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Original Article

A comparative study to evaluate the effects of antibiotics, plant extracts and fluoride-based toothpaste on the oral pathogens isolated from patients with gum diseases in Pakistan

Um estudo comparativo para avaliar os efeitos de antibióticos, extratos vegetais e creme dental à base de flúor sobre os patógenos orais isolados de pacientes com doenças gengivais no Paquistão

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Abstract

Oral diseases caused by various microorganisms are common around the world. Scientific research has now been focusing on novel medicines to overcome bacterial resistance and antibiotics side effects; therefore, the current study was designed to assess the efficacy of certain antibiotics, toothpaste, and medicinal plant extracts (*Ajuga bracteosa* and *Curcuma longa*) versus the bacterial pathogens isolated from the human oral cavity. A total of 130 samples were collected from Khyber Teaching Hospital Peshawar, Pakistan, among those 27 species isolated, and eight bacterial species were identified from the samples. Among all the bacterial species, *Staphylococcus aureus* (29.62%) and *Proteus mirabilis* (22.2%) were found to be more prevalent oral pathogens. In comparison, the least pervasive microbes were *Proteus vulgaris*, *Shigella sonnei*, *Escherichia coli* and *Aeromonas hydrophila*. The study also suggested that dental problems were more prevalent in males (41-50 years of age) than females. Among the eight antibiotics used in the study, the most promising results were shown by Foxicillin against *A. hydrophila.* The survey of TP1 revealed that it showed more potent antagonist activity against *Proteus vulgaris* as compared TP2 and TP3 that might be due to the high content of fluoride. The *Curcuma longa* showed more significant activity than *Ajuga bracteosa* (Stem, leaves and root) extracts. The data obtained through this study revealed that antibiotics were more effective for oral bacterial pathogens than toothpaste and plant extracts which showed moderate and low activity, respectively. Therefore, it is suggested that the active compounds in individual medicinal plants like *Curcuma longa* and *Ajuga bracteosa* could replace the antibiotics when used in daily routine as tooth cleansers or mouth rinses.

Keywords: *Ajuga bracteosa*, *Curcuma longa*, toothpaste, antibiotics, oral pathogens.

Resumo

As doenças bucais causadas por vários microrganismos são comuns em todo o mundo. A pesquisa científica agora tem se concentrado em novos medicamentos para superar a resistência bacteriana e os efeitos colaterais dos antibióticos; portanto, o presente estudo foi desenhado para avaliar a eficácia de certos antibióticos, pasta de dente e extratos de plantas medicinais (*Ajuga bracteosa* e *Curcuma longa*) contra os patógenos bacterianos isolados da cavidade oral humana. No total, 130 amostras foram coletadas do Khyber Teaching Hospital Peshawar, Paquistão, entre essas, 27 espécies foram isoladas e oito espécies bacterianas foram identificadas a partir das amostras. Entre todas as espécies bacterianas, *Staphylococcus aureus* (29.62%) e *Proteus mirabilis* (22.2%) foram os patógenos orais mais prevalentes. Em comparação, os micróbios menos difundidos foram *Proteus vulgaris*, *Shigella sonnei, Escherichia coli* e *Aeromonas hydrophila*. O estudo também sugeriu que os problemas dentários eram mais

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prevalentes em homens (41-50 anos de idade) do que em mulheres. Entre os oito antibióticos usados no estudo, os resultados mais promissores foram mostrados pelo Foxicillin contra *A. hydrophila*. A pesquisa de TP1 revelou que ele mostrou atividade antagonista mais potente contra *Proteus vulgaris* em comparação a TP2 e TP3, o que pode ser devido ao alto teor de flúor. A *Curcuma longa* apresentou atividade mais significativa em relação aos extratos de *Ajuga bracteosa* (caule, folhas e raiz). Os dados obtidos neste estudo revelaram que os antibióticos foram mais eficazes para os patógenos bacterianos orais do que os dentifrícios e os extratos vegetais que apresentaram atividade moderada e baixa, respectivamente. Portanto, sugere-se que os compostos ativos em plantas medicinais individuais como *Curcuma longa* e *Ajuga bracteosa* possam substituir os antibióticos quando usados na rotina diária como limpadores de dentes ou enxaguatórios bucais.

Palavras-chave: *Ajuga bracteosa*, *Curcuma longa*, pasta de dente, antibióticos, patógenos orais.

1. Introduction

The human mouth serves as the best habitat for numerous bacterial species due to its alkaline condition, favourable to most bacteria and fungi. Many of these microbes are involved in developing oral diseases (Gholizadeh et al., 2016). According to recent research, the source of various systemic diseases is the oral cavity where these microbes thrive. Their toxins gain access to distant body sites to cause infections or inflammation. Due to the advancements in microbiology, a diverse group of oral pathogens has been identified directly involved in infections at different sites of the body (Han and Wang, 2013).

