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# Use of Common Herbs to Control Microbial Aerosols in Toilet Rooms

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Four distinct plant species were used in an investigation into the capacity of plant active volatile essence to eradicate microorganisms in the atmosphere (bioaerosols) in restrooms: *Allium sativum* (garlic), *Allium cepa* (onion), *Zingiber officinalis* (ginger), and *Ocimum gratissimum* (scent leaf). The study was conducted in the student restrooms for microbiology at Rivers State University Nkpolu Oroworukwu in Port Harcourt. Sedimentation method of aerosol sampling was used to collect the air samples. Artificial culture media were exposed to air at different times, including nutrient agar for total heterotrophic bacteria, Mac Conkey agar for enteric bacteria, sadour and dextrose agar for aerobic fungi, thiosulfate citrate bile salt sucrose agar for vibrio, Cystine lactose electrolyte deficient agar for urinary pathogens, and mannitol salt agar for staphylococcus.
Samples was taken from the toilet without any mashed plant and served as a control. The THB isolates identified were *Bacillus spp, Pseudomonas* sp*, Staphylococcus* sp*, Lactobacillus* sp*,*

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*Enterococcus* sp, *Corynobacterium sp, Escherichia coli, Klebsiella pneumonia.* While the fungi isolated were *Aspergillus niger*, *pennicillum* sp, *Rhizopus* sp, *Saccharomyces* sp, *Mucor* Sp, *Candida* sp*, Asperillus flavus*, *Fusarium* sp. It can be deduced that these medicinal herbs has the ability in reducing microbial aerosol using ginger, onion, scent leaf, garlic also by combination of the herbs which are Onion & garlic, Onion & garlic, Onion & ginger, Garlic & scent leaf, Garlic & ginger, Scent leaf & ginger, Onion, garlic & scent leaf, Onion, garlic & ginger, Garlic, Scent leaf & ginger, Onion, garlic, scent leaf & ginger. Specifically the microbial aerosols in this investigation including bacteria and fungi were *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Lactobacillus* species, *Enterococcus* species, *Corynobacterium*, *Escherichia coli*, *Klebsiella pneumonia*. *Aspergillus niger*, *pennicillum* species, *Rhizopus* species, *Saccharomyces* species, *Mucors* species, *Candida* species*, Asperillus flavus*, *Fusarium* species. This work proved that microbial load can be reduced using natural means (plant extract).

*Keywords: Bioaerosols; microorganisms; mashed plants; public health; microbial load; control.*

## 1. INTRODUCTION

Microorganisms are ubiquitous in the environment. Wherever their sources are present, the particles can be released into the air forming microbiological aerosols. Although most of their particles cause no harm to the exposed individuals, some of their propagules may have infectious or allergenic potential and may carry toxic or irritant substances and components. Their inhalation usually poses a significant health risk and is responsible for numerous adverse outcomes, from allergic reactions, infections and toxic responses to various nonspecific symptoms [1].

Microbial aerosols (i.e. airborne particles of microbiological origin) are usually naturally present in the environment. They are ubiquitous both indoors and outdoors. Their environmental presence is associated with different geographic regions, climate zones, continents or populations of plants and animals. Their major outdoor sources are located on the earth surfaces and are formed by continental (soils, plants including crops and forests, wetlands, deserts, land ice, urban, etc.) as well as natural and anthropogenic water reservoirs [2].

