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| RESEARCH ARTICLE

Concentration-Dependent Antimicrobial Activity of Neem and Bitter Gourd Extracts: A Sustainable Approach to Mitigate Pollution in Dhaka

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| ABSTRACT

Air pollution in Dhaka city is increasingly severe, surpassing levels observed in many comparable urban areas. As lifestyle and dietary patterns shift, the selection of health-promoting vegetables and natural food components has become particularly important. Bitter gourd and neem extracts possess well documented antibacterial properties and are associated with improved liver and kidney function. Chronic exposure to polluted air heightens the risk of respiratory complications, a vulnerability further intensified by smoking. Therefore, avoiding tobacco use and incorporating natural protective foods into the daily diet may help strengthen immunity and mitigate the adverse health effects of rising air pollution.Aqueous leaf extracts of Azadirachta indica (neem) and Momordica charantia (bitter gourd) were tested against road dust particles collected from Dhaka city. Both extracts showed strong antimicrobial and antifungal activity and Gram staining confirmed the presence of mixed microbial species in Dhaka air pollution. A biochemical study involving 20 people from Rajshahi and 20 long term Dhaka residents indicted that the extract alone or combined, may help improve kidney, liver and lipid parameters. Chest X rays of six Dhaka residents also showed signs of respiratory effects linked to polluted air. These findings highlight the antimicrobial potential of the extracts and health risks associated with microbially contaminated urban air. The aim of this study is to evaluate the antibacterial and antifungal activities of aqueous extracts of Azadirachta indica (neem) and Momordica charantia (bitter gourd) and to assess their potential as dietary options for people in living Dhaka city to help counter pollution related health effects and support lung, liver and kidney function.

| KEYWORDS

Bitter gourd, Neem, pollution protection, Natural remedy, Urban pollution, City health

| ARTICLE INFORMATION

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Introduction

Air pollution is a major environmental issue affecting urban areas worldwide, with severe consequences for human health, climate and ecosystem. Dhaka, capital of Bangladesh, consistently ranks among most polluted cities due to rapid urbanization, industrial expansion and vehicular emissions. [1] The excessive release of particulate matter PM2.5, PM10, Carbon monoxide, nitrogen oxides, sulfer dioxide and volatile organic compounds has significantly degraded air quality, leading to an increase in respiratory diseases, cardiovascular disorders and other health complications. [2] chemical disinfectants are commonly used to control microbial contamination in environment but excessive use can cause environmental hazards and contributed to antimicrobial resistance. [3] With growing concerns over adverse effects of synthetic chemicals, there is a pressing need for sustainable and eco-friendly alternatives. Traditional medicinal plants have long been utilized for their therapeutic and antimicrobial properties. Among these, *Azadirachta Indica* (Neem) and *Momordica Charantia* (bitter gourd) have shown remarkable efficacy in controlling microbial growth and eliminating airborne pathogens. [4 The initiating events in thrombus formation involve platelet activation, aggregation and adherence. Circulating microparticles, cell fragments smaller than 1 μm released from cells in response to injury, activation or apoptosis are thrombogenic and can express tissue factor, an important initiator of the coagulation cascade. [5] People with type 2 diabetes may be particularly susceptible to cardiovascular effects of air pollution because they have accelerated development of atherosclerotic vascular disease and its complications, mediated in part by enhanced oxidative stress. [6] Diabetes is associated with baseline platelet activation and increased platelet responsiveness to agonists. Some air pollution epidemiology studies indeed suggest that diabetes confers an increased risk for health effects from air pollution. [7] Exposure to air pollution has a significant detrimental impact on health, particularly regarding blood biomarkers. Epidemiological studies indicate a strong association between air pollution and risk of developing cardiovascular diseases, respiratory conditions, neurological disorders, and metabolic dysfunctions. [8] Research has shown that air pollution exposure can significantly affect liver functions related blood markers. [9] For instance, established a significant correlation between gamma-glutamyl transferase (GGT) levels and PM2.5 exposure, with GGT serving as a marker of oxidative stress. Discovered positive associations between exposure levels of PM2.5, PM10, nitrogen dioxide (NO2), sulfur dioxide (SO2), CO and ozone (O3) and alanine aminotransferase (ALT). Specifically, PM10 exposure was positively correlated with aspartate aminotransferase (AST) while CO and O3 exposure exhibited a negative correlation with AST. Identified a significant relationship between exposure to air pollutants and logarithmic increase in ALT and AST levels, particularly noting that long term exposure to PM10 and CO is a risk factor elevated liver function in alcoholic adults. [10] Neem contains bioactive compounds such as azadirachtin, nimbin and quercetin which exhibit strong antibacterial, antifungal and antiviral properties. [11] Bitter gourd, rich in flavonoids, phenolics and alkaloids has been recognized for its potent antimicrobial and antioxidant effects. [12] Studies have demonstrated effectiveness of these plant extracts in inhibiting bacterial growth and enhancing air purification. [13] WHO announced that 99% of world's population breathes air that does not meet their guidelines for good air quality, emphasizing effects this can have on populations health and death related disease. This prompted creation of clean air (Human Rights) Bill, intended to protect right to breathe clean air and increase of various campaigns to promote lowering of emissions. [14] This study aims to investigate antimicrobial potential of neem and bitter gourd extracts and evaluate their application as natural disinfectants for air quality improvement and public health benefits.

