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Collection of Samples from Various Microenvironments Analyse and Quantify the Ambient Microbial Air Quality in The Restrooms of Dehradun City for Study of Antibiotic Resistance of These Various Isolated Microbes from Contaminated Environment and Antimicrobial Activity of Apple Leaves and Bark Ethanolic Extract on the Isolated Microbes.

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ABSTRACT:
The microbiological air quality research in Dehradun public bathrooms highlighted the possible health and environmental risks associated with high-traffic areas. It emphasizes the need to address the spread of germs and their relationship to various illnesses. The study's results also provide significant evidence for more extensive conversations about antibiotic resistance and help continue efforts to address this rising public health concern. Furthermore, investigating ethanolic extracts from apple leaves and barks for antibacterial activity is an excellent possibility for creating natural antimicrobial drugs. The study's findings indicate that plant-derived extracts can limit microbial development, offering long-term alternatives to standard antimicrobial treatments. In conclusion, the research discusses antibiotic resistance in bacteria and the antibacterial activity of apple leaves and bark ethanolic extract on isolated microorganisms.

1. Introduction:

Public restrooms serve as high-traffic areas where numerous individuals come into contact, potentially spreading bacteria. While public health efforts addressing drug resistance have primarily concentrated on hospitals and healthcare settings, communal spaces like public restrooms are not exempt from the presence of antibiotic-resistant bacteria. Studies suggest that many bacteria, including those carrying resistance genes, can persist on surfaces in these settings due to users' inadequate cleaning or hygiene practices. Human behavior, as well as drug misuse or overuse in the community, may influence the presence of antibiotic-resistant bacteria in public restrooms. When people carrying resistant germs use these facilities, they can release these bacteria into the environment, contributing to the restroom's microbial ecosystem. [1]

Additionally, improper disposal of antibiotics or their presence in wastewater may introduce resistant strains into the sewage system, potentially affecting public restrooms and other shared spaces. Public toilets serve as crucial reservoirs, indirectly contributing to the spread of resistant strains if these germs survive on surfaces or in wastewater and spread to other individuals. Although concrete evidence of transmission or increases in drug resistance specific to public restrooms may be limited, these locations can act as reservoirs or transfer points for bacteria, including resistant strains, especially if hygiene measures are inadequate. [2]

A comprehensive strategy is required to address the potential impact of medication resistance in public restrooms. This strategy should encompass enhancing sanitation procedures, promoting excellent hand hygiene, maintaining regular cleaning and disinfection schedules, and educating the public about responsible antibiotic usage. For a more profound understanding of public spaces like restrooms' role in the spread of drug resistance, studies investigating the dynamics of bacterial communities, including antibiotic-resistant strains, in

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these environments are crucial. Ongoing research and monitoring activities are essential to monitor and prevent the spread of antibiotic resistance in public settings. [3,4]

Bloodstream infections (BSIs) are the primary public health issue leading to self-limited illnesses that result in death globally. Both intravascular and extravascular infections can cause sepsis, and various fungal and bacterial species are capable of causing extravascular bloodstream infections.[5,6]

The World Health Organization reports that these infections affect 49 million people globally, leading to over 11 million deaths annually. Each year, the US reports an estimated 200,000 cases of bacterial and fungal bloodstream infections, with fatality rates ranging from 20 to 50%. Factors predisposing individuals to BSIs vary depending on age, gender, and underlying medical conditions. [7] Approximately half of all global sepsis cases occur in adolescents and children, with high child mortality rates in sub-Saharan Africa posing a significant challenge. In older populations, various medical treatments can also lead to BSIs, with factors such as diabetes, renal failure requiring dialysis, hepatic cirrhosis, broad-spectrum antibiotic usage, gender, age, and malignancy being primary risk factors. Decreased immunity, a more comprehensive range of medical treatments, and the spread of drug-resistant bacteria in crowded hospital settings raise the risk of BSIs among hospitalized patients. [8]

Bacterial infections are still the main cause of bloodstream infections (BSIs), but the occurrence of fungal infections has increased, with Candida being responsible for many cases. However, a wide range of organisms, including *Staphylococcus aureus*, *Enterococcus* species, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas* species, *Acinetobacter baumannii*, and various species of *Candida,* can cause BSIs. A systematic study on bloodstream infections in Ethiopia found that the occurrence ranged from 12.84% to 18.15%, with S. aureus and E. coli being the most frequently diagnosed bacteria. Blood culture remains the standard method for detecting bacteria and fungi in bloodstream infections. However, the misuse of antibiotics has exacerbated the situation by increasing the prevalence of microorganisms resistant to multiple treatments.[9]