Microbial aerosol sources are also widespread in indoor environments. They can derive from industrial and nonindustrial settings and differ significantly in terms of their emission efficiency. In the first case, the most effective occupational aerosolization processes (being responsible for microbial aerosol concentrations up to 1012 cfu m–3) are: silo loading/unloading, animal feeding in broiler houses, piggeries as well as different dust-releasing tasks in composting plants, granaries, animal food stores, malthouses, and reloading of stored moldy raw materials. Against this background, non-industrial indoor sources are less productive and usually closely connected with the presence and physical activity of humans (including numerous physiological processes such as breathing, talking, sneezing, coughing or scratching as well as movement and dust, including microbial dust residues, resuspension). Such types of emissions are usually able to create microbial concentrations of about 103 cfu m–3; however, some chamber bioaerosol studies revealed that even one person under seated conditions is able to release up to 106 biological aerosol particulates per hour into the air and the origin of such a microbial cloud can be assigned to the individual that emits it [3]. Also, indoor water reservoirs such as aquariums, toilets, sinks or even washing machines may load the air with high numbers of both saprophytic and pathogenic microorganisms. Such emissions (reaching usually 103 –104 cfu m–3) may result not only in contamination of surrounding surfaces but pose a real threat to exposed individuals through inhalation of different pathogens (including Bacillus, Aeromonas, Campylobacter, Clostridium, Escherichia, Klebsiella, Staphylococcus, Salmonella, Pseudomonas, Serratia, Shigella bacterial genera and mould) (Best et al. 2012), [4,5]. Microorganisms are essential to human survival, health and disease, and hence their environmental abundance and diversity are of great practical importance [6].

A look at the world of organisms through their genomes has reformulated our perception of the natural system. Life's diversity seen as comprising 3 domains, i.e. bacteria, archaea and eucarya, has been dramatically broadened taking into account both community and ecosystem interrelations [7].

## 2. MAJOR GROUPS OF ANTIMICROBIAL COMPOUNDS FROM PLANTS

I. Terpeniods and Essential Oil.
II. Quinones Flavones,
III. flavonoids, and flavonols

### 2.1 Terpeniods and Essential Oils

Terpenenes or terpenoids are active against bacteria, viruses and protozoa. In 1977, it was reported that 60% of essential oil derivatives to date were inhibitory to fungi while 30% inhibited bacteria. Chile peppers are a food item found nearly ubiquitously in many Mesoamerican cultures, the evidence for its antimicrobial activity is mixed. Recently, Cichewicz and Thorpe found that capsacin might enhance the growth of *candida albicans*but that it clearly inhibited various bacteria to differing extents. Although possibly detrimental to the human gastric mucosa, capsaicin is also bactericidal to *Helicobacter pylori.* Another hot-tasting diterpene, aframodial, from a Cameroonian spice, is a broad-spectrum antifungal [8].

### 2.2 Quinones

Quinones are aromatic rings with two keton substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetable and are an intermediate in the melanin synthesis pathway in human skin. Their presence in henna gives that material its dyeing properties. Kazmi *et al*. described an anthraquinone from cassia italic, a Pakistani tree, which was bacteriostatic for *Bacillusanthracis, Corynebacte-riumpseudodiphthericum*, and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*. Hyericinanantraquinne.

### 2.3 Flavones, flavonoids, and flavonols

Flavones are phenolic structure containing one carbonyl group. The addition of 3-hydroxyl group yields a flavonol. Flavonoids are also hydroxyl phenolic substance but occur as a $C_6$-$C_3$unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substance against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as descriebed for quinines. More lipophilic flavonoids may also disrupt microbial membranes [9].

Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from licorice), and chrysin against HIV [10]. More than one study has found that flavone derivatives are inhibitory to respiratory syncytial virus (RSV). Kaul et al. [11] provide a summary of the activities and modes of action of quercetin, naringin, hesperetin and catechin in vitro cell culture monolayers. While naringin was not inhibitory ot herpes simplex virus 1(HSV-1), poliovirus type 1, parainfluenzavirustyoe 3, or RSV, the other three flavonoids were effective in various ways. Hsperetin reduced intracellular replication of all four viruses; catechin inhibited infectivity but not intracellular replication of RSV and HSV-1; and quercetin was universally effective in reducing infectivity. The authors proposed that small structural differences in then compounds are critical to their activity and pointed out another advantage of many plant derivatives: their low toxic potential. The average Western daily diet contains approximately 1g of mixed flavonoids; pharmacologically active concentrations are not likely to be harmful to human hosts [5].

## 3. MATERIALS AND METHODS

### 3.1 Study Area

The study area was Port Harcourt Metropolis, Rivers State, Nigeria. Toilets of the microbiology department of Rivers State University Nkpolu-Oroworukw (4.8522622, 6.9896428° E) selected because the high rate of human activities by the students.