Materials and Methods:

(I) Plant Material Collection and Authentication

Fresh leaves of *Azadirachta Indica* (Neem) and *Momordica Charantia* (Bitter gourd) were collected from local market in Bangladesh in October 2025. The plans specimens were authenticated by taxonomic department of Botany, University of Dhaka, under code CPP/DU-03-2025. The collective leaves were washed, blended separately and stored in tightly sealed containers at refrigeration's temperature (5 ℃) for future use. [15]

(II) Extract Preparation

Fresh leaves (300 g) of *A. indica* and *M. Charantia* were washed with potable water and cut into small pieces. The leaves were blended together with a small amount of distilled water to obtain a homogenous mixture. The extract was filtered and the filtrate was evaporated using a rotary evaporator. The final yield of the aqueous extract was 42g which was stored at 5℃ until further analysis.[16]

(III) Preparation of Extract Concentrations for Antibacterial Assay

A serial dilution method was used to determine the minimum inhibitory concentration (MIC) of each extract. A total of 100 mg of the crude extract was dissolved in 5mL of sterile distilled water, followed by serial dilution to obtain different concentrations.

(IV) Antibacterial Activity Assessment by Disc Diffusion Method

The antibacterial activity of the plant extracts was evaluated using the Kirby-Bauer disc diffusion method. Filter paper discs were sterilized and impregnated with different concentrations of the extracts. The discs were aseptically placed on agar plates inoculated with test bacteria. The plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured in centimeters. [17]

(V) Preparation of Test Samples

Aqueous extracts (100 mg) were accurately weighed and dissolved in 5 mL of distilled water to obtain a final concentration of 20 µg/µL.

(VI) Application of Discs

Two types of discs were used for antibacterial screening:

- **Sample Discs:** Impregnated with 200 µg/µL and 300 µg/µL extract concentrations.
- **Standard Antibiotic Discs:** Kanamycin (30 µg/disc) and Ciprofloxacin (30 µg/disc) [19].

(a) Sample Disc Application Sterile filter paper discs were placed on agar plates using sterile forceps. Each disc was impregnated with 10 µL of the test sample (20 µg/µL) under aseptic conditions. Two additional discs containing 25 mg and 40 mg of crude extract were applied [20].

(b) Standard Disc Application Kanamycin (30 µg/disc) and Ciprofloxacin (30 µg/disc) were used as positive controls. The antibiotic discs were placed on the labeled sections of the Petri dishes. The plates were incubated at 37°C for 16–18 hours, and antibacterial activity was assessed by measuring the inhibition zones [21].

