COMPARATIVE STUDY OF ANTIBIOTICS, TOOTHPASTE AND PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS ISOLATED FROM HUMAN GUMS.

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ABSTRACT

This study was conducted to find out the effectiveness of some antibiotics, toothpastes, and medicinal plant extracts against the bacterial species isolated from human gums. A total of 125 samples were collected. The male and female patients were with the age from 15-60 years. A total of 27 species were isolated and identified. Among those bacterial species, Streptococcus mutans was most prevalent 29.62%, followed by Lactobacillus acidophilus 22.22%, Streptococcus sobrinus 18.51% and S aureus 14.81% respectively. While Micrococcus spp. 3.7%, Streptococcus sanguinis 3.7%, Actinomyces viscosus 3.7% and Aeromonas were least prevalent. To observe the most effective treatment for gum infections, the isolated strains were subjected to the sensitivity tests against antibiotics, toothpastes and plant extracts. The maximum zone of inhibition formed by Sulfamethoxazole was found against Staph aurus 33mm, while the minimum zone by Amoxicillin was observed against Streptococcus sobrinus (6mm). The maximum zone of inhibition by Toothpaste 1 (Fluoride and salt) was against Micrococcus spp. 34mm. The maximum zone of inhibition by Curcuma longa against S. mutans was 22mm, while the minimum zone by Ajuga bracteosa against S. mutans was 11mm. The data obtained through this study revealed that antibiotics were more effective for the treatment of oral bacterial pathogens as compared to toothpaste and plant extracts with moderate and low activity, respectively. Therefore, it is strongly suggested that use of antibiotics during infection and toothpaste with fluoride and salt contents in daily routine could help in the elimination of bacterial pathogens to maintain oral health.

Keywords: Ajuga bracteosa, Curcuma longa, Toothpaste, Antibiotics

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INTRODUCTION

Microbial investigations have revealed that diverse array (trillions) of microorganisms resides on different parts of the body. One such favorable place for these microbes is the human mouth. (Prasad et al., 2007). The
human oral cavity possesses a microbial population which is very complex and distinctive. The oral microbes have direct relationships with other microorganisms and with the human host in niche-specific associations. The disruption of homeostasis that ensures a balanced microbial composition or dysbacteriosis can contribute to numerous oral infections, notably dental caries and gum diseases (Balachandran et al., 2020).

According to Human oral microbiome database (HOMD), there are 150 genera of microorganisms identified as common causes of oral diseases among them Streptococcus, Haemophilus, Actinomyces, and Prevotella have higher abundance than any other genera. (Guo et al., 2018). More than 700 microbial species have been found to present inside a human oral cavity by non-culturing technique. Most of the bacteria of the oral cavity are benign and like in other parts of the body; they are in a symbiotic relationship. However, few can cause many gum diseases. Due to changes in home environmental conditions, though, the mucosal surface microbiota shows compositional differences with that present in dental plaque (Zaura et al., 2015).

Among other various identified bacteria species from the oral cavity, Lactobacillus and Streptococcus spp. are the dominant one. The most common opportunistic pathogen among all is Streptococcus mutans (Burton et al., 2011; Gamboa et al., 2004). However, Staphylococcus aureus is a significant pathogen prefer mouth and hands as main reservoirs for propagation and colonize than other several anatomical sites in the human body (Tanomaru et al., 2008).

Streptococcus mutans is the major cause of tooth decay is a Gram-positive, non-spor forming, catalase-negative, non-motile, facultative anaerobic cocci (David et al., 2011). The Mutans streptococci include a group of seven species, of which Streptococcus mutans and Streptococcus sobrinus are the principal species obtained from saliva and plaque of human (Loesche et al., 1986). Prevention of dental caries can be achieved by proper and regular oral care along with repetitive mechanical means of oral hygiene practices (Gurenlian et al., 2007). The purpose of this research study was to identify bacteria responsible for gums diseases in the Pakistani population and to analyze their susceptibility against various antibiotics, popular toothpaste brands containing fluoride and plant extracts which were thought to be effective against many diseases for centuries.