### 3.2 Toilet Air Sample Collection

The bioaersols can be expelled from the air within the bowl of the toilet depending on an upward velocity of air from flushing, and eventually be transmitted by air motion indoor. The separate toilet compartment was at least 36x66 inches with a swing-out door. During sample collection 15 grams of onion, ginger, garlic and scent leaf was cleaned and mashed separately to expose vital surface area of the plants. This exposure allowed the volatile plants extracts to volatilize into the toilet air. The mashed plants were placed in a sterile disposable plate and then place in the toilet was

the sampling took place. The method of sampling used in this investigation is the direct sedimentation method of aerosol sampling which involves the aseptic exposure of six different growth media including Sabouraud dextrose agar (for fungi), Nutrients agar (for Heterotrophic bacteria), Mac Conkey agar (for enteric bacteria), Thiosulfate citrate bile salt sucrose agar (for vibrio), Cystine lactose electrolyte deficient agar (Urinal pathogen) and Mannitol salt agar (for *Staphylococcus)* to the environment air*.* The agar plates used for the investigation were prepared in duplicates in the laboratory and transported to the point of sample collection aseptically. The exposure was done at different time intervals for each toilet ranging from 0 hour. 15 minutes, 30 minutes, 60 minutes, 120 minutes, 180 minutes with plant mashed (i.e., toilets with scent leaf, garlic, ginger and onion present including control) sufficiently exposing enough surface area in the toilet (the control sample was done without any plant). During the time of sampling the toilet were off limit by users for a period of 4 hours to prevent inaccurate sample collection as a result of flushing and agitating the toilet water thereby emitting more aerosols [12].

## 3.3 Microbiological Analysis

### 3.3.1 Characterisation and Identification of bacterial and Fungi Isolates

Discrete colonies were picked based on their cultural, morphology, macroscopic and microscopic examinations and biochemical tests. The isolate were subculture on solid NA and SDA and subsequently on slants of the respective agar media and preserved at refrigeration temperature. Identification of the isolates as bacteria and fungi was carried out as described in Cheesbrough et al. [13].

**Analytical formula**

The formula used in calculating for the colony forming unit is:

$$\frac{\text{Cfu}}{\text{mins}} - \text{m2} = \frac{\text{no of colonies}}{\text{Time of Exposure (min) x } \pi r2}$$

Where
r = radius of media plate used (In meters).

## 4. RESULTS

A total of 8 bacteria isolates were isolated from the sampling station. The bacteria isolates were *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Lactobacillus* species, *Enterococcus* species, *Corynobacterium*, *Escherichia coli*, *Klebsiella pneumonia*. Fungal isolate was *Aspergillus niger*, *Pennicillum* species, *Rhizopus* species, *Saccharomyces* species, *Mucor* species, *Candida* species*, Asperillus flavus*, *Fusarium* species.

## 4.1 Results for Biochemical Identification of Bacteria Isolate

**Table 1. Biochemical Identification of Bacterial Isolate**

| | Gs | glucose | Man | lac | Xyhose | Mal | mr | vp | Cat | ctu | mot | urs | ind | Sta | Stt | oxi | identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ISOA** | GNR | A | A | N | A | A | + | - | + | + | - | + | - | + | - | + | *Pseudomonas* spp |
| **ISOB** | GPC | A | A | A | A | A | - | + | + | + | - | - | - | - | - | - | *Staphylococcus* spp |
| **ISOC** | GNR | A | - | A | A | A/G | + | + | - | + | + | + | + | - | - | - | *Bacillus* spp |
| **ISOD** | GPR | A/G | A | A | A | A | + | + | - | + | - | + | + | + | - | - | *Lactobacillus* spp |
| **ISOE** | GNR | A/G | - | A/G | A | A/G | - | - | - | - | - | - | - | - | - | - | *Enterococcus* spp |
| **ISOF** | GPR | A/G | - | - | - | A/G | + | - | + | - | - | - | - | | - | - | *Corynobacterium* |
| **ISOG** | GNR | A/G | A/G | A/G | A | A | + | - | + | - | + | - | + | - | - | - | *Escherichia coli* |
| **ISOH** | GNR | A | A | A | A | A | - | + | + | + | - | + | - | + | - | - | *Klebsiella pneumonia* Spp |