(VII) Blood Sample Collection & Biochemical test

The biochemical assessment was conducted at a diagnostic laboratory in Dhaka city and outside of Dhaka city specially Rajshahi city. A total of 20 participants who had been residing in Dhaka for at least one year were enrolled in the study. Biochemical parameters were measured at baseline (before extract consumption) and again after 20 days of daily intake of combined plant extracts. To minimize potential confounding factors, individuals with hematological disorders, pregnancy or any known malignancy were excluded from study. Only participants without chronic illness and willing to comply with study procedure were included. Demographic and clinical information, including age and gender was recorded. Laboratory investigations performed for each participant included fasting blood glucose (FBG), Random blood sugar, Liver function markers (SGPT, SGOT and bilirubin) and lipid profile components (total cholesterol, HDL, LDL and triglycerides). [22] All biochemical tests were conducted following standard laboratory protocols.

(VII) Hematological Analysis

Complete blood count (CBC) analysis was performed using a Sysmex XP-100 fully automated hematology analyzer. The analyzer generated values for white blood cells (WBC), Red blood cells (RBC), Hemoglobin (Hb) Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), plateletcrit (PCT) and differential leukocyte counts (lymphocytes, neutrophils, basophils and eosinophils). To verify hematological parameters, peripheral blood smears were prepared using Leishman stain and examined microspically with an Olympus microscope. Although the instrument provides all CBC parameters, hemoglobin concentration was primarily used for routine screening of chosen Dhaka and Rajshahi

city person where an Hb level below 9.0g/dL was flagged for repeat testing. The same EDTA anticoagulated blood samples were also utilized for glycated hemoglobin (HbA1C) measurement, which was performed using a Dimension ExL-200 fully automated chemistry analyzer following standard operating procedures. [23]

(VIII) X-ray Analysis:

Chest X-ray images were obtained using a Somatom DRH system (Siemens, Erlangen, Germany) without contrast administration. Scans were performed at 10-mm intervals with 2-mm slice thickness, using 310 mAs, 125 kVp and imaging time of 4 seconds. Images were reconstructed using a 512x512 matrix with a bone reconstruction algorithm. Standard parenchymal (1600/400) and mediastinal (350/50) windows settings were used for image evaluation. Each X-ray scans was examined for the presence, distribution and extent of radiological abnormalities, including ground glass attenuation, nodular high attenuation areas, consolidation, non-septal linear opacities, Septal lines, honeycombing and architectural distortion. The extent of involvement was assessed separately in three anatomical thoracic zones. The upper zone was defined as region above the main carina, the middle zone between the main carina and the inferior pulmonary veins and the lower zone below the inferior pulmonary veins. Disease extent in each zone was visually estimated and scored accordingly. In cases with X-ray abnormalities, prone-position imaging was performed to confirm interstitial involvement. All X-ray scans were reviewed in random order by two radiologists blinded to clinical data. The radiologists evaluated the images jointly and reached a consensus for each case. [24]

(IX) Data Analysis:

Statistical analyses were performed using IBM SPSS Statistics version 20 (IBM Corp, Chicago, IL, USA). Descriptive statistics were generated for all biochemical and hematological parameters and results were presented as mean± Standard Deviation (SD). Comparative analyses were conducted to evaluate differences in fasting blood sugar (FBS), Random blood sugar (RBS), HbA1c, HDL, LDL, total cholesterol, fasting triglycerides (TG), SGPT, SGOT, bilirubin, creatinine, platelet count, WBC, hemoglobin (Hb%), mean platelet volume (MPV), platelet distribution width (PDW) and hematocrit (HCT). Binomial logistic regression analysis was used to explore independent associations between the measured parameters. Positive and negative predictive values were calculated using established cut off points for each test variable. A p-value <0.05 was considered statistically significant.

