

*Original Research Article*

**USE OF COMMON HERBS TO CONTROL MICROBIAL AEROSOLS IN TOILET ROOMS.**

**ABSTRACT**

An investigation on the ability of plants active volatile essence to eliminate microorganisms in the atmosphere (bioaerosols) in toilet room was carried using four different types of plants; *Allium sativum* (Garlic), (*Allium cepa*) (Onion), *zingiber officinalis* (Ginger) and *Ocimum gratissimum* (Scent leaf). The investigation was carried out in the Microbiology Student Toilets, located inside the Rivers State University Nkpolu Oroworukwu, Port Harcourt. The method of air sampling employed was sedimentation method of aerosol sampling where the artificial culture medium; nutrient agar (for total heterotrophic bacteria), MacConkey agar (for enteric bacteria), sadourand dextrose agar (for aerobic fungi), Thiosulfate citrate bile salt sucrose agar (for vibrio), Cystine lactose electrolyte deficient agar (Urinal pathogen) and Mannitol salt agar (for *staphylococcus*) were exposed to air at different time intervals to ascertain the reduction rate of total heterotrophic bacteria, enteric bacteria, aerobic fungi, vibrio, Urinal pathogen and *Staphylococcus*. Samples were 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, *Enterococcus* sp, *Corynebacterium* sp, *Escherichia coli*, *Klebsiella pneumoniae*. While the fungi isolated were *Aspergillus niger*, *penicillium* sp, *Rhizopus* sp, *Saccharomyces* sp, *Mucor* sp, *Candida* sp, *Aspergillus flavus*, *Fusarium* sp. The evaluation of *Staphylococcus* in the analysis of herbs treatment in 180mins (cfu/min-m<sup>2</sup>) is as follows; Ginger (0) < Onion & Ginger (0) < Scent leaf & Ginger (0) < Garlic & ginger (3.01) < Scent leaf (3.02) < Onion & scent leaf (3.03) < Onion, garlic, scent leaf & ginger (3.04) < Garlic (3.05) < Garlic, scent leaf & ginger (3.06) < Onion, garlic & ginger (3.06) < Onion, garlic & scent leaf (3.07) < Garlic & scent leaf (3.07) < Onion (3.07) < Onion & garlic (3.10). In Urinal pathogen in 180mins (cfu/min-m<sup>2</sup>) was as follows; Garlic, scent leaf & ginger (0) < Garlic, scent leaf & ginger (0) < Onion, garlic, Scent leaf & ginger (3.03) < Garlic & ginger (3.04) < Scent leaf & ginger (3.06) < Onion, garlic & scent leaf, (3.07) < Onion & ginger (3.07) < Scent leaf (3.09) < Onion (3.14) < Ginger (3.24) < Onion & scent leaf (3.25) < Onion & garlic (3.30) < Garlic, scent leaf (3.43) < Garlic (3.63). In Aerobic fungi in 180mins (cfu/min-m<sup>2</sup>) is as follows; garlic scent leaf (0) < onion (3.11). Onion, garlic, scent leaf & ginger (3.13) < Onion, garlic & scent leaf, (3.18) < Garlic, scent leaf & ginger (3.22) < Onion, garlic & ginger (3.22) < Onion & ginger (3.25) < Garlic & ginger (3.33) < Ginger (3.42) < Onion & scent leaf (3.43) < Onion & garlic (3.46) < Garlic (3.61) < Scent leaf (3.73). In Enteric bacteria in 180mins (cfu/min-m<sup>2</sup>) was as follows; Onion, garlic & scent leaf (0) < Onion & ginger (3.03) < Onion & scent leaf (3.06) < Ginger (3.13) < Onion & garlic (3.14) < Garlic scent leaf, (3.15) < Onion, garlic, scent leaf & ginger (3.18) < Garlic ginger (3.18) < Scent leaf (3.22) < Scent leaf & ginger (3.25) < Onion (3.25) < Garlic, scent leaf & ginger (3.33) < Onion, garlic & ginger (3.33). In Total heterotrophic bacteria in 180mins (cfu/min-m<sup>2</sup>) is as follows; Onion, garlic, scent leaf (3.01) < Onion & ginger (3.03) < Ginger (3.06) < Onion & scent leaf (3.06) < Ginger (3.06) < Onion, garlic & ginger (3.13) < garlic, scent leaf & ginger (3.13) < Onion & ginger (3.21) < Garlic & scent leaf (3.22) < Garlic & ginger (3.28) < Onion, garlic, scent leaf & ginger (3.36) < Onion & garlic (3.39) < Garlic scent leaf (3.15) < Onion & ginger (3.21) < Garlic & scent leaf (3.22) < Garlic & ginger (3.28) < Onion, garlic, scent leaf & ginger (3.36) < Onion & garlic (3.39) < Onion &

