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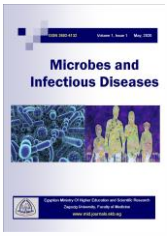

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Original article

The comparative antimicrobial effects of castor, garlic, beniseed and bitter cola extracts on microorganisms isolated from hospitals' wards

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ABSTRACT

Background: There is high incidence of nosocomial in developing countries and the bacteria responsible are becoming more resistant to commercially available antibiotics, hence, the need for this research. **Methods:** Isolation and identification of microorganisms from different wards of Ilara-Mokin health centers was done using standard methods. The bacteria isolated were identified and subjected to susceptibility test using four extracts and standard antibiotics for comparative study. **Results:** A high bacterial load of 52.00 ± 3.46 cfu/ml obtained was from the toilet in maternity ward, while the least bacterial load of 2.67 ± 0.67 cfu/ml was obtained from the floor of the male ward. A total of twelve (12) bacteria were isolated and identified, while nine (9) fungi were equally isolated and identified. The susceptibility of the bacteria isolates to ethanol extracts of castor, garlic, beniseed and bitter cola showed that garlic extract is most effective on the isolates having the highest diameter of zone of inhibition, *Bacillus licheniformis* with a diameter of 18.20±2.05 mm. **Conclusion:** The garlic ethanol extract was the most effective on the isolates while *Pseudomonas* was the most unsusceptible to all the extracts. The extracts tested in this work comparatively exceeded the potency of antibiotics, however, the Gram-negative bacteria are not very susceptible to the extracts. Therefore, these extracts can be used in development of novel antibiotics, especially to help curb resistance of pathogenic microorganisms implicated in nosocomial infections.

Background

Medically, hospital acquired infection or nosocomial, have been defined by various authors in different ways as dim fit probably based on their studies. However, in broad terms, it is an infection whose development is aided or favored by the hospital environment. This may be due to the fact

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that the infectious agents are shed off in large amount by patient on admission or taking treatment for various diseases, injuries or infections. They are acquired by either a patient during a hospital visit (or when hospitalized) or hospital staff [1].

According to [2] nosocomial have been said to be responsible for 1.7 million hospital-associated infections presently in the United States and this figure is expected to triple by 2021. It is also known to be responsible for 25,000 deaths in Europe annually from all types of microorganisms including bacteria [2]. These nosocomial infections, if not checked will continue to increase and cause resistance to antibiotics due to their contacts with different antibiotics in the hospital environment. They can cause severe pneumonia and infection of the urinary tract, blood stream and other parts of the body as they have been implicated.

These nosocomial infections are more commonly encountered in African countries and in Nigeria in particular. According to [3], this is primarily due to factors such as hospital hygiene / cleanliness, a level which most rural health care facilities are yet to attain in developing countries due to personal hygiene of patients, overcrowding hospital wards and illiteracy. These nosocomial infections usually prove difficult to treat with antibiotics the pathogens are used to contacts with most of the antibiotics and disinfectants used within the hospital environment. Equally, antibiotics resistance is fast spreading to more Gram positive and Gram-negative bacteria that can infect people within the hospital environment [4].

Since this hospital acquired infections are constantly spreading like wild fire, especially in developing countries and from person-person in cases of air-borne infections or beddings-person contact, it is an important public health issue of high interest. Ondo State has so many primary health centers in each community to cater for the immediate health needs of children, pregnant women, and adults within the community which they are located. These primary health centers should be constantly checked to ensure they don't house pathogenic bacteria and fungi either as reservoirs for these bacteria and fungi or as route of infection for patients, staff and visitors to the hospital [5].

Many different pathogens have been implicated as causative agents of these nosocomial infections. The infecting bacteria or microorganisms vary among different locations, hospitals, patient populations, different health care set-tings, different facilities, and different countries. However, microbial flora from the health care environment (endemic or epidemic exogenous environmental infections) occur as a result of several types of microorganisms' ability to survive well in the hospital environment such as, in water, damp areas, and occasionally in sterile products or disinfectants (*Pseudomonas*, *Acinetobacter*, *Mycobacterium*); included items such as linen, equipment and supplies used in care; in food and in fine dust and droplet nuclei generated by coughing or speaking (particles smaller than 10 µm in diameter remain in the air for several hours and can be inhaled in the same way as fine dust).

People at the various health centers, especially the care givers are therefore prone to being the main reservoir and source of microorganisms, main transmitter, notably during treatment and receptor for microorganisms in these sites of the health centres. Thus, becoming a new reservoir and can therefore contribute to the spread of infections within a particular community [4]. Worse still, is the fact that these microorganisms have become resistant to the common antibiotics. Hence, the need for appropriate control to protect and maintain the health status of people within the community using alternative local but effective drugs.