Results and Discussion

(I) Determination of Zone of Inhibition

After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in centimeters using a scale.

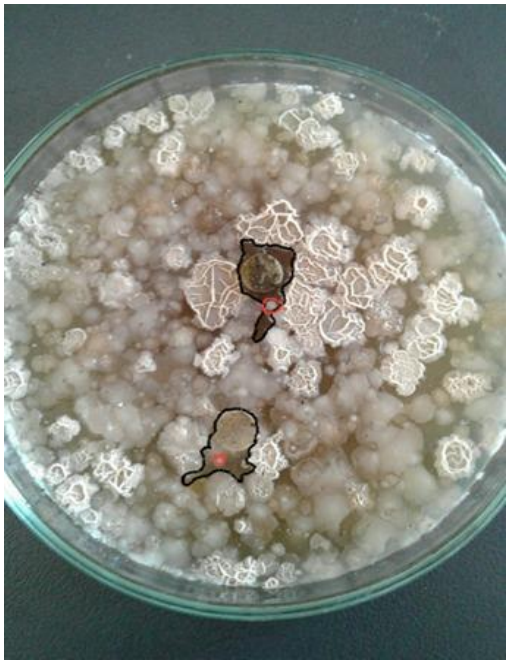
(II) Observations

Application of Extracts on Airborne and Road Particles

The antibacterial potential of *Azadirachta indica* and *Momordica charantia* leaf extracts was tested against airborne and road contaminants. The formation of inhibition zones around the applied extracts indicated microbial growth suppression.

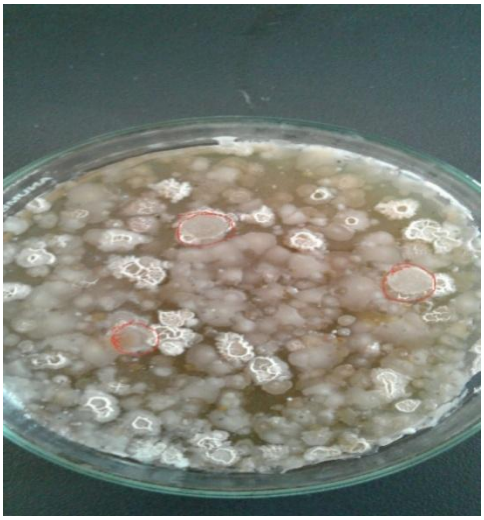
Figures 1.1: Applying 25mg and 200µg extract on road particles

Figures 1.2: Applying 25mg and 200µg extract on contaminated air.

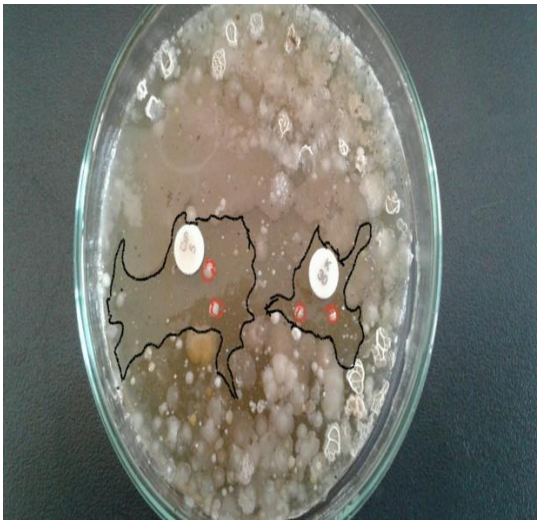


Figures 1.3: Application of 40 mg and 300 µg extract on road particles.

Figures 1.4: Application of 40 mg and 300 µg extract on contaminated air.



Figures 1.5: Applying 20µg, 40µg and 60µg extract on the contaminated air.



Figures 1.6: Applying 30µg of Ciprofloxacillin and 30 µg of Kanamycin antibiotics on the contaminated air.

In the images, the **black areas** represent the **zones of inhibition**, indicating microbial suppression, while the **red areas** denote microbial growth.