scent leaf (3.40) < Onion (3.49) < Scent leaf(3.51) < Scent leaf & ginger (3.54)<Garlic (3.76). This work proved that microbial load can be reduced using natural means (plant extract).

**Keywords:** Bioaerosols, Microorganisms, Mashed Plants, Public health, microbial load, Control.

## **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. (Górny, *et al.*, 2020).

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.(Burrows *et al.*, 2009).

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 10<sup>12</sup> cfu m<sup>-3</sup>) are: silo loading/unloading, animal feeding in broiler houses, piggeries as well as different dust-

releasing tasks in composting plants, granaries, animal food stores, malhouses, 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  $10^3$  cfu m<sup>-3</sup>; however, some chamber bioaerosol studies revealed that even one person under seated conditions is able to release up to  $10^6$  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 (Bhangar et al., 2016). 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  $10^3$ – $10^4$  cfu m<sup>-3</sup>) 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 molds) (Barker and Jones, 2005; Best et al., 2012; Stapleton et al., 2013). Microorganisms are essential to human survival, health and disease, and hence their environmental abundance and diversity are of great practical importance (Bisenet *al.*, 2012).

## **MATERIALS AND METHODS**

### **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.

### **Toilet Air Sample Collection**

The bioaerosols 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.

### **Microbiological Analysis**

#### **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.*, 2006).

### **Analytical Formula**

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

$$\text{Cfu/mins-m}^2 = \frac{\text{no of colonies}}{\text{Time of Exposure (min)} \times \pi r^2}$$

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

### **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, *Corynebacterium*, *Escherichia coli*, *Klebsiella pneumonia*. Fungal isolate was *Aspergillus niger*, *Penicillium* species, *Rhizopus* species, *Saccharomyces* species, *Mucor* species, *Candida* species, *Asperillus flavus*, *Fusarium* species.

## **RESULTS FOR BIOCHEMICAL IDENTIFICATION OF BACTERIA ISOLATE**

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

	Gs	glucose	Man	lac	Xylose	Mal	mr	vp	Cat	ctu	mot	urs	ind	Sta	Stt	oxi	identifica tion
<b>IS OA R</b>	G N	A	A	N	A	A	+	-	+	+	-	+	-	+	-	+	<i>Pseudomonass</i> pp
<b>IS OB C</b>	GP C	A	A	A	A	A	-	+	+	+	-	-	-	-	-	-	<i>Staphylococcus</i> spp
<b>IS OC R</b>	G N R	A	-	A	A	A/ G	+	+	-	+	+	+	+	-	-	-	<i>Bacillus</i> spp

<b>IS</b>	GP	A/	A	A	A	A	+	+	-	+	-	+	+	+	-	-	<i>Lactobacillus</i>
<b>OD</b>	R	G															pp
<b>IS</b>	G	A/	-	A/	A	A/	-	-	-	-	-	-	-	-	-	-	<i>Enterococcus</i>
<b>OE</b>	N	G		G		G											spp
	R																
<b>IS</b>	GP	A/	-	-	-	A/	+	-	+	-	-	-	-		-	-	<i>Corynebacteri</i>
<b>OF</b>	R	G				G											um
<b>IS</b>	G	A/	A/	A/	A	A	+	-	+	-	+	-	+	-	-	-	<i>Escherichia</i>
<b>OG</b>	N	G	G	G													<i>coli</i>
	R																
<b>IS</b>	G	A	A	A	A	A	-	+	+	+	-	+	-	+	-	-	<i>Klebsiellapneu</i>
<b>OH</b>	N																moniaSpp
	R																