Garlic is a well-known plant that have been classified in the allium (onion) family which have very high antimicrobial properties and antioxidant strength. Many authors have worked on it and classified as closely related to onions, shallots and leeks that forms bulbs. It is segmented and each segment which is called garlic bulb is called a clove [6]. There are different species with different number of cloves depending on the size. However, averagely, there are about 10–20 cloves in most of the species' single bulb. Garlic plant have been grown in many parts of the world and used for different purposes. Generally, garlic is a popular ingredient in cooking industries for seasoning and this may be due to its strong smell and delicious taste depending on individuals. However, throughout ancient history, the main use of garlic was for its health and medicinal properties cannot be overemphasized as many authors have pointed out [7]. Its use was well documented by many major civilizations, including the Egyptians, Babylonians, Greeks, Romans and Chinese. Scientists now know

that most of its health benefits are caused by sulfur compounds formed when a garlic clove is chopped, crushed or chewed [8]. Perhaps the most famous of those is known as allicin. However, allicin is an unstable compound that is only briefly present in fresh garlic after it's been cut or crushed [9].

Bitter kola which is actually very bitter to taste, is often known as bitter cola or Garcinia kola. This plant is often found more in Central and Western Africa than any other region of the world. The bitter cola fruit have long been valued for its medicinal properties by people who eat it and use it for different purposes [10]. Bitter kola seeds also have been described to have a sharp, bitter flavor that eases into a slight sweetness as its being chewed. They are and mostly eaten raw or in its raw form and occasionally may be processed into other products. Bitter kola has been used in traditional African medicine for centuries to cure different ailment. Although, this is not documented, it is considered to have many beneficial properties, including being able to help fight bacterial and viral infections. In terms of taste, the plant is well named among the plants with edible and pleasant taste [11]. The edible seeds have a notably bitter taste, while there is also a slight sweetness to them, especially after chewing it according to [7]. When consumed, bitter kola have been confirmed to offer many nutritional benefits, and studies have shown that the plant is high in carbohydrates, fat, protein, vitamin c, calcium, potassium, iron and caffeine according to [12].

Sesame, which is also known or popularly called beniseed is gotten from the sesame plant that well grown in northern and central Nigeria. They are well known to be highly rich in several important compounds and nutrients that add value to the overall human health. The presence of a good number of nutrient-dense condiments in beniseed that provides a lot of benefits to the health and total well-being of man include vitamins, mineral contents and other enzyme activators. The seeds also have been said to serve several other culinary purposes in different countries of the world according to [6].

Sesame (beniseed) is equally proven to contains phytonutrients as well as phytosterols which helps in lowering the level of cholesterol in the body by reducing its rate of absorption and can as well help in weight loss as it burns body fats and fatty deposits in the human body as outlined by [7]. Phytosterols, which are known to be plant sterols that are comparable to cholesterol but conversely functions actively in the intestine to reduce as well as displace the level of cholesterol absorption. Research has shown that sesame has the highest content of this phytosterol and that is why it ranks the highest in lowering cholesterol as outlined by several authors such as [12].

Castor oil, which on several occasion and in several countries have been used as medicine for centuries has not been well documented scientifically. Castor seeds without the hull have also been used for different therapeutic and prophylactic purposes such as birth control, constipation, leprosy, and syphilis by several traditional herbal practitioners. Castor oil is used as a laxative for constipation, to start labor in pregnancy, and to start the flow of breast milk. Some people apply castor seed paste (a traditionally prepared liquid of castor seed soaked and grinded into paste) to the skin as a poultice for inflammatory skin disorders, boils, carbuncles, pockets of infection (abscesses), inflammation of the middle ear, and migraine headaches as documented by [13].

Castor oil equally have been specifically used topically to soften skin, bunions and corns; and to dissolve cysts, growths, and warts in human skin infection therapy. It is also applied to the skin for osteoarthritis. Some women put castor oil inside the vagina for birth control or to cause an abortion [7]. Castor oil have been used even till date in the eyes to soothe membranes irritated by dust or other materials in areas of the world where sand dunes often affect children's eyes.

The present research is a comparative study of the antimicrobial properties of castor oil, garlic, beniseed and bitter cola plants' extracts on microorganisms isolated from hospitals' wards.

Methods

Sample collection

The major instrument of collection was the sterile swab sticks containing 1 ml of sterile distilled water which was used to swab the different experimental surfaces of beddings (such as pillows and bed sheets), toilet seat surfaces, floor and door handles of male, female and children wards from the following primary health centers in Ilara-Mokin town:

1. Primary health center, Express (PHCE);
2. Primary health center, Wuraola (PHCW);
3. Primary health center, Post office (PHCP).

Sample were collected in triplicates from each surface.

