DETERMINATION OF ANTIBACTERIAL ACTIVITY OF NICOTIANA TABACUM AGAINST BACTERIA ISOLATED FROM TEETH GUMS OF SMOKERS AND NON-SMOKERS

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ABSTRACT

OBJECTIVES

This study aimed to determine the antibacterial activity of Nicotiana tabacum against bacteria isolated from the gums of smokers and non-smokers.

METHODOLOGY

For this study, 100 gum samples were collected from dental clinics in Peshawar using sterile disposal swabs. The samples were transferred to Abasyn University in Peshawar, streaked on Nutrient agar plates. The obtained cultures were sub-cultured and processed for further identification by Gram staining and biochemical tests.

RESULTS

It was found that, out of a total of 100 samples, 60 were positive (35 non-smokers and 25 smokers), and 40 were negative. Among 60 samples, 12 species were identified, of which M. mucilaginous (24% of the smoker) and S. aureus (44% of the non-smoker) showed high prevalence. After the analysis of both the dried and chewed N. tabacum's antibacterial activity, it was observed that dried tobacco extract showed maximum activity against S. hyicus (16.33 ± 0.57), M. mucilaginous (16.33 ± 0.57) and least activity against E. coli (10.7 ± 0.46). In contrast, chewed tobacco extract showed maximum activity against S. cohnii (15.33 ± 0.57), while the remaining isolates were resistant.

CONCLUSION

The outcome of the studies concluded that the prevalence of bacteria isolated from smoker's samples was higher and more pathogenic than in non-smoker's samples.

KEYWORDS: *S. Aureus, M. Mucilaginous, E. Coli, S. Hyicus, S. Cohnii, Antibacterial Activity*

How to cite this article:
Majid M, Farooq S, Ilyas A, Zoreen A, Ishaq MS, Khan A, Et al. Determination of Antibacterial Activity of Nicotiana Tabacum Against Bacteria Isolated From Teeth Gums of Smokers and Non Smokers. J Wazir Muhammad Inst Paramed Tech. 2022;2(2): 13-18

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INTRODUCTION

Since the beginning of time, the earth has provided us with therapeutic substances. It is recognised that the plant universe contains an endless supply of bioactive components that are extremely useful in treating several illnesses. Ingredients from several medicinal herbs have previously shown potential in combating drug-resistant microbial species. The term "medicinal plant" refers to a variety of plant species employed in homoeopathy, a few of which have healing uses (Rasool, 2012).[1] Numerous antibacterial, antifungal, and anti-inflammatory activities have been reported by naturally occurring substances. Tobacco plants may generate grease and bio-methane and have 30 – 40% fatty vegetable oils. Citric acid, which could be utilised to make colours and polishes, is found in tobacco. Several studies claim that seed extracts have antimicrobial properties against S. aureus (Sharma et al., 2016).[2] CBT's antifungal properties were first discovered in 1990. The IC50 of alpha-

and beta-CBT-doil on blue mould has been measured and might suppress their growth (Yan et al., 2019).[3] The genus Nicotiana contains 76 species worldwide, making it the sixth-largest group in flowering plants.[4] The common mammalian micro-biome is made up of about 250 bacteria. Tension, diet, inheritance, and human ageing are often determinants of a typical micro-flora makeup (Orji et al., 2018).[5] Neisseria, streptococci, Actinomycetes, Prevotella, and Veillonella, are the gram-positive and negative anaerobic bacteria found on the basal surfaces of the teeth (Chawla et al., 2018).[6] The study of oral bacteria, including its relationships with the person or other mouth pathogens, is known as oral microbiology. The habitat in the mouth promotes the development of traits (Chowdhury et al., 2019).[7] By interacting with food particles and sputum, various oral bacteria, including S. aureus, Streptococcus mutans, and Lactobacillus acidophilus, form a waxy coating on the teeth. These microorganisms emit acids that damage teeth by creating cracks and tooth rot. Tooth decay is more common worldwide due to poor oral health care (Cine et al., 2017).[8] The current study aimed to evaluate the antibacterial activity of Nicotiana tabacum against bacteria isolated from the gums of smokers and non-smokers.