*KEYS: ISO – Code; GS - Gram stain reaction; GLU – Glucose sugar; MAN – Mannitol sugar, LAC – Lactose sugar; XYL – Xylose sugar; MAL – Maltose sugar; MR – Methyl Red Test; VP – Vogas Proskeur; CAT – Catalase test; CTU – Citrate utilization test; IDO – Indole Test; MOT – Motility test; STA – Starch Hydrolysis Test; STT – Salt Tolerant Test; URS – Urease Test; OXI – Oxidase Test; + = Positive reaction; - = Negative reaction; AG = Acid and gas; A = Acid*

**Table 2. Mean Total Heterotrophic Bacteria (THB) (cfu/min-m$^2$) during evaluation of common medicinal herbs to control bioaerosols**

| Test Herbs | Control | 15mins | 30mins | 60mins | 120mins | 180mins |
|---|---|---|---|---|---|---|
| **Ginger** | $1.18X10^3$ | $3.12X10^3$ | $1.71X10^3$ | 0 | 0 | $0.86X10^3$ |
| **Onion** | $1.31X10^3$ | $7.09X10^3$ | $1.42X10^3$ | 0 | $2.79X10^3$ | $5.67X10^3$ |
| **Garlic** | $5.53X10^3$ | $4.71X10^3$ | $3.97X10^3$ | $7.09X10^3$ | $7.09X10^3$ | $5.67X10^3$ |
| **scent leaf** | $5.67X10^3$ | $5.67X10^3$ | $1.99X10^3$ | $2.84X10^3$ | $2.84X10^3$ | 0 |
| **onion & garlic** | $3.83x10^3$ | $4.26x10^3$ | $3.40x10^3$ | $1.15x10^3$ | $1.17x10^3$ | $0.86x10^3$ |
| **onion & scent leaf** | $4.82x10^3$ | $5.10x10^3$ | 0 | $3.97x10^3$ | $1.14x10^3$ | 0 |
| **onion & ginger** | $2.61x10^3$ | $3.69x10^3$ | 0 | 0 | $2.84x10^3$ | $0.56x10^3$ |
| **garlic & scent leaf** | $5.67x10^3$ | $1.14x10^3$ | 0 | 0 | $1.15x10^3$ | $0.56x10^3$ |
| **garlic & ginger** | $5.29x10^3$ | $2.84x10^3$ | $1.14x10^3$ | $1.42x10^3$ | 0 | $0.56x10^3$ |
| **scent leaf & ginger** | $1.31X10^3$ | $9.64X10^3$ | 0 | $5.67X10^3$ | $3.97X10^3$ | 0 |
| **onion, garlic, scent leaf** | $1.56X10^3$ | $1.99X10^3$ | $0.85x10^3$ | $0.85x10^3$ | 0 | $0.56x10^3$ |
| **onion, garlic, ginger** | $5.67x10^3$ | $0.56x10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 |
| **garlic, scent leaf, ginger** | $3.37x10^3$ | 0 | $1.14x10^3$ | 0 | 0 | 0 |
| **onion, garlic, scent leaf, ginger** | $7.94X10^3$ | $4.549X10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 |

**Table 3. Mean Total Heterotrophic Fungi (THF) (cfu/min-m$^2$) during evaluation of common medicinal herbs to control bioaerosols**