Sterilization of glass wares

All the glass ware used such as conical flasks (of different sizes), reusable glass Petri dishes, measuring cylinder, McCartney bottles and test tubes were washed properly with cleaning agent and sterilized using oven at a temperature of 180^0C for 2 hours [14].

Media preparation

The different media to that was used (Nutrient agar, Eosin-Methylin Blue agar, McConkey agar, Mueller Hinton agar and salmonella-shigella agar), the media were carefully prepared according to the manufacturers' instructions before sterilizing at 121^0C for 15 minutes in an autoclave, with exception of salmonella -shigella agar as outlined by [14]. The method of [1] was employed for subculturing and biochemical tests (such as citrate test, coagulase, catalase, etc.) on isolates.

Fermentation of sugar

Different types of sugar were used such as lactose, mannitol, arabinose, maltose, glucose, and fructose according to the methods outlined by [1]. The sugars were weighed and diluted in sterilized cold water with 0.01 sodium chloride and the phenol red as indicator, before dispensing them into clean test tube and sterilizing them accordingly. They were then inoculated aseptically after allowing them to cool down according [1].

Antibiotic Susceptibility Testing

a) Standardization of inoculum

This was done using standard plate count and absorbance methods. The organism in broth was standardized using McFarland standard of 0.5 and the absorbance taken. 1 ml of the standardized broth was then cultured and the number of colonies counted after 24 hours. The number of colonies was then expressed in standard form and used for all other assays using [15] as guide.

b) Susceptibility testing

Antibiotic susceptibility of the isolates was determined by disc diffusion assay following CLSI guidelines [15]. The strains were tested for susceptibility against amoxycillin, cephazolin, gentamicin, amikacin, cefotaxime, ceftazidime, ciprofloxacin, netilmicin and piperacillin. They are commercial antibiotic prepared and marketed by Optu disc, United States of America. All the strains were tested twice without any discrepancy between results.

c) Reading and interpretation of results

The strains were scored as sensitive, intermediate (moderately sensitive) or resistant according to Clinical and Laboratory Standard Institute [15] guidelines.

Extraction from castor, garlic, beniseed and bitter cola

One hundred grams each of castor, garlic, beniseed and bitter cola was pounded in mortar and soaked in 300 ml each of the extracting solvent (ethanol) inside a transparent container (500 ml conical flask) for 72 hours in different flasks. The extracts were sieved using different sterile injection filter and allowed to settle before drying using rotary evaporator respectively according to the method of [16].

Susceptibility of the extracts on isolates

The antibacterial sensitivity testing of the extracts was done using the agar cup plate diffusion method according to [1] from a standard culture after boring a hole in the agar using 4 mm cock borer triplicates.

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 26 using one-way analysis of variance (ANOVA).

Results

Results obtained from the bacterial isolation from the various parts of the different wards in Primary health centers visited showed the following. Express health centre had the highest bacterial load of 52.00 ± 3.46 cfu/ml that was obtained from toilet situated in maternity ward of the health centre. On the other hand, the bacterial load of 2.67 ± 0.67 cfu/ml obtained from the floor of the male ward of the health centre was the minimum. On a general note, the toilets of all the wards of the health centres visited were the ones that recorded the highest bacterial load obtained throughout this research work with the male wards' toilet recording the least bacterial load consistently. On a comparative ground, it was observed that the floor of all the health centres of the wards had the least bacterial load. Also, worthy of note is the fact that there was no surface from which bacteria was not isolated in this basic health centers. Result obtained for the bacterial load from the basic health center is displayed in **figure (1).**

Figure 2 displays or shows the mean bacterial load from Primary health center, Wuraola.

The bed sheet from this health center recorded the highest bacterial load of 28.33 ± 3.18 cfu/ml. the pillow from the paediatric ward had the least bacterial load of 1.67 ± 0.33 cfu/ml. generally, the bacterial load from the paediatric ward of this health center was low for all the surfaces evaluated. The same pattern of bacterial load was recorded for Primary health center, Post office, except that the toilet recorded the highest bacterial load of 19.00 ± 2.65 cfu/ml. the bacterial load was least for all the surfaces evaluated in the paediatric ward of this health center. This result is shown in **figure (2).**

Figure 4 shows a comparative analysis of bacterial isolations from the various wards of the three (3) health centres where isolation was done from.