Oral microorganisms are usually non-pathogenic opportunistic commensals engineered to promote oral health and to give protection against pathogenic bacteria (Yumoto et al., 2019). In several studies, it was observed that some bacteria, and yeast, resides in dental biofilms and cause mouth infections and dental caries. Almost, 1,000 different bacterial species-level taxa have now been identified after several molecular and cultural studies which find oral environment favourable for their growth. A single human being may have 100-200 taxa in his/her mouth at any particular time. Some of the genera of Gram-positive bacteria like *Enterococcus*, *Lactobacillus*, *Streptococcus*, and *Staphylococcus* are more prevalent as a microbial niche in the oral cavity, among others.

In contrast, notable genera of Gram-negative bacteria include, *Dialister*, *Fusobacterium*, *Filifactor*, *Porphyromonas*, *Prevotella*, *Parvimonas*, *Tannerella*, and *Treponema*, are detected in endodontic lesions and periodontal diseases (Rossoni et al., 2019). Human oral Streptococci, are known to be the first microorganisms to colonize the over the mouth surfaces and are dominant among other microorganisms in the oral cavity. These microbes have lately drawn scientists' interest as they are involved in certain systemic diseases, including endocarditis, brain haemorrhage, certain infections, intestinal inflammation, and autoimmune disorders. Antibiotics have always been the choice to cure bacterial infections. However, antibiotic resistance has emerged as a significant problem in recent years. Various studies have reported increased resistance by oral streptococci strains to commonly used antibiotics like penicillins, aminoglycosides and few have reported against chloramphenicol and vancomycin. (Pasquantonio et al., 2012). To overcome such a prevailing threat and to treat oral infections, multiple approaches have been adopted. One such promising approach is the utilization of antibiotics in combination like metronidazole and amoxicillin to treat severe and chronic periodontitis. (Soares et al., 2012).

Tooth cleaning with Brush or twigs and flossing are considered the most efficient methods to maintain oral hygiene (Lemes et al., 2018). Use of Fluoride based toothpaste is known to be the best way to control dental carries these days. The oral healthcare industry's intense marketing of fluoride toothpaste was a significant factor in their expanded use. Since the 1980s, in the developing world, almost all types of commercially manufactured toothpaste contain fluoride. Fluoride derivatives, such as sodium fluoride, amine fluoride, sodium monofluorophosphate and stannous fluoride, have been used alone or combined in toothpaste formulations and must adhere to some specific formulations, especially abrasive systems (which make up roughly half of the entire formula of toothpaste). The use of fluoride for population-based prevention of dental caries has been endorsed officially by WHO since the late 1960s. Fluoride toothpaste as a method to prevent dental caries is recommended with the consensus between scientists and public health experts, which has proven to be a significant decline in caries prevalence worldwide (Petersen and Ogawa, 2016).

Lately, researchers have shown great interest in organic products (especially from vascular plants) owing to their antimicrobial and antioxidant properties. Antioxidants scavenge reactive oxygen species (ROS) and are useful as drugs against many infectious and non-infectious diseases. (Mothana et al., 2012).

Ajuga bracteosa is an essential medicinal plant in regions of the Himalayas. The therapeutic value comes from the presence of several pharmacologically active substances like flavonol glycosides, iridoid glycosides, neo-clerodane diterpenoids, phytoecdysones, and ergosterol-5,8- endoperoxides. *Ajuga Bracteosa* is known for its conventional applications and has displayed numerous therapeutic activities including antimicrobial, anti-inflammatory, antitumor, cardiotonic function, anti-arthritic, antioxidant activity and *in vitro* secondary metabolite production for pharmaceutical items (Hussain and Yamin, 2016).

Curcuma longa or turmeric is one of the very commonly used spices in Sub-continent, China and South Asia (Pillay et al., 2019). Turmeric is produced from the *Curcuma longa* rhizomes of family Zingiberaceae. It is prevalent for its use in seasoning, food preservation, and as a colouring pigment. Turmeric has been used in herbal medicine. Its many bioactivities such as anti-HIV, anti-carcinogenic, anti-inflammatory, antibiotic, anti-diabetes, and antibacterial, effects have already been confirmed in several clinical tests (Sabir et al., 2021). Curcumin or curcuminoids are the most studied constituent of turmeric. Most of the turmeric bioactivities are recognized as being related to curcumin. However, 2-5% of turmeric comprises curcumin, essential ingredients such as sesquiterpenes, polyphenols, triterpenoids, sterols, and diterpenes have also been found in turmeric. High doses of turmeric (0.5-1.5 g/day/person) can be tolerated with no adverse effects. The potential of the antimicrobial ability of a certain amount of turmeric extracts has recently been demonstrated that can be tolerated without any harmful effect. (Kalaycioğlu et al., 2017).

The current study was aimed to assess the best practice to maintain oral health. For this purpose, the efficacy of various antibiotics, medicinal plant extracts and locally available toothpaste were checked against bacterial strains isolated from the patients' oral cavity.