**KEYS**

ISO - Code	+	=	Positive reaction
GS - Gram stain reaction	-	=	Negative reaction
GLU – Glucose sugar	AG	=	Acid and gas
MAN – Mannitol sugar	A	=	Acid

- LAC – Lactose sugar
- XYL – Xylose sugar
- MAL – Maltose sugar
- MR – Methyl Red Test
- VP – VogasProskeur
- 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

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

Test Herbs	Control	15mins	30mins	60mins	120mins	180mins
<b>Ginger</b>	1.18X10 <sup>3</sup>	3.12X10 <sup>3</sup>	1.71X10 <sup>3</sup>	0	0	0.86X10 <sup>3</sup>
<b>Onion</b>	1.31X10 <sup>3</sup>	7.09X10 <sup>3</sup>	1.42X10 <sup>3</sup>	0	2.79X10 <sup>3</sup>	5.67X10 <sup>3</sup>
<b>Garlic</b>	5.53X10 <sup>3</sup>	4.71X10 <sup>3</sup>	3.97X10 <sup>3</sup>	7.09X10 <sup>3</sup>	7.09X10 <sup>3</sup>	5.67X10 <sup>3</sup>
<b>scent leaf</b>	5.67X10 <sup>3</sup>	5.67X10 <sup>3</sup>	1.99X10 <sup>3</sup>	2.84X10 <sup>3</sup>	2.84X10 <sup>3</sup>	0
<b>onion &amp; garlic</b>	3.83x10 <sup>3</sup>	4.26x10 <sup>3</sup>	3.40x10 <sup>3</sup>	1.15x10 <sup>3</sup>	1.17x10 <sup>3</sup>	0.86x10 <sup>3</sup>
<b>onion &amp; scent leaf</b>	4.82x10 <sup>3</sup>	5.10x10 <sup>3</sup>	0	3.97x10 <sup>3</sup>	1.14x10 <sup>3</sup>	0
<b>onion &amp; ginger</b>	2.61x10 <sup>3</sup>	3.69x10 <sup>3</sup>	0	0	2.84x10 <sup>3</sup>	0.56x10 <sup>3</sup>
<b>garlic &amp; scent leaf</b>	5.67x10 <sup>3</sup>	1.14x10 <sup>3</sup>	0	0	1.15x10 <sup>3</sup>	0.56x10 <sup>3</sup>

garlic & ginger	5.29x10 <sup>3</sup>	2.84x10 <sup>3</sup>	1.14x10 <sup>3</sup>	1.42x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>
scent leaf & ginger	1.31X10 <sup>3</sup>	9.64X10 <sup>3</sup>	0	5.67X10 <sup>3</sup>	3.97X10 <sup>3</sup>	0
onion, garlic,scent leaf	1.56X10 <sup>3</sup>	1.99X10 <sup>3</sup>	0.85x10 <sup>3</sup>	0.85x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>
onion, garlic,ginger	5.67x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0
garlic,scent leaf, ginger	3.37x10 <sup>3</sup>	0	1.14x10 <sup>3</sup>	0	0	0
onion, garlic, scent leaf, ginger	7.94X10 <sup>3</sup>	4.549X10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0

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

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

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

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

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

Test Herbs	Control	15mins	30mins	60mins	120mins	180mins
Ginger	3.71X10 <sup>3</sup>	1.14X10 <sup>3</sup>	0.85x10 <sup>3</sup>	1.14X10 <sup>3</sup>	2.84x10 <sup>3</sup>	0.56x10 <sup>3</sup>
Onion	1.17x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.85x10 <sup>3</sup>	3.97X10 <sup>3</sup>
Garlic	1.59X10 <sup>3</sup>	4.82X10 <sup>3</sup>	5.67X10 <sup>3</sup>	8.51x10 <sup>3</sup>	4.25x10 <sup>3</sup>	0.56x10 <sup>3</sup>
scent leaf	3.40X10 <sup>3</sup>	1.14x10 <sup>3</sup>	1.14x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	1.14x10 <sup>3</sup>
onion & garlic	1.70x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>
onion & scent leaf	1.13x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>
onion & ginger	3.41X10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>

garlic & scent leaf	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>
garlic & ginger	1.14x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>
scent leaf & ginger	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>	0
onion, garlic,scent leaf	3.96X10 <sup>3</sup>	0.85x10 <sup>3</sup>	0.56x10 <sup>3</sup>	1.42x10 <sup>3</sup>	0	0
onion, garlic,ginger	1.14x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0	0
garlic,scent leaf, ginger	0	0	0	0	0	0
onion, garlic, scent leaf, ginger	1.42x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0	0

