antibacterial activity.

Listerine contains thymol, methyl salicylate, and menthol, which have demonstrated effectiveness against gingivitis and plaque [29, 29].

Minto mouthwash contains Sodium mono fluoro phosphate, Eucalyptus oil, and other ingredients. It has moderate antimicrobial activity against *Bacillus subtilis* and is least effective against other bacterial strains. Its low antimicrobial activity compared to most other tested mouthwash is due to the presence of sodium mono fluoro phosphate as an active ingredient. The result of the current study can be correlated with Prasanth et al., 2011 [30] who reported that the formulations containing sodium fluoride and sodium mono fluoro phosphate as an active ingredient have antimicrobial activity but less than

other formulations which contain ingredients like Chlorhexidine and triclosan. Fluorides are widely used in various oral health products including toothpaste and mouth rinses because they aid in caries prevention.

Teh et al., 2015 [5] reported that the In-vitro study has limitations: the experiment performed in Invitro is not subjected to identical conditions in the oral cavity. In this research antimicrobial activity of mouthwashes was determined by the Invitro method, so results may not be representative of the actual behavior of mouthwashes in vivo Pires et al., 2007 [31] reported that an antimicrobial agent is unlikely to function in vivo if it does not exhibit any activity in Invitro.



Figure 1: Zone of inhibition produced by EnziClor, Oral-B, Listerine and Protect Mouthwashes against *Escherichia coli*. (CT= Negative control, E1=EnziClor, O3=Oral-B, L4=Listerine, P5=Protect).



Figure 2: Zone of inhibition produced by Oral-B, EnziClor, and Minto mouthwashes against *Micrococcus luteus*. (CT=Negative control, O3=Oral-B, E1=EnziClor, M7=Minto).

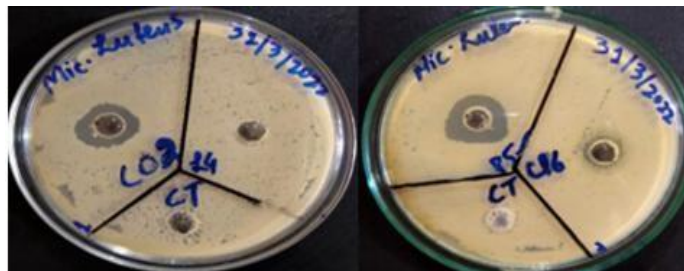


Figure 3: Zone of inhibition produced by Colgate Plax, Listerine, Protect and Closeup mouthwashes against *Micrococcus Luteus*. (CT=Negative control, CO2=Colgate plax, L4=Listerine, P5=Protect, Cl6=Closeup).

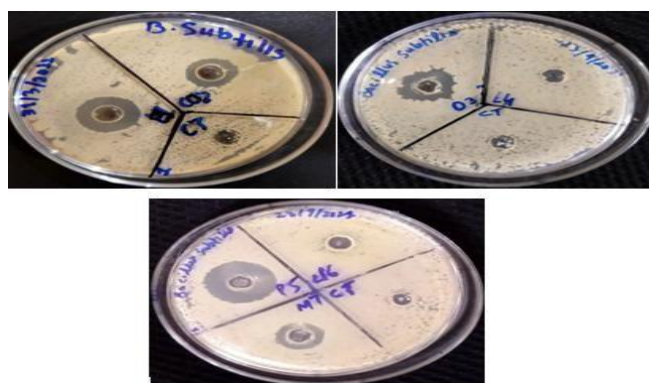


Figure 4: Zone of inhibition produced by commercially available mouthwashes against *Bacillus Subtilis* (CT=Negative control, E1=Enziclor, CO2=Colgateplax, O3=OralB, L4=Listerine, P5=Protect, CL6=Closeup, M7=Minto).



Figure 5: Zone of inhibition produced by Enziclor, Close-up, and Colgate Plax against *Bordetella bronchiseptica* (CT= Negative control, E1=Enziclor, CO2=Colgate plax, CL6=Closeup).