The results obtained in the course of the isolation from the different sampling locations showed a total of twelve (12) different bacteria distributed across the locations. They are *Salmonella typhi, Bacillus licheniformis, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas putida, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Serratia marcescen, Proteus vulgaris* and *Micrococcus luteus*. The fungal isolates from the location were equally identified. A total of nine (9) fungi were identified and they are *Aspergillus niger, Aspergillus flavus, Penicillium italicum, Fusarium oxysporium, Muco mucedo, Rhizopus stolonifer, Aspergillus fumigatus, Aticulospora inflata and Pithomyces species*. The results of the bacterial identification result are outlined in **tables (1, 2),** while the results of the macroscopic and microscopic process of identification of the fungi are presented in **table (3).**

Table 4 shows the total number of isolates from each health center. A total number of eight (8) isolates were obtained from Primary health center, Express (PHCE) and Primary health center, Post office (PHCP) respectively. This number represents the highest number of isolates from all the health centers. The least number of isolates of five (5) was obtained for Primary health center, Wuraola (PHCW). The isolates were subjected to different identification processes.

Table 5 shows the distribution of isolates from various basic health centers evaluated. Worthy of note is the fact that *Pseudomonas* was isolated from two of the centers while *Staphylococcus aureus* and *Staphylococcus epidermidis* was isolated from all the basic health centers.

The distribution table showed that the toilets and the bed sheet housed most of the isolates in this work. All the isolates were present in the toilets and only two out of the eight were not isolated from the bed sheets. This is shown in **table (6).**

Tables 7a and **7b** are the results of the diameter of zones of inhibition of the various extracts on the bacteria isolates. In all the extracts, the garlic extract exhibited the highest diameter of zones of inhibition against all the Gram-negative and Gram-positive bacteria respectively. The other three (3) extracts tend to exert their effect selectively; either on the Gram-positive or Gram-negative bacteria, but generally more on the Gram-positive bacteria. The garlic extract exerted the highest diameter of zone of inhibition on the *Bacillus* genus with the highest diameter of zone of inhibition on *Bacillus licheniformis* with a diameter of 18.20±2.05 mm. The garlic extract also exerted the highest diameter of zone of inhibition on *E. coli*.

The bitter cola extract exerted its highest diameter of zones of inhibition against *Staphylococcus aureus* with a zone diameter of 12.00±0.00 mm, while the *Pseudomonas* species were not susceptible to it. Generally, all the Gram-negative *Enterobacteriaceae* were not too susceptible to it. Sesame extract on the other hand exerted its highest diameter of zone of inhibition on Gram-positive *Bacillus* species. Although, none of the bacteria isolates was completely resistant to the sesame extract, the Gram-negative bacteria were more susceptible to the extract. The castor oil also exerted a higher effect on the Gram-positive bacteria than the Gram-negative bacteria. The castor oil exerted its highest diameter of zone of inhibition of 16.40±1.20 mm on *Staphylococcus aureus*, while its minimum zone of inhibition of 4.00±0.00 mm was on *Pseudomonas putida*.

Table 8 shows the result of the sensitivity of commercial antibiotic discs on the bacterial isolates from the health centres. Generally, most of the bacteria were not susceptible to the antibiotics while majority of the antibiotics recorded very low sensitivity on the bacteria. Augmentin was sensitive on and had effect on Gram-positive bacteria, while Gram-negative bacteria were not susceptible to it. Ofloxacin had the highest diameter of zone of inhibition against *Staphylococcus epidermidis* with a zone diameter of 22.00±1.40 mm, but was not sensitive at all on *S. aureus* and *Serratia*. Serratia marcescen generally was not susceptible to most of the antibiotics. *E. coli* was not susceptible to most of

the antibiotics except nalidixic acid to which the diameter of zone of inhibition recorded was 10.00±0.40 mm.

Table 1. Colonial Morphology of bacteria Isolated from water samples.

Isolate no	Pigmentation /color	Shape	Edge	Optical character	Consistency	Colony surface	Spore form	Gram's reaction	Motility
1	White	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+
2	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Positive	+ve rod	+
3	Cream	Circular	Entire	Transparent	Butyrous	Rough	Negative	-ve rod	+
4	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Negative	+ve cocci	+
5	White	Irregular	Undulate	Opaque	Granular	Smooth	Negative	+ve cocci	-
6	White	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+
7	White	Irregular	Undulate	Opaque	Granular	Smooth	Negative	-ve rod	+
8	Light yellow	Irregular	Lobate	Translucent	Viscid	Smooth	Negative	-ve rod	+
9	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Positive	+ve rod	+
10	Greenish	Circular	Entire	Opaque	Viscid	Smooth	Negative	-ve rod	+
11	White	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+
12	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Negative	+ve rod	+

Key: += Positive; - = Negative

Table 2. Biochemical and sugar fermentation reactions of isolates.

Keys: Cat= catalase, Oxi= oxidase, Ind= indole, H_2S= Hydrogen Sulphide, Nit red= nitrogen reduction, Ure= urease, Lact= lactose, Fruc= fructose, Malt= maltose, Gala= galactose, Glu= gluctose, Arab= arabinose, Raf= rafinose, Man= manitol, MR=methyl red, VP= Voges-proskauer, - = negative, + = positive.