METHODOLOGY

Using sterile disposal swabs, one hundred gum samples were collected from patients at different dental clinics in Peshawar. The labelled samples were immediately transferred to the Microbiology Laboratory at Abasyn University Peshawar and streaked on Nutrient agar. After that, the plates were incubated at 37^{o}C for 24 hours. The cultures obtained on each plate were sub-cultured. The first step after sub-culturing was the identification of bacteria. To characterise the phenotype of a bacterium, a microscopic examination is essential. The staining process distinguishes bacteria based on the composition of their cell membranes. Staphylococci dye blue to purple due to their thick peptidoglycan coating, whereas gram-negative colonies dye red to pink and have a thin peptidoglycan cell wall (Smith and Hussey, 2005).[9] Biochemical Test: Depending on the differences in the biochemical characteristics displayed by several strains of bacteria, biochemical tests were used to determine the microorganisms. The following is a list of numerous biochemical experiments employed for Staphylococci and gram-negative bacterial detection (Shoaib et al., 2020).[10] Catalase Test: The presence of microbes that generate catalase was analysed using catalase testing. The hydrogen peroxide was neutralised by catalase, generated by facultative anaerobes and obligatory aerobes, and bubbling appeared. As a result, they signified a successful test (Facklam and Elliott, 1995).[11] Coagulase Test: This test was performed to determine whether bacteria could produce the coagulase enzyme. The enzymes will cause the blood fluid to clot (Holt et al., 1994).[12] Urease Test: The urease test identified bacteria that can produce the urease enzyme. The enzyme hydrolyses urea into NH_3 and CO_2 (Shoaib et al., 2020).[10] Oxidase Test: An oxidase test was performed to identify bacteria with the capacity to synthesise the oxidase enzymes. The electron donor would be oxidised by oxidase, resulting in a deep purple colour (Win et al., 2006).[13] Indole Test: Bacteria having the ability to produce tryptophanase were determined by the indole test. The enzymatic reaction produced indole gas, verified by Kovac's reagents (MacFaddin, 2000).[14] Triple Sugar Iron Test: The TSI test was used to distinguish Enterbacteriacea members based on differences in carbohydrate fermentation patterns and hydrogen sulfide production (Harley, 2005).[15] Preparation of Plant Extract: The tobacco leaves, dried and chewed, were shade-dried at room temperature for one month in the microbiology laboratory and converted into powder. Leaves were shade-dried because their bio-active components were not damaged. The grinded material of dried tobacco (115.76 mg) was soaked in methanol (500 mL), whereas chewed tobacco (41.63 mg) was soaked in methanol (250 mL). The methanol extracts were dried in a vacuum using a rotary evaporator (Buchi Labortechnik AG, Switzerland) (Shekins et al., 2016).[16] Currently, the best extracting solvent is methanol because of its strong polarity and potential for high-leaf extract (Hassim et al., 2014).[17] Antibacterial Activity of Extract: The antimicrobial effect of Nicotiana tabacum extracts, both dried and chewed, was assessed using Muller Hinton Agar. The extract (made with a minute quantity of DMSO with leaf extract) was introduced into the wells made by a borer of 6mm diameter, which already has bacteria. The plates were then incubated for 16 hours at 37^{o}C. The results were analysed, and the zone of inhibition was measured in mm. DMSO and distilled water were negative controls, and Ciprofloxacin (5µg) was used as a positive control. All the tests were performed in triplicate (Bouyahya et al., 2016).[18]

RESULTS

Out of 100 samples, 60 were positive (35 were non-smokers and 25 were smokers), while 40 were negative samples, as shown in figures 1 and 2. 12 species were isolated after biochemical testing (Table 1). Staphylococcus aureus, Staphylococcus xylosus, Staphylococcus cohnii, Staphylococcus hyicus, Streptococcus salivarius, Streptococcus faecium, Streptococcus agalactiae, Streptococcus thermophilus, Micrococcus mucilaginous, Micrococcus luteus, Cellobiosococcus spp, and Escherichia coli, the overall prevalence of these species, as well as from smokers and non-smokers samples were summarised in table 2.

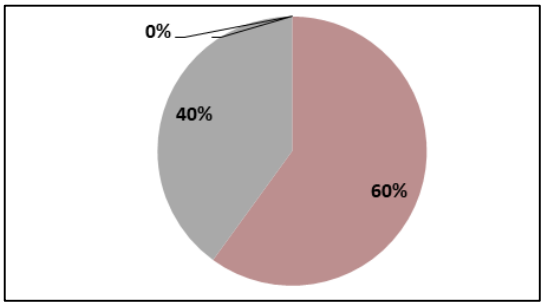

Figure 1: Overall Prevalence of collected samples, in which 60 were positive and 40 were negative.

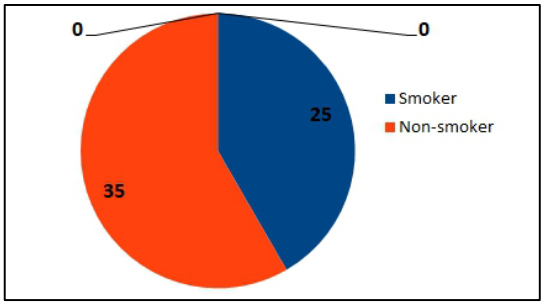

Figure 2: Prevalence of Positive samples out of 100 samples, 35 were non-smokers, and 25 were smokers.