1.1. Contributions

1. According to the author's knowledge, there were no scientific studies done to explore the antibiogram of Curcuma longa and A. Bracteosa against the oral bacterial pathogens before this study. Hence, this study provides a new insight for researchers;
2. The use of fluoride beyond the limit could also be dangerous for public health, according to WHO. Use of medicinal plant extracts in powder or mouth rinses could be a possible solution to fluoride overdose;
3. The study strongly supports the use of bioactive compounds found in *A. bracteosa* and *C. longa* with toothpaste or mouth rinses could minimize the risk of infections;
4. The prevalence of the oral pathogens in Khyber Pakhtunkhwa (KPK) province of Pakistan, according to age and gender; was not done previously so far. Oral infections are more common in this region as the people use an excessive amount of tobacco, Naswar snuffing, and beetle nuts which are also the major cause of mouth ulcers and cancer.

2. Methodology

2.1. Ethical statement

This study was approved by the Research and Ethics Committee, University of Agriculture, Peshawar-Pakistan. The plant's extracts were provided by the Department of Botany, University of Peshawar-Pakistan. No plant was un-necessarily uprooted, cut or harmed during the study. The medicinal plants used in this study are registered with the herbarium of the Department of Botany, University of Peshawar-Pakistan.

2.2. Sample collection and management

This experimental study was conducted at the Microbiology Department of Shaheed Benazir Bhutto Women University Peshawar, Pakistan from Mar 15, 2019, to Jun 15, 2020. A total of 300 samples were collected from indoor patients at Khyber Teaching Hospital in Peshawar, Pakistan but 130 had showed gums diseases rest were excluded . Samples were collected using sterile swabs under standard microbiological practices. The samples were processed within 05 hours after collection and streaked on Nutrient agar (Sigma-Aldrich) plates subjected to incubation 24 hours at 37 ° C. Following incubation, mixed colonies were obtained on each plate. The colonies were picked and purified by repeated subculturing for specific bacterial growth by using differential media (i.e., Blood Agar, Mannitol Salt Agar (MSA), MacConkey Agar and Chocolate Agar (Sigma-Aldrich) for the growth of bacterial species. The pure colonies were identified using Gram Staining and other biochemical tests such as Oxidase, Indole, TSI, Citrate, Urease and Catalase (Collee and Marr, 1996). The colonies were sent to First Fungal Culture Bank of Pakistan for further identification.

2.3. C. Longa rhizome extraction

Curcuma longa plant (registration ID: Bot.20205 (PUP)) was collected from Charsadda, KP, Pakistan. The rhizomes of *C. longa* were dried and then crushed to make powder, and 25 grams of dry powder was put into the Soxhlet apparatus for extraction of soluble bioactive molecules from the rhizome by using various solvents (like chloroform, benzene, petroleum ether, methanol and water). It was then concentrated in the rotary evaporator under low pressure as described by Omar et al. (2020) and Ali et al. (2020). The condensed extract was discharged to sterilized collecting tube.

2.4. A. bracteosa leaves, stem and root extraction

Ajuga bracteosa (registration ID: Bot.20204 (PUP)) was collected from Dir district, KP, Pakistan. The plant was identified at the Centre of Biodiversity and Taxonomy, Department of Botany, University of Peshawar, Pakistan. After washing, the plant was dried in the shade, grounded with mortar. The ground material was extracted with ethanol and n-hexane by using a Soxhlet apparatus. After extraction, the sample was evaporated by using a rotary evaporator. The extracts were collected into Eppendorf tubes, dried and screened for their antibacterial activities.

2.5. Evaluation of toothpaste

Three local toothpaste brands (available in the local markets of Peshawar, KP, Pakistan) were selected to assess their antimicrobial activity against bacterial pathogens isolated from oral cavities of different patients. The toothpaste solutions were made by combining the estimated quantity of toothpaste (2.0 gm) in determining volume (2 mL) of sterile distilled water to give 1:1 dilution; they were further diluted sterile distilled water and developed four separate dilutions of 1:2, 1:4, 1:8, and 1:16. Nutrient agar plates were prepared to assess toothpastes' antimicrobial activity against the Klebsiella pneumonia, Streptococcus aureus, Staphylococcus pyogenes *Proteus mirabilis*, *Proteus vulgaris*, *Shigella sonnei*, *Escherichia coli* and *Aeromonas hydrophila*.

2.6. Characterization based on antibiotic, toothpaste and plant extract sensitivities

2.6.1. Antibiotic sensitivity

For antibiotic sensitivity testing, well diffusion test was performed. The following antibiotics cefoxitin (FOX), sulfamethoxazole (SXT), oxacillin (OX), vancomycin (VA), moxifloxacin (MXF), amoxicillin (AMC), ampicillin (AMP), azithromycin (AZM) with weight/volume ratio were selected to check the antimicrobial activity. The distilled water was run as a control because all the six dilutions (5, 10, 15, 20, 25, and 30 µg/uL) of the antibiotics were made in distilled water. Thes tock solution of the antibiotics was made in distilled water according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) (Jorgensen et al., 2007). Distilled water was run as a negative control.