Isolate no	Cat	Oxi	Ind	H_2S	Nit red	Ure	Lact	Fruc	Malt	Gala	Glu	Arab	Raf	Man	MR	VP	Identified organism
1	-	-	-	-	+	-	-	+	-	+	+	-	+	-	+	+	*Salmonella typhi*
2	-	+	+	-	-	-	+	-	+	-	-	+	+	-	-	+	*Bacillus licheniformis*
3	-	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	*Pseudomonas putida*
4	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	*Staphylococcus epidermidis*
5	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	*Staphylococcus aureus*
6	-	-	+	+	-	+	-	+	-	-	+	-	-	-	+	-	*Proteus vulgaris*
7	-	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	*Escherichia coli*
8	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	*Serratia marcescens*
9	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	*Bacillus subtilis*
10	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	*Pseudomonas aeruginosa*
11	-	-	-	-	+	-	+	+	-	+	+	-	+	-	+	+	*Klebsiella pneumoniae*
12	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	*Micrococcus luteus*

Table 3. Macroscopic and microscopic identification of isolated fungi from the water samples

Morphological characteristics	**Microscopic Examination**	**Suspected fungi**
A velvety filamentous white colour of growth that sporulate black powdery spores was observed using hand lens.	The long septate hyphae with conidiophore bearing brown spores and philia at its apex was very clear.	*Aspergillus niger*
The colour observed was brownish smooth circular and raised colony of growth	Presence of arthrospore were seen with rounded end	*Aspergillus fumigatus*
The colour seen is pink fluffy hyphae and spreading colonies which is creamy around the edges of growth.	Septate hyphae that a sickle-like chlamydospores at the hyphae was seen.	*Fusarium oxysporium*
Dark green, velvety and spongy edges	The spores were brush-like with bearing structures	*Penicillium italicum*
A pale to dark brown colour having cottony texture and very slow sporulation is observed	Spores are multicellular and deeply pigmented with presence of both transverse and longitudinal divisions, spore shape is club shaped.	*Pithomyces species*
Pale to dark brown colour and few sporulation	Spores are multicellular and deeply pigmented, presence of both transverse and longitudinal divisions as an oval shape	*Articulospora inflata*
A white and woolly aerial growth was observed. It darkens as it sporulate	Non-septate hyphae with straight sporangiophore were seen with many spherical spores on top	*Mucor mucedo*
Long hyphae growth was seen which sporulated within two days to turn into black spore colour.	Non-septate, branched mycelium with round shaped sporangia was seen in large numbers	*Rhizopus stolonifera*
It had a greenish smooth circular and raised colony of hyphal growth	Presence of arthrospore spores with rounded endand oval tips.	*Aspergillus flavus*

Table 4. Total number of isolates identified from each basic health center.

S/N	**Health Center**	**No. of isolates**
1	Primary health center, Express (PHCE)	8
2	Primary health center, Wuraola (PHCW)	7
3	Primary health center, Post office (PHCP)	8

Table 5. Distribution of all the isolates from various health centers.

Center	1	2	3	4	5	6	7	8	9	10	11	12
PHCE	+	+	+	+	-	+	-	+	+	-	-	+
PHCW	+	+	+	+	+	-	-	-	+	-	+	-
PHCP	+	+	+	+	+	+	+	+	-	+	+	-

Keys: +=Present / Isolated; -=Absent / Not isolated

Table 6. Distribution of isolates in surfaces swabbed.

S/N	Surface	1	2	3	4	5	6	7	8	9	10	11	12
1	**Bed sheet**	-	+	+	+	-	+	+	-	-	+	+	+
2	**Door handles**	+	+	+	-	+	-	-	-	+	+	+	-
3	**Floor**	+	+	-	-	+	+	+	-	+	+	-	-
4	**Pillow**	-	+	+	-	-	-	+	-	-	+	+	-
5	**Toilets**	+	+	+	+	+	+	+	+	+	+	+	+

Keys: 1= *Escherichia coli;* 2= *Staphylococcus aureus;* 3= *Staphylococcus epidermidis;* 4= *Proteus mirabilis;* 5= *Bacillus subtilis;* 6= *Pseudomonas aeruginosa;* 7= *Klebsiella pneumoniae;* 8= *Candida albicans*; 9= *Serratia marsescen*, 10=*Salmonella typhi*;11= *Bacillus licheniformis;* 12=*Pseudomonas putida.*

Table 7a. Diameter of zones of inhibition of garlic extracts on bacteria isolates.