Antibacterial activity of Dried Tobacco Extract: Agar well diffusion was performed to determine the antibacterial activity of dried tobacco extract against isolated species. The extract was effective against S. hyicus (16.33 ± 0.57), M. mucilaginous (16.33 ± 0.57), S. faecium (15.5 ± 0.86), S. aureus (15.33 ± 0.57), M. luteus (15.33 ± 0.57), S. salivarius (15.1 ± 0.76), S. thermophilus (14.83 ± 0.76), S. cohnii (14.83 ± 0.76), S. agalactiae (12.6 ± 0.57), S. xylosus (10.8 ± 0.28), Cellobiosococcus spp. (10.8 ± 0.28) and E. coli (10.7 ± 0.46). Ciprofloxacin antibiotic was used as a positive control against isolates S. thermophilus (26mm), S. faecium (25mm), S. hyicus (23mm), M. luteus (21mm), S. aureus (20mm), S. xylosus (20mm), S. cohnii (20mm), S. salivarius (20mm), S. agalactiae (20mm), Cellobiosococcus spp (20mm), E. coli (20mm), M. mucilaginous (18mm). DMSO was used as a negative control that showed no zone of inhibition, as shown in Table 3. Antibacterial activity of Chewing Tobacco Extract: Agar well diffusion was performed to determine the antibacterial activity of chewing tobacco extract. It showed activity against three species, S. cohnii (15.33 ± 0.57), M. luteus (15.1 ± 0.76) and S. faecium (10.7 ± 0.46), while the rest of the isolates were found resistant. Ciprofloxacin was used as a positive control against isolates S. thermophilus (26mm), S. faecium (25mm), S. hyicus (23mm), M. luteus (21mm), S. aureus (20mm), S. xylosus (20mm), S. cohnii (20mm), S. salivarius (20mm), S. agalactiae (20mm), Cellobiosococcus spp (20mm), E. coli (20mm) and M. mucilaginous (18mm). DMSO was used as a negative control that showed no zone of inhibition, summarised in Table 4.

Table 1: Identification of Bacteria Based on Biochemical Tests

S.No	Isolates	Gram staining	Catalase	Coagulase	Oxidase	Citrate	Indole	Urease	TSI	H_2S Gas	Gas
1	E. coli	-	+	-	-	-	+	-	A/A	-	+
2	S. aureus	+	+	+	-	+	-	+	A/A	-	-
3	S. xylosus	+	+	-	-	-	-	+	A/A	-	+
4	S. cohnii	+	+	-	-	-	-	+	A/A	-	-
5	S. hyicus	+	+	+	-	+	-	+	k/A	-	-
6	S. salivarus	+	-	+	-	-	-	+	A/A	-	-
7	S. faecium	+	-	+	-	-	-	-	A/A	-	-
8	S. agalactiae	+	-	-	-	-	-	-	A/A	-	-
9	S. thermophilus	+	-	+	-	-	-	+	A/A	-	-
10	M. mucilaginosus	+	-	+	+	-	-	+	A/A	-	-
11	M. luteus	+	+	-	+	+	-	+	K/A	-	-
12	Cellobiosococcus spp	+	+	-	+	-	-	+	K/A	-	-

Table 2: Overall Prevalence of Bacteria Isolated From the Smoker and Non-Smoker Samples

S.No	Isolates	Overall prevalence of bacteria isolated from 60 samples	Prevalence of Bacteria Isolated from Smoker Samples	Prevalence of Bacteria Isolated from Non-Smoker Samples
1	E. coli	03%	-	06%
2	S. aureus	28%	08%	44%
3	S. xylosus	10%	08%	11%
4	S. cohnii	04%	08%	-
5	S. hyicus	07%	-	11%
6	S. salivarus	10%	08%	11%
7	S. faecium	10%	08%	11%
8	S. agalactiae	03%	08%	-
9	S. thermophilus	03%	08%	-
10	M. mucilaginosus	10%	24%	-
11	M. luteus	07%	16%	-
12	Cellobiosococcus spp	05%	04%	06%

Table 3:Antibacterial Activity of Dried Tobacco Extract

S.No	Isolates	Zone Diameter Interpretive Criteria (Nearest Whole mm)			
		Tobacco Extract (dried tobacco)	Positive Control (CIP)	Negative Control (DMSO)	Solvent Methanol
1	S. aureus	15.33 ± 0.57	20mm	0	10
2	S. xylosus	10.8 ± 0.28	20mm	0	05
3	S. cohnii	14.8 ± 0.7	20mm	0	09
4	S. hyicus	16.33 ± 0.57	23mm	0	06
5	S. salivarius	15.1 ± 0.76	20mm	0	05
6	S. faecium	15.5 ± 0.86	25mm	0	06
7	S. agalactiae	12.6 ± 0.57	20mm	0	04
8	S. thermophilus	14.83 ± 0.76	26mm	0	06
9	M. mucilaginous	16.3 ± 0.57	18mm	0	04
10	M. luteus	15.33 ± 0.57	21mm	0	06
11	Cellobiosococcus spp	10.8 ± 0.28	20mm	0	05
12	E. coli	10.7 ± 0.46	20mm	0	06