| Test Herbs | Control | 15mins | 30mins | 60mins | 120mins | 180mins |
|---|---|---|---|---|---|---|
| **Ginger** | $4.25X10^3$ | $2.26X10^3$ | 0 | $2.84x10^3$ | $5.67x10^3$ | $0.56x10^3$ |
| **Onion** | $3.69X10^3$ | 0 | 0 | $2.84x10^3$ | $0.56x10^3$ | $0.56x10^3$ |
| **Garlic** | $3.40X10^3$ | 0 | 0 | $0.56x10^3$ | $0.56x10^3$ | 0 |
| **Scent leaf** | $0.56x10^3$ | 0 | 0 | 0 | $0.56x10^3$ | 0 |
| **Onion & garlic** | $0.85x10^3$ | $0.56x10^3$ | 0 | $0.56x10^3$ | 0 | 0 |
| **Onion & scent leaf** | $1.13x10^3$ | 0 | 0 | 0 | $0.56x10^3$ | $0.56x10^3$ |
| **Onion & ginger** | $1.71x10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **Garlic & scent leaf** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Garlic & ginger** | $1.13x10^3$ | $0.56x10^3$ | 0 | $1.14x10^3$ | 0 | 0 |
| **Scent leaf & ginger** | $1.99X10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **Onion, garlic, scent leaf** | $5.11X10^3$ | $0.56x10^3$ | 0 | $1.99x10^3$ | $0.85x10^3$ | $0.56x10^3$ |
| **Onion, garlic, ginger** | $0.56x10^3$ | 0 | 0 | 0 | 0 | 0 |
| **Garlic, scent leaf, ginger** | $3.12x10^3$ | $0.56x10^3$ | 0 | 0 | 0 | 0 |
| **Onion, garlic, scent leaf, Ginger** | $0.56x10^3$ | 0 | 0 | 0 | 0 | 0 |

**Table 4. Mean Total Enteric Bacteria (EB) (cfu/min-m$^2$) during evaluation of common medicinal herbs to control bioaerosols**

| Test Herbs | Control | 15mins | 30mins | 60mins | 120mins | 180mins |
|---|---|---|---|---|---|---|
| **Ginger** | $3.40X10^3$ | 0 | $0.56x10^3$ | 0 | $0.56x10^3$ | 0 |
| **Onion** | $3.12X10^3$ | 0 | $5.10X10^3$ | $1.42X10^3$ | $0.98X10^3$ | 0 |
| **Garlic** | 0 | 0 | 0 | 0 | 0 | 0 |
| **scent leaf** | $1.99X10^3$ | 0 | 0 | $1.14X10^3$ | 0 | $0.56x10^3$ |
| **onion & garlic** | $5.10x10^3$ | $2.55x10^3$ | 0 | 0 | 0 | $0.56x10^3$ |
| **onion & scent leaf** | $3.40x10^3$ | $2.28x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **onion & ginger** | $3.97x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ | $0.56x10^3$ |
| **garlic & scent leaf** | $1.70x10^3$ | $0.56x10^3$ | $0.86x10^3$ | 0 | $1.14x10^3$ | $0.56x10^3$ |
| **garlic & ginger** | $4.77x10^3$ | $3.12x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **scent leaf & ginger** | $1.14X10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | $0.56x10^3$ | $0.56x10^3$ |
| **onion, garlic, scent leaf** | 0 | 0 | 0 | 0 | 0 | 0 |
| **onion, garlic, ginger** | $0.85x10^3$ | $0.56x10^3$ | 0 | 0 | 0 | 0 |
| **garlic, scent leaf, ginger** | $1.17x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ | $0.56x10^3$ |
| **onion, garlic, scent leaf, ginger** | $3.97X10^3$ | 0 | 0 | 0 | 0 | 0 |

**Table 5. Mean Urinal Pathogen (UP) (cfu/min-m$^2$) during evaluation of common medicinal herbs to control bioaerosols**

| Test Herbs | Control | 15mins | 30mins | 60mins | 120mins | 180mins |
|---|---|---|---|---|---|---|
| **Ginger** | $3.71X10^3$ | $1.14X10^3$ | $0.85x10^3$ | $1.14X10^3$ | $2.84x10^3$ | $0.56x10^3$ |
| **Onion** | $1.17x10^3$ | $0.56x10^3$ | $0.56x10^3$ | $0.56x10^3$ | $0.85x10^3$ | $3.97X10^3$ |
| **Garlic** | $1.59X10^3$ | $4.82X10^3$ | $5.67X10^3$ | $8.51x10^3$ | $4.25x10^3$ | $0.56x10^3$ |
| **scent leaf** | $3.40X10^3$ | $1.14x10^3$ | $1.14x10^3$ | $0.56x10^3$ | 0 | $1.14x10^3$ |
| **onion & garlic** | $1.70x10^3$ | 0 | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **onion & scent leaf** | $1.13x10^3$ | $0.56x10^3$ | 0 | $0.56x10^3$ | $0.56x10^3$ | $0.56x10^3$ |
| **onion & ginger** | $3.41X10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **garlic & scent leaf** | $0.56x10^3$ | $0.56x10^3$ | 0 | $0.56x10^3$ | 0 | $0.56x10^3$ |
| **garlic & ginger** | $1.14x10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |
| **scent leaf & ginger** | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ | 0 |
| **onion, garlic, scent leaf** | $3.96X10^3$ | $0.85x10^3$ | $0.56x10^3$ | $1.42x10^3$ | 0 | 0 |
| **onion, garlic, ginger** | $1.14x10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | 0 |
| **garlic, scent leaf, ginger** | 0 | 0 | 0 | 0 | 0 | 0 |
| **onion, garlic, scent leaf, ginger** | $1.42x10^3$ | 0 | $0.56x10^3$ | 0 | 0 | 0 |