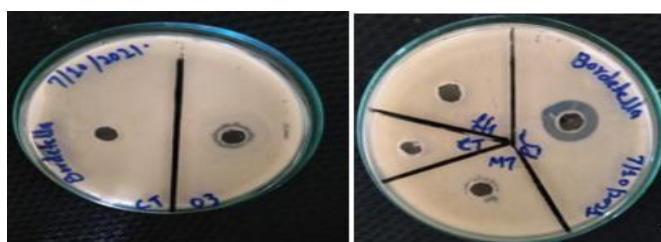


Figure 6: Zone of inhibition produced by Enziclor, Close-up, and Colgate Plax against *Bordetella bronchiseptica* (CT= Negative control, E1=Enziclor, CO2=Colgate plax, CL6=Close up).



Figure 7: Zone of inhibition produced by commercially available mouthwashes against *Bacillus pumilus* (CT=Negative control, E1=Enziclor, CO2=Colgateplax, O3=OralB, L4=Listerine, P5=Protect, CL6=Closeup, M7=Minto).

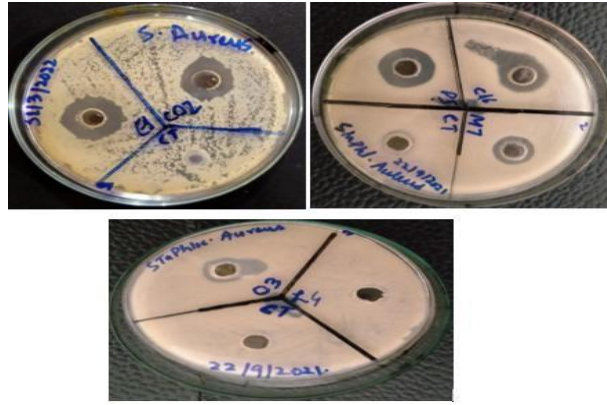


Figure 8: Zone of inhibition produced by commercially available mouthwashes against *Staphylococcus aureus* (CT= Negative control, E1=Enziolor, CO2=Colgate plax, O3=Oral B, L4=Listerine, P5=Protect, CL6=Closeup, M7=Minto).

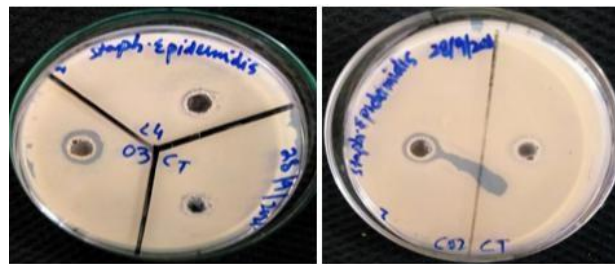


Figure 9: Zone of inhibition produced by Listerine, Oral-B, Colgate-Plax against *Staphylococcus epidermidis* (CT=Negative control, CO2=Colgate plax, O3=Oral B, L4=Listerine).

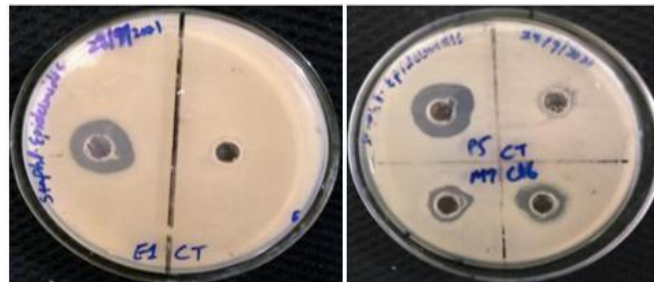


Figure 10: Zone of inhibition produced by Enziolor, Protect, Close-up, and Minto against *Staphylococcus epidermidis* (CT= Negative control, E1=Enziolor, P5=Protect, CL6=Close-up and M7=Minto).