2.6.2. Toothpaste sensitivity

For the toothpaste sensitivity testing, agar well diffusion method was used. Three different amounts of each toothpaste were made at full strength (50 mg) and 1:1 dilution. Fresh cultures from the sample were taken in broth, and the turbidity was made with McFarland Standards (0.5%). The zone of inhibition around the toothpaste containing wells showed the antimicrobial activity against the bacteria. The maximum zone formed by the toothpaste dilutions against bacteria were considered as most effective. In contrast, medium zone formed against bacteria were regarded as intermediate toothpaste, and no zone of inhibition showed the resistance of bacteria to that particular toothpaste. The distilled water was run as a negative control.

2.6.3. Plant extract sensitivity

For plant extract sensitivity testing, agar well diffusion method was used. The fresh cultures from the samples were taken and tested against *A. bracteosa* (registration ID: Bot.20204 (PUP)) and *C. longa* (registration ID: Bot.20205 (PUP)) extracts. Leaf, stem and root of *A. bracteosa* and rhizome of *C. longa* were used in crude form and diluted with Dimethyl Sulfoxide (DMSO) according to the standard procedure. The zone of inhibition around the wells showed the antimicrobial activity against the bacteria. The maximum zone formed by plant extracts against bacteria was considered either sensitive, intermediate or resistant to a particular plant extract. The plant extract was used to detect the antimicrobial pattern of *E. coli*, *S. aureus*, *S. pyogenes*, *K. pneumonia*, *P. mirabilis*, *P. vulgaris*, *S. sonnei* and *A. hydrophila* species. The DMSO was taken as a negative control to check the activity.

2.7. Statistical analysis

Mean antimicrobial activities of plant extracts were calculated for each bacterium (95% Confidence Interval). Pearson Correlation between dose and the antibacterial activity of plant extracts was calculated with respect to P = .05 with 95% Confidence Interval. R program version 1.3.959, Graph Pad Prism version 8.4.3, were used to analyze all the data.

3. Results and Discussion

It is well studied that the invasion of many bacteria in the oral cavity may cause various bacterial infections. Therefore, it is incredibly essential to allow early diagnosis of various pathogenic potential microbes. The identified bacteria and their strength in an oral environment could help predict the accurate progression of various periodontal diseases (Shrivastava et al., 2020).

The present study was conducted to identify various bacterial strains responsible for gum diseases in the Pakistani population. The study also aimed to check the antimicrobial activity of some antibiotics prescribed by the dentists, including a few popular toothpaste brands and two plant extracts believed to be effective against certain diseases. In this study, patients of different age groups having primary, mixed and permanent dentition have participated as shown in the Figure 1 . Both genders were included in the study. However, we found that males were more affected than female patients and the possible reason could be the frequent use of Naswar (moist, powdered tobacco snuff), locally-made cigarettes and beetle nuts etc. which are mostly used by males in KPK province. The *S. aureus* and *S. sonnei* were the most common microbes in males, as shown in Figure 2.

The 130 collected samples were collected from the patients, including male and females, age ranging from 1 to 90 years (Figure 1). Total 27 microbial colonies were obtained from 130 oral swabs sent to the laboratory for further identification, as shown in Table S1 and Figure S1. Among these microbes, only eight bacterial species were isolated after clinical screening tests. These bacterial species

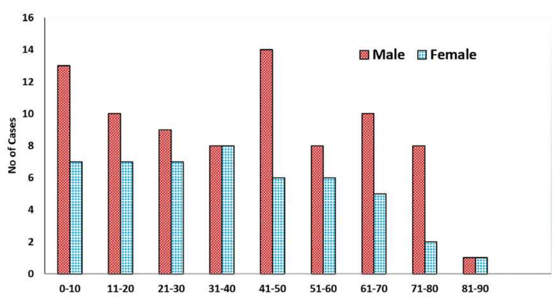

Figure 1. Age-wise and gender wise incidence of oral disease.

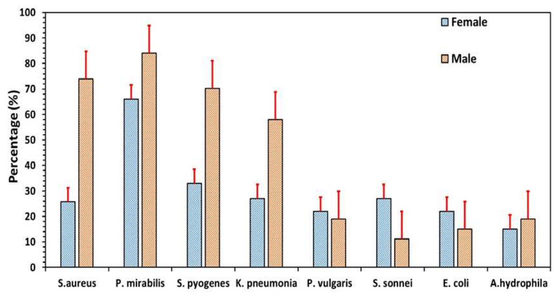

Figure 2. Gender-wise prevalence of oral pathogens.