In a literature review on non-healthcare toilet cleanliness, there's mainly a discussion of the bacteria associated with the faeces, or the skin, or both. On the other hand, Hospital-based studies have mainly concentrated on pathogens like Staphylococcus aureus and MRSA. [10] However, recent studies bring forth new perspectives that human associated bacteria might also contribute to the poop-free microbial diversity of public toilets which is normally linked to the cohorts of individuals. [11] To add on, other investigations done outside the hospital setting with varying microbial populations also discovered a high incidence of antibiotic-resistant bacteria in restrooms for public use. There are cells that show evidence and possibility of surviving or adapting to this type of environment even in the presence of nutrient starvation. [12]

Plants have over 8,000 polyphenolic chemicals; some polyphenols, including phloretin and quercetin, have amazing antiviral/antimicrobial action, stopping the adhesion and proliferation of bacteria and boosting immune responses. Apple trees are growing extensively in various climates worldwide, and apple trees (*Malus domestica*) are among the main fruits sold on the market. Among apples' most plentiful phenolic compounds are chlorogenic acid, phloretin, phloridzin, epicatechin, quercetin, and procyanidin B2. [13] We gather large quantities of dried twigs and apple leaves during the pruning season to improve the quality of the apple output and produce large-scale byproducts of tremendous value as polyphenolic chemicals with potent antioxidant capacity. The main chemicals in apples and products made from apples are phloretin and phloridzin (phloretin 2′-O-glucoside), which comprise more than 90% of the soluble phenolics in the leaves and barks.[14]

Furthermore, low-toxic or non-toxic flavonoids have enormous potential for treating antibiotic-resistant bacteria. Therefore, the current study aims to determine presence of antibiotic-resistant microbes found in public rest rooms and investigate the antibacterial action of apple leaf and bark extract against antibiotic-resistant bacteria obtained from public toilets.

2. Materials and Methods

Collection of Plants Material : Fresh specimens of leaves and bark, , were collected from Himachal Pradesh, Dharkot region (*(Coordinates 30.2792° N, 78.2092° E)* (**Figure 1**), from apple orchards (March, 2024) and authenticated using Burke Museum Herbarium Image Collection (on 25/March/2024) by Dr. Waseem Khan, Siddhartha Institute of Pharmacy, Dehradun.

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Figure 1 Collection of apple leaves and barks.

Preparation of extract: We sorted the plant samples into leaves and bark and then washed them in 70:30 ethanol. [15] We dried the plant samples in the shade for seven days. The dried samples were ground to a fine powder using a cutting mill at 1000 rpm with a 2 mm bottom sieve, reaching a final particle size of <1 mm. [16]

We combined 2 grams of finely powdered leaf powder and bark with 50 millilitres of ethanol, then individually soaked them in an airtight container. After that, they were periodically vortexed for 48 hours on a shaker set to 50 rpm. Using Whatman No. 1 filter paper, they filtered the solutions after 48 hours, collecting the filtrate in falcon tubes (**Figure 2**) and stored at 4°C. [17]

Figure 2 SOLVENT EXTRACTS OF APPLE LEAVES AND BARK

Site of Sample Collection

Samples were obtained and collected from different Restrooms in Dehradun (Coordinates- 30.3165° N, 78.0322° E) on a single day April (24 /04/2024)

Site 1 Public Restrooms at Rajpur Road and ISBT

Site 2 Public Restrooms at Hospitals at Shimla Bypass Road

Site 3 Public Restrooms at Clock Tower and Railway Station

Ten surfaces (door handles into and out of the restroom, handles into and out of a restroom stall, faucet handles, soap dispenser, toilet seat, toilet flush handle, floor around the toilet and floor around the sink) in four male and three female restrooms. [18]

Surfaces were sampled using sterile, cotton-tipped swabs as described previously. [12] A total of twenty-one swab samples to be collected from the seven rest rooms in all. The moistened swab sticks were used to swab each surface back and forth in zigzag manner and the swab stick was immediately returned to the vial. Samples were transferred in the sample collection tube in Normal Saline. [19]

Culture of isolated microbes: The dried surface of a sterile culture plate was inoculated by swab streaking over the entire sterile media surface (nutrient agar media, blood agar media and XLD Media) [13]. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.[14,20]

Characterisation of bacteria:

The isolated bacteria were identified by following standard microbiological methods as stated in Bergey's

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Manual of Systematic Bacteriology (Buchanan et al.,1970).[15, 21]

Biochemical characterization of pathogens

Different biochemical tests were performed for bacterial identification including IMViC (indole test, methyl red test, Voges-Proskauer test, citrate utilization test), sugar fermentation (glucose, lactose, sucrose, mannitol), motility, oxidation/fermentation, oxidase, catalase, urease, coagulase and triple sugar iron.[15,22]

Antimicrobial resistance detection by Disc Diffusion method:

The Kirby-Bauer disk diffusion susceptibility test aims to ascertain the susceptibility of facultative anaerobic and pathogenic aerobic bacteria to different antimicrobial agents. Susceptibility to antibiotics was determined by the DD method on media using a bacterial suspension with the turbidity adjusted to a 0.5 McFarland standard. Plates were incubated at 35 uC for 24 h. Results were interpreted according to CLSI (2005) guidelines.[18,23]

Antimicrobial activity detection by Disc Diffusion method of apple extracts:

The dried extracts were reconstituted to 20% in dimethyl sulfoxide (DMSO) to the final concentration of 100 mg/mL for the bioassay analysis. A 100 μL volume of each extract was propelled directly into the wells (in triplicate) of the inoculated agar plates for each test organism. The plates were allowed to stand for 1 h at room temperature (40∘C) for diffusion of the extract into agar and incubated at 37°C and 25°C for 24 h and 72 h, respectively. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone was greater than 8 mm. The experiments were performed in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated.[19,24]

3. Results and Discussion

The study's goals and objectives regarding microbiological air quality in toilets in Dehradun city are pertinent to public health and environmental microbiology. Initially, collecting and analysing samples from various microenvironments facilitate quantifying and assessing ambient microbial air quality. This data offers essential insights into the possible hazards associated with various toilet facilities and practices for bacterial and fungal contamination, including electric dryers, soap types, and contact surfaces. The research attempts to identify particular regions or activities that may facilitate microbial transmission and health concerns in public bathrooms by evaluating bacterial and fungal counts, percentage distributions, kinds of isolates, and their prevalence. Secondly, examining the antibiotic resistance profiles of isolated microorganisms is essential for understanding the extent and significance of antimicrobial resistance in a local setting. Antibiotic resistance constitutes a worldwide health issue, and research on environmental reservoirs of resistant microorganisms, such as public bathrooms, is essential. [25]

Understanding which medicines are effective against isolated bacteria is crucial for guiding antibiotic therapy and stewardship. This knowledge not only aids in the development of measures to prevent antibiotic-resistant pathogens but also enhances public health responses to microbial illnesses. The third aspect of our study, which explores the antibacterial properties of apple leaf and bark ethanolic extracts against isolated microorganisms, is particularly promising. This research aligns with the growing interest in natural antimicrobial options and suggests that these extracts could be used to develop new antibacterial agents or disinfectants for public toilets. By combining traditional medicinal plant knowledge with modern scientific methods, we are paving the way for sustainable urban microbial risk management solutions. [26]

Our work on microbial air quality and antibiotic resistance in public toilets is not just about scientific discovery; it's about contributing to a larger discourse on environmental microbiology and antimicrobial treatments. By integrating these findings with public health policy and practice, we aim to enhance sanitation and health in urban areas like Dehradun. The studies show that ethanolic extracts of apple leaves and bark can kill bacteria, and they also reveal the extent of antibiotic resistance in bacteria like Salmonella, E. coli, and Klebsiella. In the face of germ resistance and the search for effective therapies, these discoveries are invaluable. They underscore the importance of our collective efforts in multidisciplinary microbiological research and the potential of plant-derived chemical therapeutics in combating antibiotic resistance. [27]

We can see different types of Gram-positive (Bacillus spp.) and Gram-negative (Salmonella, Escherichia coli, and Klebsiella) bacteria from different sources by using Gram staining and biochemical identification (Table 1 and Table 2). Each species has distinct biochemical features that help identify it and determine its pathogenic potential and treatment alternatives. Biochemical experiments demonstrate that Salmonella spp. and Escherichia coli have different metabolic pathways and may be susceptible to certain antimicrobials. In phytochemical studies (Table 3), apple leaf and bark ethanolic extracts include alkaloids, flavonoids, and phenols. Pharmaceutically, these chemicals are

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antioxidant, antibacterial, and anti-inflammatory. These contents suggest that apple extracts may treat bacterial infections and require further study of their mechanisms of action. These extracts selectively inhibit Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi (Table 6). Apple leaf ethanolic extract inhibits Pseudomonas aeruginosa, showing its potential as a natural antibacterial. [28]

Apple bark extract is somewhat active against Salmonella typhi. These findings show that various bacteria respond differently to plant extracts and recommend tailored infection treatment. Meanwhile, Salmonella, E. coli, and Klebsiella (Tables 3, 4, and 5) show worrying drug resistance tendencies. Multiple antibiotic classes show resistance, suggesting multidrug-resistant strains in these bacterial populations. These discoveries help us comprehend microbial pathogenesis, antibiotic resistance, and plant-derived chemical therapeutics. In light of antibiotic resistance, they emphasize the significance of multidisciplinary microbiological research, combining phytochemistry, microbiology, and pharmacology to find new therapies. To improve clinical results, further research should clarify these plant extracts' modes of action and possible synergies with conventional antibiotics. [15,20]

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