**METHODS**

This experimental study was conducted at Nistar Medical University and Microbiology Department of Abasyn University Peshawar from Mar 15, 2020, to Jun 15, 2020. A total of 125 samples were collected from indoor patients at Nistar and Alfalah Dentistry Departments, Pakistan. Samples were collected using sterile swabs under standard microbiological practices. The samples were processed within 05 hours after collection and streaked on Nutrient agar plates which were subjected to incubation for 24 hours at 37 °C. After incubation, mixed colonies were obtained on each plate. The colonies were picked and purified by repeated sub-culturing for specific bacterial growth and used differential media (i.e. Blood Agar, Mannitol Salt Agar (MSA), MacConkey Agar and Chocolate Agar) for the growth of bacteria species. Further identification of the pure colonies was made by using Gram Staining and other biochemical tests such as Oxidase, Indole, TSI, Citrate, Urease and Catalase. (Ilyas et al., 2016). Characterization on the basis of Antibiotic, Toothpaste and Plan Extract Sensitivities.
**Antibiotic Sensitivity:** For Antibiotic Sensitivity testing, Kirby Baur disc diffusion test was performed according to the CLSI guidelines. Amoxicillin (5µg), sulfamethoxazole (25µg), oxacillin (5µg), vancomycin (30µg), moxifloxacin (5µg), amoxicillin (30µg), ampicillin (10µg), azithromycin (15µg) were selected to check the susceptibility as described by (Abdulhaq et al., 2020).

**Toothpaste Sensitivity:** For Toothpaste Sensitivity testing agar well diffusion method was used. Three different concentrations of each toothpaste were made at full strength (50 mg) and 1:1 dilution. The fresh cultures from the sample were taken in broth, and the turbidity was made with McFarland Standards. The zone of inhibition around the wells showed the antimicrobial activity according to standard procedure. The zone of inhibition formed by toothpaste against bacteria was considered as sensitive toothpaste while medium zone formed against bacteria were considered intermediate toothpaste and no zone of inhibition showed the resistance of bacteria to that particular toothpaste.

**Plant Extract Sensitivity:** For plant extract sensitivity testing well diffusion method was used. The fresh cultures from the sample were taken and tested against A. bracteosa and C. longa extracts. Leaf, Stem and Roots of A. bracteosa and entire plant of C. longa were used in crude form and diluted with Dimethyl Sulfoxide (DMSO) according to standard procedure. The zone of inhibition around the wells showed the antimicrobial activity against the bacteria. The maximum zone of inhibition formed by plant extract against bacteria was considered as sensitive, intermediate and resistant to a particular plant extract. The plant extract was used to detect the plant extract susceptibility pattern of Streptococcus mutans, Staph aureus, Streptococcus sobrinus and Lactobacillus acidophilus.

**Statistical Analysis:** Mean antimicrobial activities of plant extracts were calculated against each bacterium with 95% Confidence Interval. Pearson Correlation between dose and anti-bacterial activity of plant extracts was calculated with consideration of P=0.05 with 95% Confidence Interval. All the analyses were carried through GraphPad Prism (Version 5.0) software.

**RESULTS**

From 125 infected samples, 27 pure species were obtained. The data indicates the percentage of bacterial isolates from teeth gum of patients. Eight different types of bacteria, i.e. Streptococcus mutans, Staph aureus, Streptococcus sobrinus, Lactococcus acidophilus, Micrococcus, Streptococcus sanguinis and Aeromonas species, were identified. Streptococcus mutans was isolated in the highest number (29.62%), followed by Lactobacillus acidophilus (22.22%), Streptococcus sobrinus (18.51%) and S. aureus (14.81%) respectively. While Micrococcus spp. 3.70%, Streptococcus sanguinis 3.70%, Actinomyces viscosus 3.70% and Aeromonas 3.70% were present at the lowest percentage.

**Antibiotic Sensitivity Test:** In this study, the identified eight species antibiogram patterns were analyzed against eight different antibiotics. Among all used antibiotic SXT (sulfamethoxazole) showed maximum effect against all tested bacterial strains. The maximum zone of inhibition was produced against Staph aurus (33mm) while the minimum zone was observed against S. sobrinus (23mm). The most effective antibiotics were MXF (Moxifloxacin), FOX (Foxicillin), and AMC (Amoxicillin).