S/N	Bacteria	Garlic extract	Bitter cola extract
1	***Salmonella typhi***	8.75±1.15	4.20±1.12
2	***Bacillus licheniformis***	18.20±2.05	10.10±1.10
3	***Pseudomonas putida***	10.00±0.00	2.00±0.00
4	***Staphylococcus epidermidis***	12.90±1.20	11.20±1.30
5	***Staphylococcus aureus***	10.25±1.58	12.00±0.00
6	***Proteus vulgaris***	12.55±0.55	6.20±0.60
7	***Escherichia coli***	14.10±1.10	8.75±1.15
8	***Serratia marcescens***	13.65±0.55	8.20±2.05
9	***Bacillus subtilis***	16.90±1.30	10.00±1.00
10	***Pseudomonas aeruginosa***	12.30±1.57	0.00±0.00
11	***Klebsiella pneumoniae***	10.20±0.60	4.25±1.58
12	***Micrococcus luteus***	12.54±1.02	10.05±0.05

Table 7b. Diameter of zones of inhibition of ginger extracts on bacteria isolates.

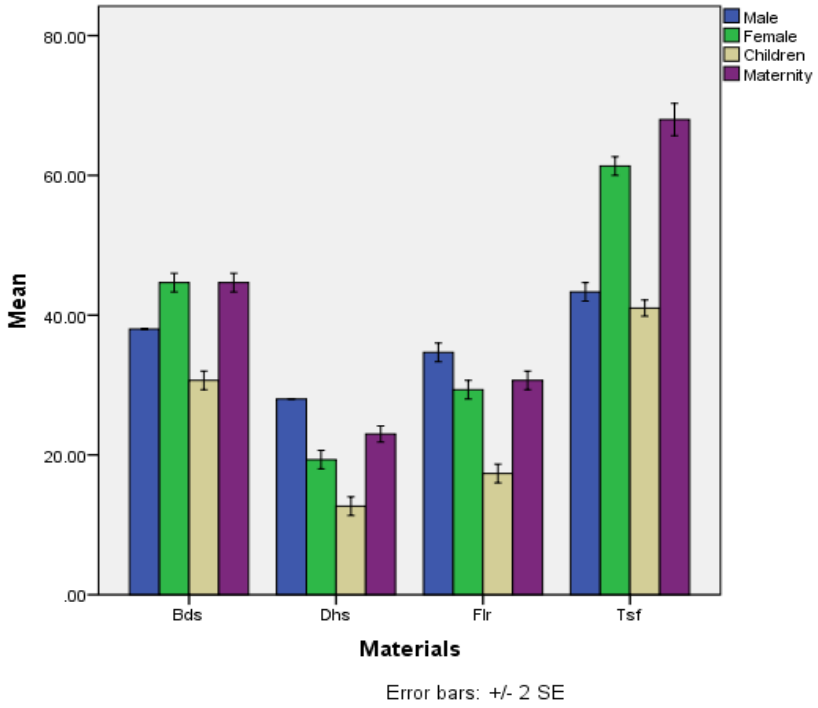
S/N	Bacteria	Sesame extract	Castor oil extract
1	***Salmonella typhi***	6.40±1.10	10.00±0.00
2	***Bacillus licheniformis***	13.10±2.10	14.20±1.30
3	***Pseudomonas putida***	4.00±0.00	4.00±0.00
4	***Staphylococcus epidermidis***	6.20±1.30	16.40±1.20
5	***Staphylococcus aureus***	4.00±0.00	15.33±1.35
6	***Proteus vulgaris***	8.40±0.20	6.00±0.00
7	***Escherichia coli***	6.25±1.05	7.33±0.33
8	***Serratia marcescens***	8.20±2.05	5.00±1.50
9	***Bacillus subtilis***	10.00±0.00	15.13±1.33
10	***Pseudomonas aeruginosa***	6.00±0.00	4.20±0.10
11	***Klebsiella pneumoniae***	8.25±1.58	6.33±0.13
12	***Micrococcus luteus***	6.05±1.05	14.17±2.13

Table 8. Diameter of zones of inhibition of commercial antibiotics used on test isolates.