Table 1.1: In vitro Antibacterial Activity of water extract of *Azadirachta indica* and *Momordica charantia* leaves.

Bacterial strains	Extract (mg)	Standard (Kanamycin) 30 µg	Standard (Ciprofloxacillin) 30 µg	Diameter of zone of inhibition in cm
Conatminated air	25	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	1.2 cm (long) 2 cm (wide)
Particle of road	25	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	1.2 cm (long) 0.7 cm (wide)
Contaminated air	40	1.5 cm (long)	1.8 cm (long)	3 cm (long)

		0.7 cm (wide)	1 cm (wide)	0.5 cm (wide)
Particle of road	40	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	1.2 cm (long) 0.7 cm (wide)

Table 1.2: In vitro Antibacterial Activity of water extract of *Azadirachta indica* and *Momordica charantia* leaves.

Bacterial strain	Extract (µg)	Standard (Kanamycin) 30 µg	Standard (Ciprofloxacillin) 30 µg	Diameter of zone of inhibition in cm
Contaminated air	200	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	Growth
Particle of road	200	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	Growth
Contaminated air	300	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	1.2 cm (long) 0.6 cm (wide)
Particle of road	300	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	0.75 cm (long) 0.8 cm (wide)
Contaminated air	20,40,60.	1.5 cm (long) 0.7 cm (wide)	1.8 cm (long) 1 cm (wide)	Growth

(III) Antibacterial Activity of Aqueous Extracts

The antibacterial activity of the aqueous extracts of *A. indica* and *M. charantia* was assessed based on the inhibition zone measurements. The extract concentration-dependent response was observed for both airborne and road particles. This observation screen is placed in figure 1.1 to 1.6 and the data was measured and placed in Table 1.1 and 1.2.

(a) Zone of Inhibition Measurements for Extracts (mg scale)

The antibacterial activity of Azadirachta indica and Momordica charantia leaf extracts was evaluated based on the zone of inhibition observed at different concentrations. In the mg scale, the antibacterial effect was more pronounced at higher concentrations (40 mg) compared to lower concentrations (25 mg). Although standard antibiotics such as Kanamycin and Ciprofloxacin exhibited stronger inhibition, the plant extracts still demonstrated significant antibacterial properties. Among the tested samples, the highest inhibition was observed in the contaminated air samples at 40 mg extract concentration.

(b) Zone of Inhibition Measurements for Extracts (µg scale)

In the µg scale, the inhibition zone increased with extract concentration, with 300 µg showing greater antibacterial activity than 200 µg. However, at lower doses (20–60 µg), microbial growth was still present, suggesting that these concentrations may not be effective against the tested bacteria. While standard antibiotics exhibited superior inhibition, the plant extracts still demonstrated notable antibacterial effects, indicating their potential as natural antimicrobial agents.

(c) Gram Stain Test Observation

Gram staining of the collected air samples revealed the presence of both Gram-positive and Gram-negative bacteria, indicating significant microbial contamination in the urban atmosphere. Which is shown in figure 2.1.

Figure 2.1: Gram stain test results showing a mixed population of bacterial species, confirming the environmental diversity of microorganisms in airborne contaminants.

(IV) Biochemical and Hematological parameter observation:

A total of 40 participants were included in this study, comprising both males and females, with ages ranging from 15 to 42 years. Among them, 20 individuals were recruited from Rajshahi, and 20 had been residing in Dhaka city for at least one year. Participants underwent a series of clinical evaluations, including liver function tests, kidney function tests, fasting blood sugar, and diabetes-related parameters, using a fully automated biochemistry analyzer. The average values of these parameters were calculated using IBM SPSS, and binomial logistic regression analysis was performed to assess statistical significance. Additionally, complete blood count (CBC) parameters were analyzed using the Sysmex XP-100 system, focusing specifically on white blood cell count (WBC), hemoglobin (Hb%), mean platelet volume (MPV), platelet distribution width (PDW), hematocrit (HCT), and mean corpuscular volume (MCV). The results indicated that both *Azadirachta indica* and *Momordica charantia* leaf extracts were associated with improvements in liver and kidney function markers, as well as several immunological parameters. Furthermore, the extracts demonstrated potential in maintaining blood glucose levels within normal ranges, suggesting possible benefits for glycemic control. Detailed numerical data for all measured parameters are presented in Table 2. Overall, these findings suggest that the administration of these plant extracts may have a supportive role in enhancing liver, kidney, and immune system functions, as well as in managing blood sugar levels. According to hematological analyses, individuals residing in Dhaka exhibited comparatively elevated liver and kidney function parameters, as well as other hematological markers, likely due to environmental pollution. Only 12 patients were able to consume Azadirachta indica and Momordica charantia leaf extracts, either alone or in combination, for 20 days. Following the intervention, their hematological profiles and liver function parameters showed improvement, indicating a reduced health risk associated with pollution exposure in Dhaka city.

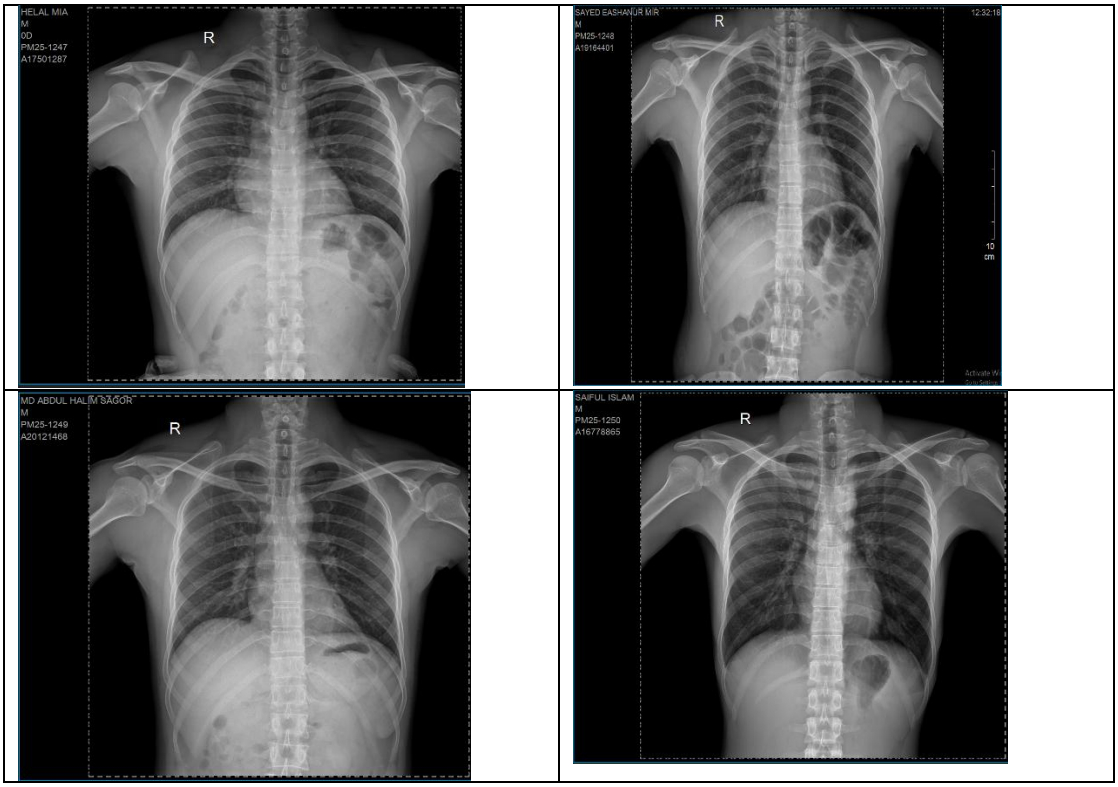
Table: 2 Biochemical and Hematological Analysis of Outside Dhaka, inside Dhaka and consultant people using bitter gourd and neem extract:

Characters/ parameter	People outside Dhaka	People Inside Dhaka	Consult to take Neem Extracts	Consult to take Bitter Gourd Extract	P=significance
Ages	15 to 42	15-40	12 persons	12 persons	0.062
Female/Male	5 female 15 male	5 female 15 male	2 female 10 male	1 female 11 male	0.061
FBS	5.1 mmol/L	6.4 mmol/L	5.3 mmol/L	5.4 mmol/L	0.211
RBS	6.2 mmol/L	6.8 mmol/L	5.6 mmol/L	6.0 mmol/L	0.016
HbA1C	5.2%	6.6%	5.9%	5.8%	0.201
HDL	34 mg/dl	32 mg/dl	52 mg/dl	49 mg/dl	0.211
LDL	113 mg/dl	119 mg/dl	96 mg/dl	99 mg/dl	0.012
Cholesterol	182 mg/dl	196 mg/dl	190 mg/dl	191 mg/dl	0.211

Fasting TG	119 mg/dl	131 mg/dl	125 mg/dl	130 mg/dl	0.210
SGPT	65 U/L	96 U/L	48U/L	56 U/L	0.019
SGOT	38 U/L	49 U/L	32 U/L	29 U/L	0.116
Bilirubin	0.04 mg/dl	0.6 mg/dl	0.05 U/L	0.04 U/L	0.211
Creatinine	1.13 mg/dl	1.19 mg/dl	1.03 mg/dl	1.06 mg/dl	0.211
Platelet count	212,000/cumm	189,000/cumm	342,000/cumm	348,000/cumm	0.018
WBC	6,900/cumm	7,800/cumm	4,900/cumm	3,700/cumm	0.219
Hb%	16 g/dl	14.2 g/dl	13.9 g/dl	15.2 g/dl	0.116
MPV	11.6 fL	12.4 fL	10.8 fL	11.1 fL	0.019
PDW	8.9%	9.2%	10.9%	14.2%	0.211
HCT	37.6%	42.6%	35.6%	33.6%	0.210
MCV	70.8 fL	80.2 fL	78.9 fL	76.2 fL	0.211

(V) **Chest X-ray report observation for lung examination as association of polluted city air response:**

Chest X-rays were performed on 12 participants to evaluate potential respiratory benefits following the use of *Momordica charantia* (Bitter gourd) and *Azadirachta indica* (Neem) leaf extracts, administered individually or in combination. Radiographic analysis indicated observable improvements in lung development and structure among the participants. Notably, 6 of the X-ray films demonstrated marked enhancement in lung capacity and overall pulmonary health, suggesting that these plant extracts may contribute to improved respiratory function. These findings highlight the potential applicability of these extracts in supporting lung health, particularly in urban environments such as Dhaka, where air pollution and respiratory challenges are prevalent.