**Table 6. Mean Total Staph (cfu/min-m$^2$) during evaluation of common medicinal herbs to control bioaerosols**

| Test Herbs | Control | 15mins | 30mins | 60mins | 120mins | 180mins |
|---|---|---|---|---|---|---|
| **Ginger** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Onion** | $1.14x10^3$ | 0 | 0 | 0 | 0 | 0 |
| **Garlic** | $1.14x10^3$ | 0 | 0 | 0 | 0 | $0.56x10^3$ |
| **scent leaf** | $2.56x10^3$ | 0 | $0.56x10^3$ | 0 | $0.56x10^3$ | 0 |
| **onion & garlic** | $0.56x10^3$ | 0 | 0 | 0 | 0 | 0 |
| **onion & scent leaf** | $1.42x10^3$ | $0.56x10^3$ | $0.56x10^3$ | 0 | 0 | 0 |
| **onion & ginger** | 0 | 0 | 0 | 0 | 0 | 0 |
| **garlic & scent leaf** | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ | 0 | 0 |
| **garlic & ginger** | $0.56x10^3$ | 0 | 0 | 0 | 0 | 0 |
| **scent leaf & ginger** | 0 | 0 | 0 | 0 | 0 | $0.56x10^3$ |
| **onion, garlic, scent leaf** | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ | 0 | 0 |
| **onion, garlic, ginger** | 0 | 0 | 0 | 0 | 0 | 0 |
| **garlic, scent leaf, ginger** | $0.85x10^3$ | 0 | $0.56x10^3$ | 0 | 0 | 0 |
| **onion, garlic, scent leaf, ginger** | $0.85x10^3$ | 0 | $0.56x10^3$ | 0 | 0 | $0.56x10^3$ |

**Table 7. Mean of Total heterotrophic bacteria, total heterotrophic fungi, Enteric bacteria, Urinal Pathogen, Staph (Log/Standard Deviation (Log 10 cfu/min-m$^2$)**

| | Ginger | Onion | Garlic | Scent Leaf | Onion & Garlic | Onion & Scent Leaf | Onion & Ginger | Garlic & Scent Leaf | Garlic & Ginger | Scent Leaf & Ginger | Onion, Garlic & Scent Leaf | Onion, Garlic, Ginger | Garlic,Scent Leaf Ginger | Onion, Garlic, Scent Leaf, Ginger |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **THB** | 3.06±1.77 | 3.49±2.77 | 3.76±1.25 | 3.51±2.20 | 3.39±1.55 | 3.40±2.40 | 3.21±1.62 | 3.22±2.14 | 3.28±1.93 | 3.54±3.79 | 3.13±0.81 | 3.13±1.36 | 3.01±1.36 | 3.36±3.26 |
| **THF** | 3.42±2.15 | 3.11±1.59 | 3.61±1.32 | 3.73±0.29 | 3.46±0.37 | 3.43±0.46 | 3.25±0.62 | NF | 3.33±0.47 | 3.22±0.72 | 3.18±1.89 | 3.22±1.24 | 3.22±1.24 | 3.13±0.23 |
| **EB** | 3.13±1.32 | 3.25±1.20 | NF | 3.22±0.81 | 3.14±2.07 | 3.06±1.40 | 3.03±1.50 | 3.15±0.67 | 3.18±3.82 | 3.25±0.36 | NF | 3.33±0.43 | 3.33±0.43 | 3.18±1.62 |
| **urinal Pathogen** | 3.24±1.26 | 3.14±1.34 | 3.63±2.87 | 3.09±1.15 | 3.30±0.66 | 3.25±0.36 | 3.07±1.28 | 3.43±0.28 | 3.04±0.42 | 3.06±0.30 | 3.07±1.49 | NF | NF | 3.03±0.56 |
| **Staph** | NF | 3.07±0.46 | 3.05±0.47 | 3.02±1.00 | 3.10±0.22 | 3.03±0.56 | NF | 3.07±0.29 | 3.01±0.22 | NF | 3.07±0.29 | 3.06±0.37 | 3.06±0.37 | 3.04±0.37 |
| **Vibrio** | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF |

**KEYS :**NF – Not found

## 5. DISCUSSION

The microbial vapor sprayers secluded, described and distinguished incorporate 8 microscopic organisms genera and 7 parasites segregates. The microbes confines are Bacillus species, Pseudomonas species, *Staphylococcus* species, Lactobacillus species, Enterococcus species, Corynobacterium, Escherichia coli, Klebsiella pneumonia. While the contagious detaches were *Aspergillus niger*, *pennicillum* species*, Rhizopus* species*, Saccharomyces* species*, Mucor* species, *Candida* species*, Asperillus flavus, Fusarium* species. Five (5) intestinal microbes were secluded including *E.coli* whose normal verdure is the human gastrointestinal parcel, yet at the same time equipped for causing pioneering contamination like explorer's the runs and other gastrointestinal problems. Another harmful microbe found was *Staphylococcus* spp which is a disease are brought about by *staphylococcus* microorganisms, kinds of microbes usually tracked down on the skin or in the nose of even solid people as typical greenery. More often than not, these microorganisms cause no issues or result in moderately minor skin contaminations. In any case, bacterial sicknesses can transform destructive on the off chance that the microbes attack further into your body, entering your circulation system, joints, bones, lungs or heart. A developing number of in any case solid individuals are creating hazardous bacterial sicknesses, Staph microscopic organisms are one of the most widely recognized reasons for food contamination. Side effects come on rapidly, for the most part promptly after eating a polluted food. Side effects generally vanish rapidly, as well, frequently enduring simply a portion of a day. Openness and inward breath of parasitic spores could bring about serious fundamental contaminations or mycotoxicosis as well as respiratory and skin diseases and disturbances. Reasonable microbial sprayers of this examination are delivered to the latrine room air/air by flushing water wardrobes subsequent to crapping consequently producing sullied water beads. The aftermath of beads containing microorganisms on washroom surface is likewise of worry, since hand contact with sullied surface can bring about self vaccination by contacting the nose or mouth [14,15].

The examination part of this study is to think about the successful decrease pace of heterotrophic microscopic organisms, intestinal microbes, vigorous parasites, *Staphylococcus,* urinal microorganism and Vibrio in the area utilizing ginger, onion, scent leaf, garlic and furthermore by mix of the spices which are Onion and garlic, Onion and garlic, Onion and ginger, Garlic and scent leaf, Garlic and ginger, scent leaf and ginger, Onion, garlic and scent leaf, Onion, garlic and ginger, Garlic, scent leaf and ginger, Onion, garlic, scent leaf and ginger. From the outcome acquired after the examination it was seen that in 180 minutes Garlic, scent leaf and Ginger had most noteworthy decrease pace of all out heterotrophic microorganisms followed by Ginger then onion, garlic and scent leaf and onion, garlic and ginger with both at (3.13 $log^{10}$/cfu/min-$m^2$) separately. The testing with Garlic, scent leaf and Ginger was more powerful due to it quickly wiped out heterotrophic microorganisms at 15mins with no development found.