were identified as *Klebsiella pneumonia*, *Streptococcus aureus*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Proteus vulgaris*, *Shigella sonnei*, *Escherichia coli* and *Aeromonas hydrophila*. Bacterial isolates from the oral cavity of patients as shown in Figure 3 which indicates the percentage of different bacterial strains; among these bacterial strains, *S. aureus* was isolated in the highest number (29.62%), followed by *P. mirabilis* (22.22%), *S. pyogenes* (18.51%) and *K. pneumonia* (14.81%). At the same time, *P. vulgaris* (3.70%), *Shigella sonnei* (3.70%), *E. coli* (3.70%) and *Aeromonas hydrophila* (3.70%) were present at the lowest percentage. In our study, *S. aureus* was the predominant isolated pathogen (29.0%) This result show compliance with study done in Yemen by Hassan et al. (2019). They found this pathogen's dominancy with a percentage of 43.1% (Hassan et al., 2019). The high frequency of S. *aureus* in mouth infections can be explained by the fact found by Adhikari et al. (2012)

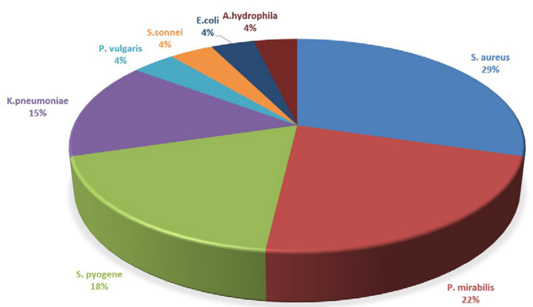

Figure 3. Frequncy of oral pathogens from gums.

that *S. aureus* often colonizes the mucous membrane or soft tissues and most commonly causes infections ranging from minor boils to life-threatening infections like bacteremia and sepsis.

3.1. Antibiotic sensitivity test

Antibiotics are commonly used in the case of caries and other dental disorders, for both medical and prophylactic purposes (Zarco et al., 2012).

In this study, the identified eight bacterial species antibiogram pattern was analyzed against eight different antibiotics, as shown in Figure S2, Figure 4 and Table S2. FOX (30 mg) was observed to be the most effective drug against *A. hydrophila,* which showed 47.9 mm zone of inhibition while S. *pyogenes* showed weaker antimicrobial activity against all the concentrations of FOX. All concentrations of SXT were found to be effective against *P. vulgaris*, while *S. pyogenes* showed minimum zone against different concentrations of SXT. *S. pyogenes* and *K. pneumonia* were resistant against all OX. S. aureus strengths showed maximum zones against all concentrations of OX, while *E. coli* showed minimum zones. Among all the bacterial strains *K. pneumonia* was resistant against vancomycin, while maximum zone was observed against *P. mirabilis* and the minimum zone of inhibition was shown by *A. hydrophila*.

MXF showed the highest activity against *P. vulgaris* and *A. hydrophila,* whereas it showed the least activity against *S. pyogenes*.

S. pyogenes was observed to be resistant at different concentrations of amoxicillin (5 mg, 10 mg and 15 mg), and started showing zone of inhibition with the increase

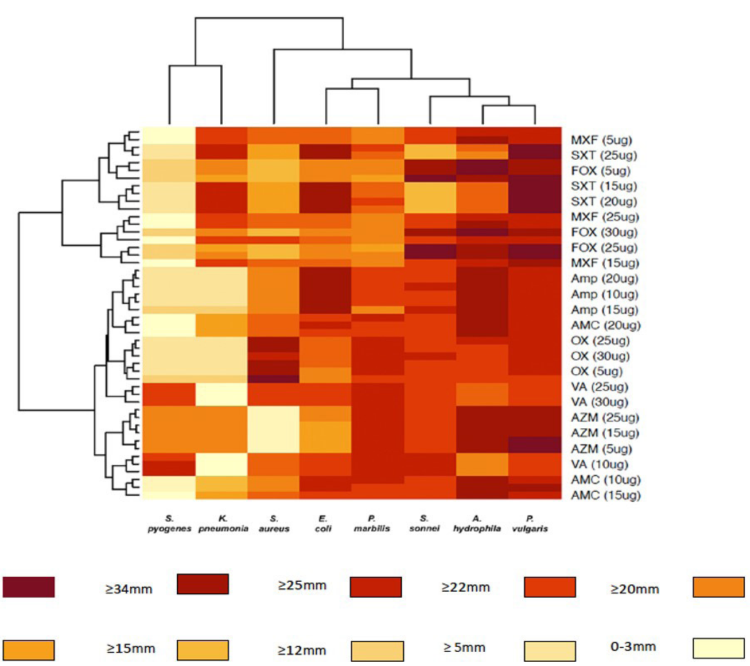

Figure 4. Antibiotic sensitivity on isolated bacterial species from teeth gums. Cefoxitin (FOX), sulfamethoxazole (SXT), oxacillin (OX), vancomycin (VA), moxifloxacin (MXF), amoxicillin (AMC), ampicillin (AMP), azithromycin (AZM). The tree is a dendrogram, which shows the hierarchy (similarity) between isolates and their activity against the different antibiotics. The graph was plotted using the R program.

of drug concentration (30 mg); it showed maximum zone of inhibition (6.8 mm) at 30 mg. Therefore, it was proved to be the most resistant pathogen among all the bacterial strains against AMC. AMC showed more potent antagonistic activity against *A. hydrophila*.