**Table 1:** Antibiotic sensitivity pattern of Isolated bacterial species from Teeth Gums.

| Microorganisms          | FOX (mm) | SXT (mm) | OX (mm) | VA (mm) | MXF (mm) | AMC (mm) | AMP (mm) | AZM (mm) |
|-------------------------|----------|----------|---------|---------|----------|----------|----------|----------|
| Staph aureus            | 21       | 33       | R       | R       | 30       | 13       | R        | 10       |
| Streptococcus mutans    | 18       | 26       | 22      | 20      | 29       | 16       | 10       | R        |
| Streptococcus sobrinus  | 15       | 23       | R       | 22      | 23       | 6        | R        | 10       |
| Lactobacillus acidophilus| 21     | 29       | 19      | 23      | 28       | 19       | 18       | 17       |
| Micrococcus spp.        | 30       | 31       | 20      | 21      | 31       | 20       | 19       | 20       |
| Streptococcus sanguinis | 30       | 25       | 18      | 22      | 30       | 18       | 18       | 14       |
| Actinomyces viscosus    | 22       | 32       | 12      | 21      | 29       | 19       | 23       | 9        |
| Aeromonas               | 32       | 28       | 18      | 18      | 31       | 21       | 22       | 19       |

**TOOTHPASTE SENSITIVITY TEST:**

The diluted and undiluted form of kinds of toothpaste revealed that antimicrobial activity was at full strength in undiluted form by showing the high number of antimicrobial agents and having linear reduction with the decrease of dilution. Overall, toothpaste I contained high amount of Fluoride and salt is diluted, and non-diluted form showed a good effect.
against all tested bacterial strains. The non-diluted one showed good activity against Micrococcus spp (34mm) while the minimum zone was seen against S. aureus (21mm). The maximum activity of diluted one was observed against Micrococcus spp. And S. mutans (26mm) by Toothpaste 2 had active ingredients Calcium and Fluoride while the minimum zone was produced against Actinomyces viscosus (14mm). Overall tooth paste 3 had calcium as active ingredient was moderate in action.

Table 2: Kinds of toothpaste sensitivities against isolated bacterial species from teeth gums

| Microorganisms                  | Toothpaste, 1 |          | Toothpaste, 2 |          | Toothpaste, 3 |          |
|--------------------------------|---------------|----------|---------------|----------|---------------|----------|
|                                | Diluted       | Non-Diluted | Diluted       | Non-Diluted | Diluted       | Non-Diluted |
| Staph aureus                   | 22            | 26        | 21            | 26        | 19            | 22        |
| Streptococcus mutans           | 17            | 21        | 26            | 30        | 19            | 25        |
| Streptococcus sobrinus         | 16            | 28        | 23            | 27        | 16            | 21        |
| Lactobacillus acidophilus      | 15            | 26        | 22            | 27        | 20            | 22        |
| Micrococcus spp.               | 16            | 34        | 26            | 29        | 21            | 26        |
| Streptococcus sanguinis        | 15            | 24        | 25            | 27        | 19            | 21        |
| Actinomyces viscosus           | 14            | 29        | 19            | 22        | 17            | 21        |
| Aeromonas spp.                 | 16            | 24        | 21            | 23        | 18            | 23        |

**Plant extracts Sensitivity test:** The methanolic extract of Ajuga bracteosa was used separately (leaf, root and stem) against tested bacterial strains. Leaf extract produced a maximum zone against Streptococcus mutans (22mm) while the minimum zone of inhibition was against Streptococcus sobrinus (12mm). The methanolic extract of C. longa produced a maximum zone of inhibition against Streptococcus mutans (22mm) while the minimum activity was observed against Lactobacillus acidophilus (15mm).