Bacteria	Aug	Amp	Ofl	Gen	Cot	Nit	Nal	Tet
Salmonella typhi	6.20±0.40	4.00±0.00	12.40±1.10	5.00±0.00	4.00±0.00	0.00±0.00	10.00±0.00	6.00±0.00
Bacillus licheniformis	10.20±0.20	7.00±0.00	16.10±0.10	2.00±0.00	4.50±0.10	0.00±0.00	8.20±0.60	4.00±0.00
Pseudomonas putida	0.00±0.00	6.00±0.00	18.00±0.00	2.00±0.00	2.00±0.00	4.00±0.00	8.00±0.00	6.11±0.13
Staphylococcus epidermidis	2.00±0.00	0.00±0.00	22.00±1.40	0.00±0.00	0.00±0.00	4.00±0.00	6.03±0.33	0.00±0.00
Staphylococcus aureus	2.00±0.00	4.50±0.10	0.00±0.00	8.20±0.60	4.00±0.00	2.00±0.00	4.10±0.20	9.05±0.55
Proteus vulgaris	2.00±0.00	2.00±0.00	4.00±0.00	8.00±0.00	6.11±0.13	2.00±0.00	4.50±0.05	5.15±0.25
Escherichia coli	0.00±0.00	0.00±0.00	4.00±0.00	6.03±0.33	0.00±0.00	0.00±0.00	10.00±0.40	5.13±0.17
Serratia marcescens	2.00±0.00	4.50±0.10	0.00±0.00	8.20±0.60	4.00±0.00	2.00±0.00	6.03±0.33	0.00±0.00
Bacillus subtilis	6.00±0.00	5.00±0.00	12.00±0.00	2.00±0.00	4.50±0.10	0.00±0.00	8.20±0.60	4.00±0.00
Pseudomonas aeruginosa	2.00±0.00	3.00±0.00	11.00±0.00	2.00±0.00	2.00±0.00	4.00±0.00	8.00±0.00	6.11±0.13
Klebsiella pneumoniae	0.00±0.00	12.00±0.00	22.00±0.00	0.00±0.00	0.00±0.00	4.00±0.00	6.03±0.33	0.00±0.00
Micrococcus luteus	2.00±0.00	0.00±0.00	10.00±0.00	2.00±0.00	4.50±0.10	0.00±0.00	8.20±0.60	4.00±0.00

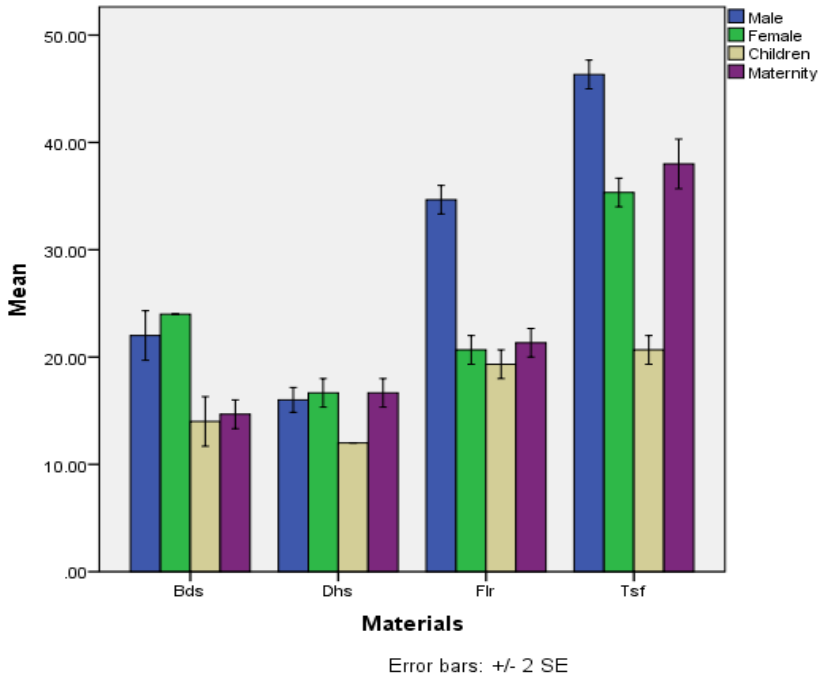
Key: Aug=Augmentin, Amp=Ampicillin, Ofl=Ofloxacin, Gen=Gentamycin, Cot=Cotrimoxazole, Nit=Nitrofurantoin, Nal=Nalidixic acid, Tet=Tetracyclin

Figure 1: Result of mean bacterial load (cfu/ml) from Primary health center, Express.



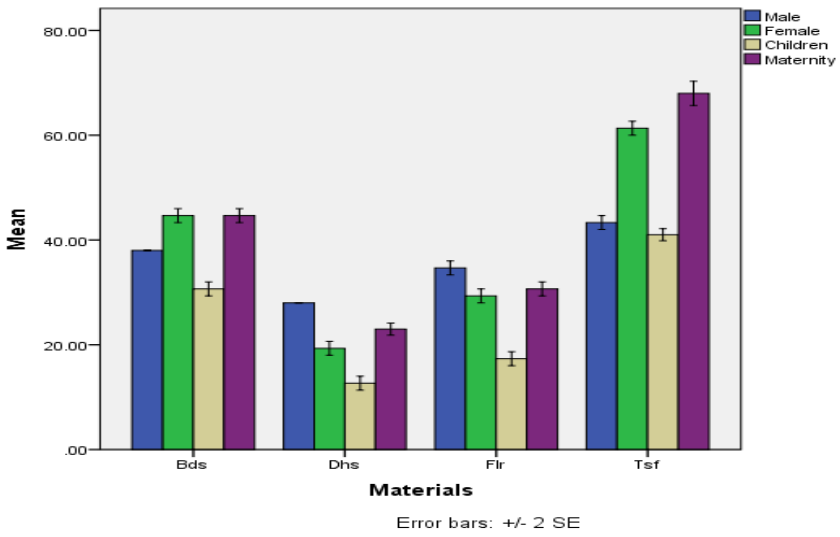
Legends: Bds=Bed sheet; Dhd=Door handles; Flo=Floor; Pil=Pillow; Toi=Toilet