Among other bacterial pathogens, *K. pneumonia* and *S. pyogenes* showed resistivity against ampicillin at all concentrations; While all concentrations of AMP were observed to be effective against *E. coli*. AMP showed maximum activity against *E. coli,* i.e., 27.6 mm at 30 mg. *S. aureus* showed the weaker activity against ampicillin at all the concentrations used.

S. aureus showed resistance against all the concentrations of AZM. Maximum activity was observed against *P. vulgaris*, and the minimum recorded against *E. coli*. VA showed strong antagonistic activity against *P. mirabilis* (23.5 mm) and weaker antagonistic activity against the *A. hydrophila* (18.5 mm).

It was observed that all the selected antibiotics showed action against the isolates expect AMP (showed zero activity towards *K. pneumonia, S. pyogenes* at any concentration) and vancomycin (showed zero activity towards *K. pneumonia*). Our finding does not match Anejo-Okopi et al. (2015) finding that dental caries pathogens showed resistance to ciprofloxacin, ampicillin, erythromycin and amoxicillin. This report also did not agree with the study of Palmer et al. (2016) that resistance of oral pathogens to antibiotics is due to excessive use or unnecessarily prescribed antibiotics for the treatment of oral infections. Narayanan et al. (2011) also observed the resistance pattern and found that oral pathogens were penicillin, bacitracin, streptomycin, vancomycin and chloramphenicol resistant. El Sherbiny (2014) found that 80% of gram-positive anaerobic cocci from dental plaque were resistant to penicillin, and 95% of them were resistant to vancomycin. Greater resistance against the antibiotics clindamycin, metronidazole, and amoxicillin has been reported in *P. gingivalis* and *A. actinomycetemcomitans* associated with periodontal disease (Khan et al., 2015).

The current study revealed the efficacy of antibiotics against oral pathogens and found that among the selected eight antibiotics, the most effective zones of inhibition were formed by FOX (30 mg / mL) against all pathogenic bacteria so it might be the best choice of antibiotic to kill oral pathogens. These eight antibiotics were most effective against the *A. hydrophila* and *P.vulgaris.*

According to statistical analysis, FOX showed nonsignificant results against *P. vulgaris and S. sonnei* while OX and AMP showed nonsignificant results against *S. aureus.* AMP also produced nonsignificant results against *P. mirabilis.* Rest of the antibiotics showed significant results against the oral pathogen, as shown in Table S2.

3.2. Toothpaste sensitivity test

The toothpaste shows its effectiveness in dental hygiene, possibly because of triclosan, chlorine, and fluoride. The herbal toothpaste, though significantly decreases oral pathogen strength after cleaning and being organic as antimicrobials, helps sustain oral health.

Three different local toothpaste brands containing fluoride as a main component were tested for the antimicrobial activity against eight various bacterial pathogens, as shown in Figure 5, Figure S3 and Table S3. The list of toothpaste ingredients mentioned on the package is listed in Table 1. Among all three types of toothpaste,

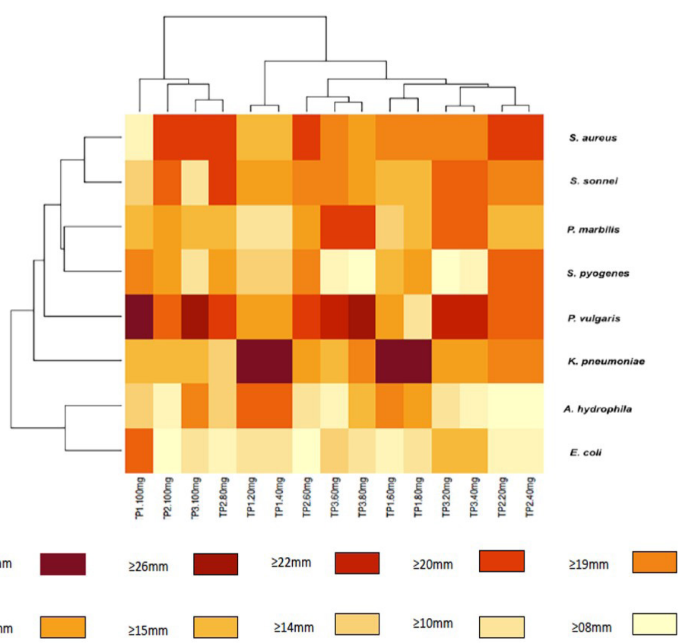

Figure 5. Heatmap showing the antimicrobial activity of different kinds of toothpaste against bacterial strain. TP1 stands for *toothpaste 1*, and the TP2 stands for *toothpaste 2*, TP3 stands for toothpaste 3. The tree is a dendrogram, which shows the hierarchy (similarity) between isolates and their activity against the Oral different pathogens. The graph was plotted using the R program.