Table 4:Antibacterial Activity of Chewing Tobacco Extract

S.No	Isolates	Zone Diameter Interpretive Criteria (nearest whole mm)			
		Chewing tobacco Extract	Positive Control (CIP)	Negative Control (DMSO)	Solvent Methanol
1	S. aureus	0	20mm	0	10
2	S. xylosus	0	20mm	0	05
3	S. cohnii	15.33 ± 0.57	20mm	0	09
4	S. hyicus	0	23mm	0	06
5	S. salivarius	0	20mm	0	05
6	S. faecium	10.7 ± 0.46	25mm	0	06
7	S. agalactiae	0	20mm	0	04
8	S. thermophilus	0	26mm	0	06
9	M. mucilaginosus	0	18mm	0	04
10	M. luteus	15.1 ± 0.76	21mm	0	06
11	Cellobiosococcus spp.	0	20mm	0	05
12	E. coli	0	20mm	0	06

DISCUSSION

Since the ancient period, plants have provided us with therapeutic compounds. It is impossible to overstate the value of plants in treating illnesses. Resistant bacteria are now a worldwide issue.[19] The antibacterial effects of tobacco leaf extracts (95% C_2H_6O and C_6H_{14}) on ascomycetes have led to the hypothesis that CBT-diol is the primary antibacterial agent.[3] This study was conducted at Abasyn University Peshawar to find out the antibacterial activity of N. tabacum against the gum's bacteria in smokers and non-smokers. In a recent study, 100 dental caries samples were collected from smokers and non-smokers from dental clinics in Peshawar using sterile disposal swabs. The labelled samples were transferred to the Microbiology Laboratory of Abasyn University for further processing. Twelve species were isolated, summarised in Table 1, where the prevalence of bacteria is also shown in the Pie Chart (Figures 1 and 2) and Table 2. Both dried and chewed tobacco leaves were shade-dried at room temperature for one month in the laboratory

and were converted into powder. Grinded material of both chewing tobacco (41.63 mg) and dried tobacco (115.76 mg) was placed in the extractor and extracted using methanol (250 mL for chewing tobacco or 500 mL for dried tobacco). The methanol extracts will be dried in a vacuum using a rotary evaporator. The extracts were introduced into the six mm-diameter wells on the plates, which already had bacterial growth. The plates were then incubated for 16 hours at 37 °C. The results were analysed, and the zone of inhibition was measured in mm. DMSO was used as a negative control, and Ciprofloxacin was used as a positive control. According to various investigations, the Nicotiana tabacum stem's methanolic extract had the highest activity against Staphylococcus aureus, with inhibitory lengths of 10.667 ± 1.527.[2] In this study, it was observed that both tobacco forms showed antibacterial activity, as described in detail in tables 3 and 4. Dried tobacco extract showed maximum activity against S. hyicus (16.33 ± 0.57) and M. mucilaginosus (16.33 ± 0.57) and minimum activity against E. coli (10.7 ± 0.46), whereas chewing tobacco extract showed maximum activity against S. cohnii (15.33 ± 0.57), M. luteus (15.1 ± 0.76) and S. faecium (10.7 ± 0.46). At the same time, the rest of the isolates were resistant. Ciprofloxacin was used as a positive control and demonstrated the greatest activity against S. thermophilus isolates (26 mm) and the least activity against M. mucilaginosus (18 mm). DMSO was used as a negative control that showed no zone of inhibition.

LIMITATIONS

This study did not collect additional samples from other sites (Skin, nasal passage). Other solvents can be used for extract preparation, negative controls, and different concentrations of tabacum extracts. As a result, other researchers are advised to use different solvents and different concentrations of extracts.

CONCLUSIONS

According to the current study's findings, it was concluded that smokers' samples had the highest concentration of harmful bacteria, such as M. mucilaginosus 24% (due to disturbance of normal flora), compared to non-smokers samples, which has 44% more S. aureus. It was observed that dried tobacco extract showed maximum activity against pathogenic bacteria S. hyicus, M. mucilaginosus, whereas chewing tobacco extract showed maximum activity against S. cohnii, while the rest of the isolates were found resistant.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

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4.	**Aqdas Zoreen** - Critical Revision
5.	**Muhammad Saqib Ishaq** – Critical Revision; Final Approval
6.	**Amjad Khan** – Critical Revision
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