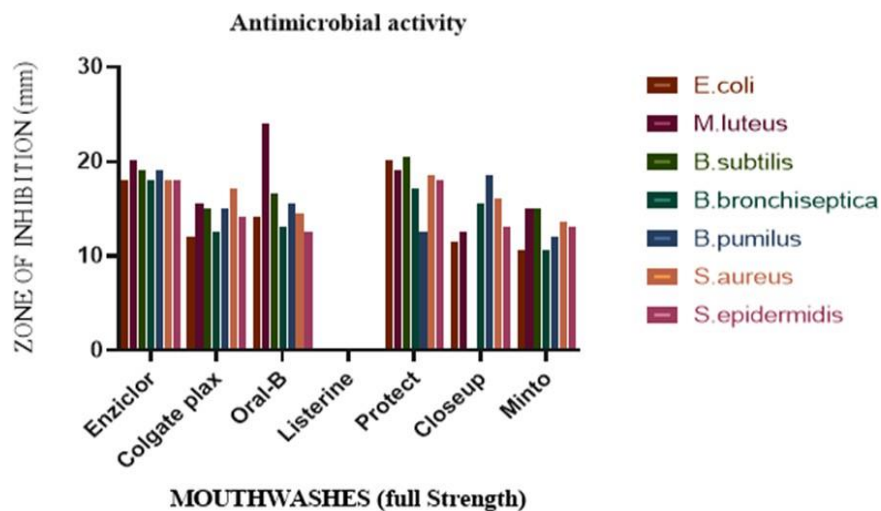


Figure 11: Antimicrobial activity of mouthwashes.

Table 2: Comparison of antimicrobial activity of seven commercially available mouthwashes against seven bacterial strains.

Mouthwashes (100 µl)	The mean diameter of the zone of inhibition (mm)						
	<i>E. coli</i>	<i>M. luteus</i>	<i>B. subtilis</i>	<i>B. bronchiseptica</i>	<i>B. pumilus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
Enziclor	18±0.0	200.0	19±0.0	18±1.4	19±0.0	18±2.8	18±0.0
Colgate plax	12±2.8	15.5±0.7	15±1.4	12.5±0.7	15±0.0	17±1.4	14±2.8
Oral-B	14±0.0	24±1.41	16.5±0.7	13±1.4	15.5±2.1	14.5±0.7	12.5±0.7
Listerine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Protect	20±0.0	19±0.0	20.5±2.1	17±1.4	12.5±2.1	18.5±0.7	18±0.0
Closeup	11.5±0.7	12.5±0.7	0.0±0.0	15.5±0.7	18.5±2.1	16±2.8	13±1.4
Minto	10.5±0.7	15±0.0	15±1.4	10.5±0.70	12±0.0	13.5±0.7	13±0.0

Table 3: Antioxidant activity of commercially available mouthwashes.

Serial No.	Name of mouthwashes (1ml)	Inhibition (%)
1	Enziclor	55.17%±0.25
2	Colgate plax	76.43%±0.10
3	Oral-B	56.60%±0.33
4	Listerine	86.68%±0.03
5	Protect	87.76%±0.05
6	Closeup	67.96%±0.00
7	Minto	71.48%±0.06
8	Ascorbic acid (Standard)	97.75%±0.06

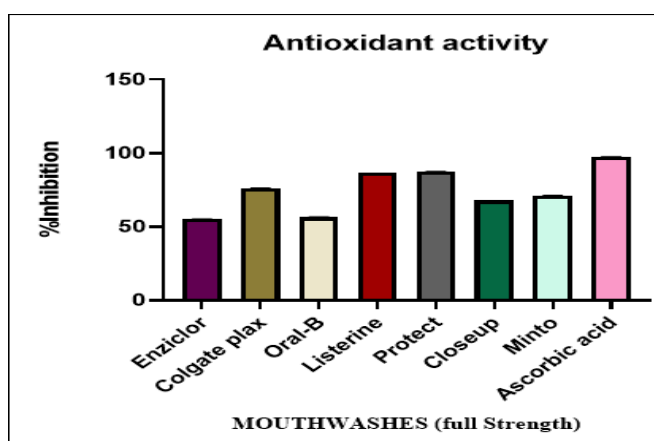


Figure 12: Antioxidant activity of commercially available mouthwashes.