TP1 showed maximum activity against various strains at different concentrations; full strength of TP1 showed Stronger antagonistic activity against *P. vulgaris* while it showed weaker antagonistic effective against *S. aureus E. coli* and *S. pyogenes*.

After TP1, TP2 was observed to be the most effective toothpaste against bacterial pathogens. It showed maximum inhibition zone zone for *S. aureus* and *P. vulgaris* while showed minimal activity against *E. coli* and *A. hydrophila*.

TP3 was observed as the least effective toothpaste as compared to the other toothpaste. TP3 showed maximum activity against *P. vulgaris,* and minimum activity was observed against *S. pyogenes*.

This study reported that toothpaste's full concentration gave the highest zone of inhibition and these inhibition zones tend to decrease down the concentration gradient. The TP1 showed maximum activity against various oral pathogens under study that might be due to a higher concentration of Flouride, i.e., 1,400 ppm. Other researchers found the same results. Apart from the addition of antimicrobial agents in toothpaste, the agent concentration is also paramount to achieving the most wanted outcome of reducing oral microbes (Odeleye et al., 2018).

The statistics showed that TP1 produced nonsignificant results against *P. vulgaris*, *S. sonnei* and *A. hydrophila*, while TP2 showed nonsignificant results against *K. pneumonia*. The TP1 and TP2 showed significant results against the rest of the oral pathogens.

3.3. Plant extracts sensitivity test

The use of plants and plant derivatives that possess preventive and therapeutic effects could contribute to oral health (Subramaniam et al., 2012). The list of the active components of plant extracts was obtained from the Botany department of University of Peshawar, Pakistan, as shown in Table 2.

Table 1. List of ingredients used in toothpaste under study.

Toothpastes	Ingredients as mentioned on the package
Toothpaste 1 (TP1)	Sorbitol, syloblanc, glycerine, sodium chloride, sodium monoflourophosphate (1400 ppm fluoride), certified food colour, sodium carboxymethyl cellulose, clove oil, menthol, eucalyptus oil, aroma, aqua, spearmint.
Toothpaste 2 (TP2)	Active ingredients:
	Potassium citrate 5.35%, Chlorohexidine digluconate 0.2%, Allantoin 0.1%, sodium benzoate 0.2%, sodium chloride 1%, sodium monoflourophosphate 0.76% (1000 ppm).
	Other ingredients:
	Dicalcium phosphate dihydrate/ hydrated silica, Glycerine, sodium saccharine, sodium lauryl sulphate, sodium lauroyl sarcosinate, cellulose gum, sodium tetraphosphate/potassium tetra phosphate, Cl-77891, flavour, purified water.
Toothpaste 3 (TP3)	Sodium monofluorophosphate 01.00%, Miswak (Salvadora persica) powder 00.58%.

The extracts of *A. bracteosa* could be useful against some multidrug-resistant pathogens as reported by Ganaie et al. (2017). The findings of Niamsa and Sittiwet in 2009 provided interesting details with the use of *C. Longa* aqueous extract used in antibiotic control (Palombo, 2011; Ganaie et al., 2017).

The methanolic extract of *A. bracteosa* (leaf, root and stem) was tested against eight different bacterial strains, as shown in Table S4, Figure S4 and Figure 6. Extracts from *A. bracteosa* leaves showed maximum activity against *K. pneumonia* (14.5 mm) at full concentration among all other plants extracts used in the study while least effect was observed against *S. sonnei* (5.4 mm) and *P. mirabilis* (5.6 mm). *A. bracteosa* stem extracts showed maximum activity against *A. hydrophila* (13.2 mm) and *S.aureus* (13.3 mm) while minimum activity was recorded against *K. pneumonia* (5.3 mm).

Ajuga bracteosa roots extract showed maximum activity against *A. hydrophila* (14.5 mm) whereas it was least effective against *E. coli* (6.2 mm). It is found that *A. bracteosa* leaf and roots were equally effective against different bacterial pathogens while stem extract showed the weaker activity against pathogens. In contrast, the minimum activity was observed against *P. vulgaris*. Shah et al. (2012), conducted a study in 2012 to investigate medicinal plant extract activity on bacterial pathogens and found that the ethanol extract of *A. bracteosa* showed no significant activity against *S. epidermidis*, moderate activity against *E. coli*, while no activity was shown against other bacterial species in his study.

Curcuma longa roots extracts showed maximum activity against *A. hydrophila* (17.5 mm) and *K. pneumoniae* (16.7 mm) while minimum activity was observed against *P. vulgaris* (9.2 mm) and *S. sonnei* (9.1 mm).