**Table.6: Mean Total Staph (cfu/min-m<sup>2</sup>) 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 <sup>3</sup>	0	0	0	0	0
Garlic	1.14x10 <sup>3</sup>	0	0	0	0	0.56x10 <sup>3</sup>
scent leaf	2.56x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0
onion & garlic	0.56x10 <sup>3</sup>	0	0	0	0	0
onion & scent leaf	1.42x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0.56x10 <sup>3</sup>	0	0	0
onion & ginger	0	0	0	0	0	0
garlic & scent leaf	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>	0	0
garlic & ginger	0.56x10 <sup>3</sup>	0	0	0	0	0
scent leaf & ginger	0	0	0	0	0	0.56x10 <sup>3</sup>
onion, garlic,scent leaf	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>	0	0
onion, garlic,ginger	0	0	0	0	0	0
garlic,scent leaf, ginger	0.85x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0	0
onion, garlic, scent leaf, ginger	0.85x10 <sup>3</sup>	0	0.56x10 <sup>3</sup>	0	0	0.56x10 <sup>3</sup>

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

	GI NG ER	ON IO N	GA RLI C	SC EN T AF	ON IO N & GA RLI C	ON IO N & SC EN T AF	ON IO N & GI EN ER	GA RLI C & SC EN T AF	GA RLI C & GI EN ER	SC EN T & GI NG ER	ONION ,GARL IC & SCENT LEAF	ONI ON, GA RLI C, GIN GE R	GARLI C,SCE NT LEAF GINGE R	ONION ,GARL IC, SCENT LEAF ,GING ER
THB	3.06 ±1.7 7	3.49 ±2.7 7	3.76 ±1.2 5	3.51 ±2.2 0	3.39 ±1.5 5	3.40 ±2.4 0	3.21 ±1.6 2	3.22 ±2.1 4	3.28 ±1.9 3	3.54 ±3.7 9	3.13±0. 81	3.13 ±1.3 6	3.01±1. 36	3.36±3. 26
THF	3.42 ±2.1 5	3.11 ±1.5 9	3.61 ±1.3 2	3.73 ±0.2 9	3.46 ±0.3 7	3.43 ±0.4 6	3.25 ±0.6 2	NF	3.33 ±0.4	3.22 ±0.7	3.18±1. 89	3.22 ±1.2	3.22±1. 24	3.13±0. 23

								7	2			4		
EB	3.13 ±1.3 2	3.25 ±1.2 0	NF	3.22 ±0.8 1	3.14 ±2.0 7	3.06 ±1.4 0	3.03 ±1.5 0	3.15 ±0.6	3.18 ±3.8	3.25 ±0.3	NF	3.33 ±0.4	3.33±0. 43	3.18±1. 62
								7	2	6		3		
URIN AL PAT HOG EN	3.24 ±1.2 6	3.14 ±1.3 4	3.63 ±2.8 7	3.09 ±1.1 5	3.30 ±0.6 6	3.25 ±0.3 6	3.07 ±1.2 8	3.43 ±0.2	3.04 ±0.4	3.06 ±0.3	3.07±1. 49	NF	NF	3.03±0. 56
								8	2	0				
STAP H	NF	3.07 ±0.4 6	3.05 ±0.4 7	3.02 ±1.0 0	3.10 ±0.2 2	3.03 ±0.5 6	NF	3.07 ±0.2	3.01 ±0.2	NF	3.07±0. 29	3.06 ±0.3	3.06±0. 37	3.04±0. 37
								9	2			7		
VIBR IO	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