Antioxidant Activity

The antioxidant activity of commercially available mouthwashes was assessed by DPPH free radical scavenging assay. Ascorbic acid was used as a standard. The result of the One-way analysis of variance (ANOVA) revealed a significant difference among means ($P < 0.0001$). The % Inhibition of DPPH values of mouthwashes range from 55.17% to 87.76% in comparison with Ascorbic acid as standard. The Protect showed the highest % inhibition ($87.76\% \pm 0.05$), followed by Listerine ($86.68\% \pm 0.03$) and Colgate Plax ($76.43\% \pm 0.10$). Touchme Minto, Closeup, Oral-B, and Enziclor mouthrinses showed % inhibition of ($71.48\% \pm 0.06$), ($67.96\% \pm 0.00$), ($56.60\% \pm 0.33$) and ($55.17\% \pm 0.25$) respectively. Ascorbic acid showed a % inhibition of ($97.75\% \pm 0.06$).

Mouthwashes have varying antioxidant activity, most likely due to the different compositions of the mouthwashes.

The researchers determined the antioxidant activity of mouthrinses and their active ingredients so it has been reported that many mouthwashes have antioxidant activity which could be due to the presence of an active component, vehicle, or both [15]. Chlorhexidine gluconate and sodium fluoride, active components of most mouthwashes have antioxidant activity. Listerine has potent antioxidant activity due to the presence of methyl salicylate. Most mouth rinses contain high ethanol concentration, so it has a role in the antioxidant activity of some mouthwashes.

The mouthwash Protect, Enziclor, Colgate Plax, and Closeup have antioxidant activity but less than

ascorbic acid. The results are supported by Garcia et al [32], who reported that chlorhexidine and sodium fluoride possess antioxidant properties but not as much as ascorbic acid.

Listerine contains methyl salicylate, eucalyptol, thymol, and menthol as active ingredients [15] reported that eucalyptol, menthol, thymol, glycerol, and panthenol have greater antioxidant activity as compared to anise and clove oil.

Mentha piperita extract has antibacterial and antioxidant activity [27]. The Closeup contains basil extract, mentha Piperita extract, cardamom oil, eucalyptus oil, and other ingredients. Basil leaves have importance in the treatment of sore throat. Basil extract possesses good antioxidant activity [33, 34]. The antioxidant activity of cardamom oil was reported by Jena et al [35] and Eucalyptus oil by Mishra et al [36].

Propylene glycol, an ingredient in Minto and Colgate Plax mouthwash has antioxidant activity reported by Bokov et al and Volkov et al [37, 38].

A study assessed the antioxidant activity of polysorbate 80 and dimethyl sulfoxide using the DPPH free radical technique. The results showed that both have antioxidant activity even at low concentrations [39, 40]. Polysorbate 80 is found in Oral-B mouthwash.

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Chlorhexidine, Sodium fluoride, methyl salicylate, mentha Piperita extract, eucalyptol, Polysorbate 80, thymol, menthol, Propylene glycol, cardamom oil, eucalyptus oil, and Basil extract have proven antioxidant activity in literature as previously described. In this study, the tested mouthwash containing these ingredients showed antioxidant activity.

CONCLUSION

It is concluded that all tested mouthwashes have antioxidant activity besides their antimicrobial efficacy. Within the limitations of research, the current study demonstrated that mouthwashes had variable antimicrobial and antioxidant activities. Protect® had the highest percentage of inhibition of antioxidant activity, whereas Enziclor® had the lowest percentage of inhibition among all mouthwashes.

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