It is found that *C. longa* was the most effective antimicrobial agent against all the oral pathogens involved in this study as the curcumin present in the extract has known to be a potent bacteriocidal for 50 years

Table 2. List of active components present in plant extracts of *A. bracteosa* and *C. longa.*

Plant Extract	Active Components
Ajuga bracteosa	Limonene,
	α−ηυμυλενε,
	β−μψρχενε,
	Elemol,
	Camphene,
	β−Χαρψοπηψλλενε,
	α−πηελλενδρενε.
Curcuma longa	Curcuminoids:
	curcumin,
	Demethoxycurcumin,
	Bidemethoxycurcumin.

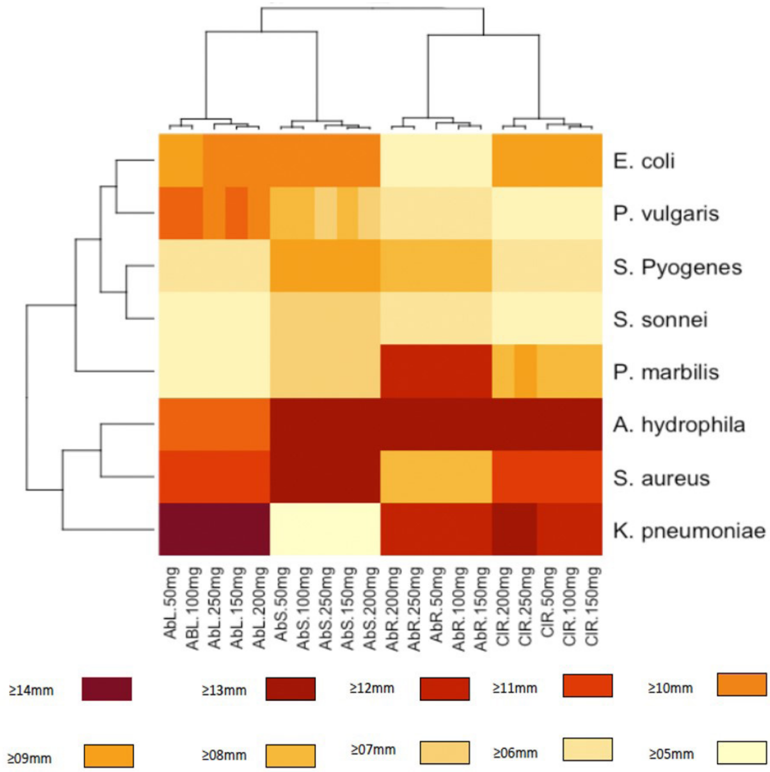

Figure 6. Heatmap showing the antimicrobial activity of the plant extracts against bacterial species isolated from gums. AbL stands for *Ajuga bracteosa* leaves, the AbS stands for *Ajuga bracteosa* stem, AbR stands for *Ajuga bracteosa* roots, and CIR stands for the *Curcuma longa* root. The tree is a dendrogram, which shows the hierarchy (similarity) between isolates and their activity against the different oral pathogens. The graph was plotted using the R program.

(Teow et al., 2016). *A. bracteosa* leaves, and stem extracts were found to be less effective against oral pathogens in comparison to *C. longa*. In the previous study, methanolic extract of *C. longa* showed significant activity against *S. epidermidis* and *B. Subtilis*, low for *S. aureus*, *K. pneumonia* and zero activity against *E. coli* (Shah et al., 2012)

4. Conclusion

Our study concluded that the use of antibiotics in case of infections is the best choice of treatment. Our research found that dental problem is more common in males than in females in the KPK province of Pakistan and mostly people 41-50 of ages were more effected. It was concluded from the observations that TP1 was the most effective toothpaste among all the other toothpaste tested against the pathogens, which might be due to fluoride in that toothpaste. Regarding plant extracts, *C. longa* showed convincing results against oral bacterial pathogens in this study, that is maybe due to the presence of curcuminoids in this plant. By comparing the results, it is revealed that antibiotics' effectiveness in case of infections cannot be denied. However, the use of fluoride-based toothpaste in daily routine could be beneficial to avoid any bacterial infections. Use of toothpaste with natural extracts could give added benefits.

5. Limitation

C. longa extract could be combined with TP1 toothpaste to check the efficacy against different oral pathogens, including fungi. Still, with these limitations, our research suggested that *C. longa* and *A. bracteosa* contains bioactive natural products that could help cure dental problems. After further investigation and identification, these plants' most effective component could be used in toothpaste or mouth rinses to maintain oral hygiene.

6. Data Availability

This does not apply to our research because of its experimental research, not a case study work, but if needed co-authors will provide it.

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Supplementary Material

The supplementary files consist of biochemical table identification of oral bacterial pathogens and pictorial representation of biochemical tests used in this study. The activity of antibiotics, toothpaste and medicinal plant extract against oral pathogens. It also contains the pictorial representation of antibiotics, three toothpaste (TP1, TP2 and TP3) and medical plant extract (Ajuga bracteosa and Curcuma longa) activity against oral pathogens which showed the maximum zone of inhibition.

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