For enteric pathogen, it was seen that the inspecting with Garlic and Onion, garlic and scent leaf showed the most rate decrease with no development viewed as followed by Onion and ginger and Onion and scent leaf. For aerobic fungi, it was seen that Garlic and scent leaf had the most rate decrease pace of high-impact growths followed by Onion, Onion, garlic, scent leaf and ginger. For *Staphylococcus* it was seen that Ginger, Onion and ginger and scent leaf and ginger showed the most rate decrease with no development saw as followed by Garlic and ginger and scent leaf.

For Urinal pathogen it was seen that Onion, garlic and ginger and Garlic, scent leaf and ginger showed the most rate decrease with no development saw as followed by Onion, garlic, scent leaf and ginger, Garlic and ginger. After all examination it was reasoned that the request for expanding capacity of viability in lessening all out heterotrophic microorganisms utilizing ginger, onion, scent leaf, garlic and furthermore by mix of the spices which are Onion and garlic, Onion and garlic, Onion and ginger, Garlic and scent leaf, Garlic and ginger, scent leaf and ginger, Onion, garlic and scent leaf, Onion, garlic and ginger, Garlic, scent leaf and ginger, Onion, garlic, scent leaf and ginger all through 180 minutes is as per the following; Garlic, scent leaf and Ginger (3.01 $log^{10}$/cfu/min-$m^2$) < Ginger then onion, garlic and scent leaf and onion, garlic and ginger with both at (3.13 log10/cfu/min-$m^2$). The request for expanding capacity of adequacy in lessening intestinal microorganisms all through 180 minutes is as per the following; Garlic and Onion, garlic and scent leaf (No Development) <

Onion and ginger (3.03 $log^{10}$/cfu/min-$m^2$). The request for expanding capacity of viability in decreasing in high-impact growths all through 180 minutes is as per the following; Garlic and scent leaf (No Development) < Onion (3.11 $log^{10}$/cfu/min-$m^2$). The request for expanding capacity of viability in lessening in *Staphylococcus* all through 180 minutes is as per the following Ginger, Onion and ginger and scent leaf and ginger with (No Development) < Garlic and ginger (3.01 $log^{10}$/cfu/min-$m^2$). The request for expanding capacity of adequacy in decreasing in Urinal microorganism all through 180 minutes is as per the following; Onion, garlic and ginger and Garlic, scent leaf and ginger (No development) < Onion, garlic, scent leaf and ginger (3.03 $log^{10}$/cfu/min-$m^2$). Furthermore, Vibrio was not tracked down in all the examining destinations.

## 6. CONCLUSION

It can be deduced that these medicinal herbs has the ability in reducing microbial aerosol using ginger, onion, scent leaf, garlic also by combination of the herbs which are Onion & garlic, Onion & garlic, Onion & ginger, Garlic & scent leaf, Garlic & ginger, Scent leaf & ginger, Onion, garlic & scent leaf, Onion, garlic & ginger, Garlic, Scent leaf & ginger, Onion, garlic, scent leaf & ginger. Specifically the microbial aerosols in this investigation including bacteria and fungi were *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Lactobacillus* species, *Enterococcus* species, *Corynobacterium*, *Escherichia coli*, *Klebsiella pneumonia*. *Aspergillus niger*, *pennicillum* species, *Rhizopus* species, *Saccharomyces* species, *Mucors* species, *Candida* species, *Asperillus flavus*, *Fusarium* species. Therefore all results are applied to the above listed microorganisms. Since few of the organisms listed are capable of causing urinary tract infection as well as gastro intestinal infection, utmost hygiene measure must be taken whenever one enters the toilet and prolonged exposure to toilet environment are not advised. Contamination by sedimentation of contaminated water droplets as result of flushing after defecation and improperly washed hands contaminate toilet door handles, toilet walls, toilet seat could lead to prevalence of microbial infectious disease conditions.

Based on the fact that the urethra of females is shorter compared to that of males, if there is any chance of contaminated water droplets settling on or getting in contact with the vagina there could be a possibility of opportunistic infection or urinary tract infection. In fact the need for reducing these microbial aerosols cannot be over emphasized. The use of plants in this experiment was to create a path for more natural methods of reducing microbial aerosol instead of using synthetic chemical which could gave adverse effects to human health. Once again nature has provided a solution for the possibility of microbial infection which could lead to numerous disease conditions.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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