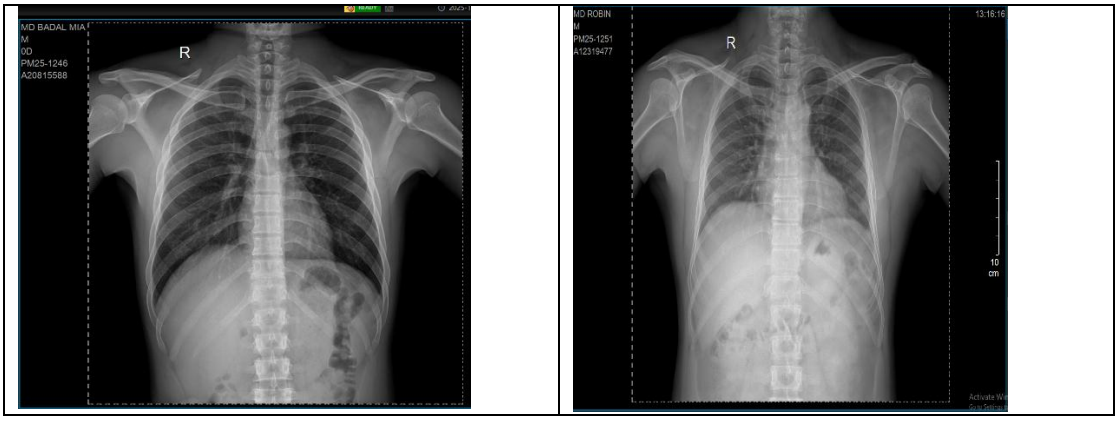


Figure 3 People consultant with bitter gourd and need extract use for month.

Discussion:

The findings of this study reveal a concerning level of microbial contamination in the urban atmosphere, with both Gram-positive and Gram-negative bacteria present in significant amounts. The predominance of Gram-negative bacteria is particularly noteworthy due to their well-known pathogenic potential and the health risks they pose to densely populated communities. Against this backdrop, the antibacterial evaluation of *Azadirachta indica* (Neem) and *Momordica charantia* (Bitter melon) leaf extracts demonstrated promising results. [25] Both plant extracts exhibited inhibitory effects against environmental pathogenic microorganisms. The antibacterial activity, as reflected by the diameters of inhibition zones, clearly increased with rising extract concentrations, indicating dose-dependent efficacy. These results support previous research highlighting the antimicrobial potential of commonly available medicinal plants and emphasize their relevance as natural, eco-friendly agents for environmental management. Given their antimicrobial capabilities, the integration of these plants into urban planning may offer an innovative approach to mitigating airborne microbial pollution. Tree-planting initiatives involving A. indica and M. charantia could contribute to cleaner air and improved environmental conditions. Furthermore, the use of blended leaf extracts by municipal authorities—for example, in road washing after rainfall—may help reduce microbial load on urban surfaces. Alongside their environmental benefits, these plants are also widely recognized for their nutritional and therapeutic value. Although anecdotal observations suggest improvements in liver, kidney, and lung function following regular consumption, such claims warrant rigorous clinical validation. [26] Nonetheless, promoting the availability and affordability of these plants could encourage their integration into daily diets, potentially supporting general well-being, especially among low-income populations.

Conclusion: This study highlights the dual significance of *Azadirachta indica* and *Momordica charantia* as both environmental and nutritional resources. Their demonstrated antibacterial activity suggests that they may serve as natural agents for reducing microbial contamination in urban air. Incorporating these plants into large scale greening programs and exploring practical applications of their extracts could form part of a sustainable strategy to combat rising pollution levels. To support public health, government initiatives should aim to increase the accessibility and affordability of these valuable plant species, enabling broader use in daily diets and community health programs. Ultimately, harnessing the antimicrobial and ecological benefits plants can contribute to building a greener, healthier and more sustainable urban environment specially like polluted city of Dhaka.

Data Availability

Not Applicable

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Compliances with Ethical Standards

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Contributions

The first draft of the manuscript was originally written by Tamanna Rashid as well as Data curation, Investigation, Methodology and Dipongkar Ray Sobuj & Md Maniruzzaman also contributed same category. Md Mahfuz Miah, Saiful Islam Arif, Md. Rakib Rased Rana made table arrangements, data collection and analysis, Methodology, Writing – review & editing, figure design and material preparation. Sabiha Akter, Kaisar Ahmad Chowdhury, Md. Karim Hossain, Aklima Akter Shima, Md Tarikul Islam Tarek, Mst. Tasnova, Md. Sadikuj Jaman worked as data collection and analysis, Methodology, Writing – review & editing. All authors read and approved the final manuscript.

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Ethics declarations

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Competing interests

The authors declare no competing interest

Patient consent for publication

Not Applicable

Ethical approval and consent to participate

Not Applicable

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