## KEYS

NF – Not found

## DISCUSSION

The microbial aerosols isolated, characterized and identified include 8 bacteria genera and 7 fungi isolates. The bacteria isolates are *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Lactobacillus* species, *Enterococcus* species, *Corynebacterium*, *Escherichia coli*, *Klebsiella pneumoniae*. While the fungal isolates were *Aspergillusniger*, *pennicillum* species, *Rhizopus* species, *Saccharomyces* species, *Mucor* species, *Candida* species, *Asperillusflavus*, *Fusarium* species. Five (5) enteric bacteria were isolated including *E.coli* whose natural flora is the human gastrointestinal tract, but still capable of causing opportunistic infection such as traveller's diarrhea and other gastrointestinal disorders. Another virulent pathogen found was *Staphylococcus* spp which is an infection are caused by staphylococcus bacteria, types of germs commonly found on the skin or in the nose of even healthy individuals as normal flora. Most of the time, these bacteria cause no problems or result in relatively minor skin infections. But staph infections can turn deadly if the bacteria invade deeper into your body, entering your bloodstream, joints, bones, lungs or heart. A growing number of otherwise healthy people are developing life-threatening staph infections,

Staph bacteria are one of the most common causes of food poisoning. Symptoms come on quickly, usually within hours of eating a contaminated food. Symptoms usually disappear quickly, too, often lasting just half a day. Exposure and inhalation of fungal spores could result in serious systemic infections or mycotoxicosis as well as respiratory and skin infections and irritations. Viable microbial aerosols of this investigation are released to the toilet room atmosphere/air by flushing water closets after defecating thereby generating contaminated water droplets. The fallout of droplets containing pathogens on bathroom surface is also of concern, since hand contact with contaminated surface can result in self inoculation by touching the nose or mouth (Hutchinson. 1956).

The analysis aspect of this study is to compare the effective reduction rate of heterotrophic bacteria, enteric bacteria, aerobic fungi, Staphylococcus, urinal pathogen and Vibrio in the location using ginger, onion, scent leaf, garlic and 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. From the result obtained after the investigation it was observed that in 180minutes Garlic, Scent leaf & Ginger had highest reduction rate of total heterotrophic bacteria followed by Ginger then onion, garlic & scent leaf and onion, garlic & ginger with both at  $(3,13 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2)$  respectively. The sampling with Garlic, Scent leaf & Ginger was more effective because of it rapidly eliminated heterotrophic bacteria at 15mins with no growth found.

For enteric bacteria, it was observed that the sampling with Garlic and Onion, garlic & scent leaf showed the most percentage reduction with no growth found followed by Onion & ginger and Onion & scent leaf.

For aerobic fungi, it was observed that Garlic & scent leaf had the most percentage reduction rate of aerobic fungi followed by Onion, Onion, garlic, scent leaf & ginger. For Staphylococcus it was observed that Ginger, Onion & ginger and Scent leaf & ginger showed the most percentage reduction with no growth found followed by Garlic & ginger and Scent leaf.

For Urinal pathogen it was observed that Onion, garlic & ginger and Garlic, scent leaf & ginger showed the most percentage reduction with no growth found followed by Onion, garlic, scent leaf & ginger, Garlic & ginger. After all investigation it was deduced that the order of increasing ability of effectiveness in reducing total heterotrophic bacteria using ginger, onion, scent leaf, garlic and 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 throughout 180 minutes is as follows; Garlic, Scent leaf & Ginger ( $3.01 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ) < Ginger then onion, garlic & scent leaf and onion, garlic & ginger with both at ( $3.13 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ). The order of increasing ability of effectiveness in reducing enteric bacteria throughout 180 minutes is as follows; Garlic and Onion, garlic & scent leaf (No Growth) < Onion & ginger ( $3.03 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ). The order of increasing ability of effectiveness in reducing in aerobic fungi throughout 180 minutes is as follows; Garlic & scent leaf (No Growth) < Onion ( $3.11 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ). The order of increasing ability of effectiveness in reducing in Staphylococcus throughout 180 minutes is as follows Ginger, Onion & ginger and Scent leaf & ginger with (No Growth) < Garlic & ginger ( $3.01 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ). The order of increasing ability of effectiveness in reducing in Urinal pathogen throughout 180 minutes is as follows; Onion, garlic & ginger and Garlic, scent leaf & ginger (No growth) < Onion, garlic, scent leaf & ginger ( $3.03 \log_{10}/ \text{cfu}/\text{min}\cdot\text{m}^2$ ). And Vibrio was not found in all the sampling sites.

## 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, *Corynebacterium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aspergillus niger*, *penicillum* 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.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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