Table 3: Antimicrobial activity of the plant extracts against bacterial species isolated from gums.

| Plant Extracts       | Streptococcus mutans | Staph aureus | Streptococcus sobrinus | Lactobacillus acidophilus | Aeromonas spp. |
|----------------------|----------------------|--------------|------------------------|---------------------------|----------------|
| Ajuga bracteosa leaf | 20                   | 17           | 12                     | 16                        | 15             |
| Ajuga bracteosa root | 19                   | 15           | 15                     | 13                        | 12             |
| Ajuga bracteosa stem | 11                   | 19           | 15                     | 14                        | 16             |
| Curcuma longa        | 22                   | 21           | 16                     | 15                        | 18             |

**Statistical analysis for the antimicrobial activity against Plant extracts:** After getting the antimicrobial activity of plant extracts against isolated bacteria, the results were further subjected to statistical analyses. Pearson Correlation between dose and anti-bacterial activity of plant extracts with consideration of 0.05 P value and 95% confidence interval was calculated. The analysis revealed that Ajuga bracteosa leaf extract was merely significant against Strep. pyogenes (p=0.0101), while other bacteria showed no significance at all. Curcuma longa was found to be significant only against K. pneumoniae (p= 0.9627) out of seven other bacterial isolates. Whereas Curcuma longa showed no significance against any of the bacterial strains under study.

**DISCUSSION**

It is well studied that the invasion of many bacteria in the oral cavity may cause various bacterial infections.
In our study S. aureus was the predominant isolated pathogen, this result agreed with the finding conducted in Yemen by Hassan A et al., (2018) who found the dominancy of this pathogen with a percentage of 43.1%. The high frequency of S. aureus in mouth infections can be explained by the fact found by Dingies MM et al., (2000) that S. aureus often colonizes the mucous membrane of the nose, where it can cause endogenous oral cavity infections.

The study observed that all the selected antibiotic show action against the isolates except Oxacillin (no activity against K. pneumonia, S. pyogenes and vancomycin (no activity against K. pneumonia). Our finding does not match with Okopi et al. (2015) finding that dental caries pathogens showed resistance to ciprofloxacin, ampicillin, erythromycin and amoxicillin. This report also does not agree with the reports of Sweeney et al., (2004) that resistance of oral flora to antibiotics is an international problem and may be due to excessive abuse of antibiotics. Maripandi et al. (2011) also observed the resistance pattern and found that oral pathogen resistant to penicillin, bacitracin, streptomycin, vancomycin and
chloramphenicol. Boyanova et al. (2004) found that 21% of gram-positive anaerobic cocci from dental plaque were resistant to penicillin, and 16% of them were resistant to clindamycin. Brook (2007) detected susceptibility of Veillonella species (from dental plaque) to beta-lactam but resistance to tetracycline and erythromycin while Campylobacter gracilis showed 100% sensitivity to erythromycin, but 50% to clindamycin, cephalothin and pipracillin. This study also reported the efficiency of antibiotics against oral pathogens and found that among the selected eight antibiotics used the most effective zones of inhibition were formed by sulfamethoxazole (25µg) against all pathogenic bacteria so it can be a choice of antibiotic to treat dental caries. This study reported that undiluted toothpaste concentration gave the highest zone of inhibition and these inhibition zones tend to decrease down the concentration gradient. Other researchers found the same result that apart from the addition of antimicrobial agents in a toothpaste, the agent concentration is also paramount to achieve the most wanted outcome of reducing oral microbes (Sadeghi and Assar, 2009; Tatikonda et al., 2014; Odeleye, F. O et al., 2018). The use of plants and plant derivatives which are known to possess preventive and therapeutic effects could contribute to oral health (Subramaniam P., et al., 2012). This study found that extract of Ajuga bracteosa produced maximum zone against K. pneumonia while the minimum zone of inhibition was against S. pyogenes while extract of C. longa produced a maximum zone of inhibition against K. pneumonia. In contrast, the minimum activity was observed against P. vulgaris. Shah at el., 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 against E. coli, while no activity was shown against other bacterial species in his study. In contrast, ethanol extract of C. longa showed significant activity against S. epidermidis and B. Subtilis, low for S. aureus, K. pneumonia and no activity against E. coli (Shah, Z., at al., 2012).

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AZ: Study Concept, design, Manuscript writing
AJ: Manuscript writing, Critical revision
AN: Concept, design, data collection, Manuscript writing
MAFZ: Manuscript drafting, Data analysis
ZN: Concept, design, data analysis
MH: Data collection
AA: Critical revision