Figure 2. Results of the mean bacterial load (cfu/ml) from Primary health center, Wuraola.



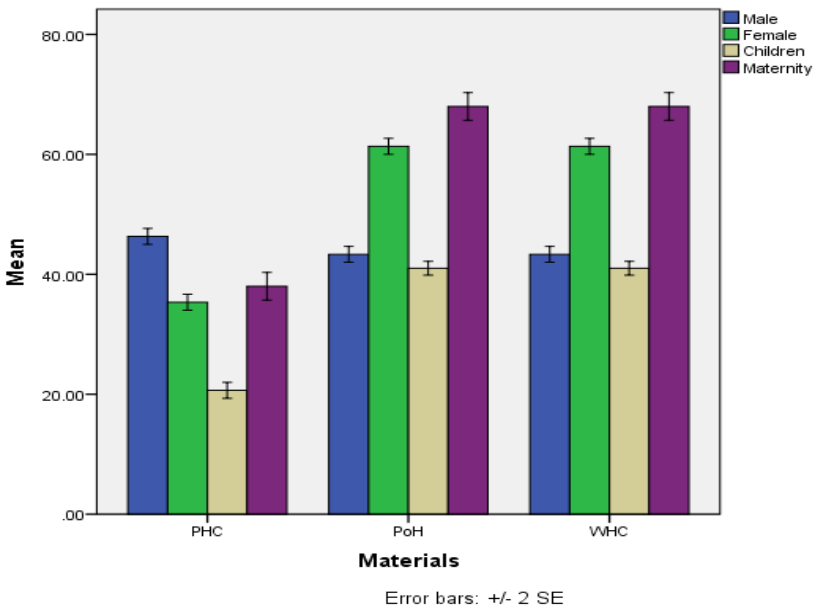
Legends: Bds=Bed sheet; Dhd=Door handles; Flo=Floor; Pil=Pillow; Toi=Toilet

Figure 3. Result showing the mean bacterial load (cfu/ml) from Primary health center, Post office.



Legends: Bds=Bed sheet; Dhd=Door handles; Flo=Floor; Pil=Pillow; Toi=Toilet

Figure 4. Comparative bacterial isolation from the various wards of the health centers.



Discussion

The findings from this research on the microbial state of the health facilities in these different health centers evaluated for their level of contamination, it is clear that the various health centers need special attention, especially in the rural areas of Nigeria as a country. Part of the need for attention is to reduce nosocomial infections which is one of the World Health Organization goals [36]. The results have revealed one clear weak-point that proper cleaning methods such as surface sterilization of all the facilities in these basic health

centers should be adopted on a regular basis for reducing the microbial loads on them which is presently lacking [17]. The results obtained for the bacterial load of the bedsheet from different wards in the different basic health centers is similar to the results obtained by [18] who recorded a sudden increase or surge in the recorded microbial load of bedsheet in pediatric ward of a hospital were children were admitted as a result of communicable disease that was considered endemic to the region. According to [19], some major reasons for the high bacterial load often obtained from pillows and beddings in different wards or units in the hospitals weather in developed or developing countries are mostly due to the fact that most of the patients are under critical health conditions and are most times not able to bath for days. Furthermore, most hospitals in developing countries as always, the case, may not have sufficient or enough of these bedding materials for daily changing and as a when due. Another reason for this common occurrence may be the concentration of the disinfectants used for cleaning which mostly overdiluted and had become very low resulting in minimal effect on microorganisms in such materials.

On the basis of the results of the isolation and determined bacterial loads from the various wards sampled or evaluated, the toilets which contain the highest bacterial load from all the basic health centers evaluated or assessed, serious and immediate requirement of special hospital managements' attention is needed in terms of washing procedures, disinfection of available water used for proper flushing immediately after use as well as for bathing by patients and caregivers in the wards respectively. The toilets on the other hand, however, are not expected to be sterile. Since, waste products are emptied on the toilets of the wards of the health centers on regular basis as long as patient and staff of the health centers remain there. However, according to [20], the admitted patients who may be on antibiotics visit the toilets frequently, therefore, the toilets must be kept very clean always to prevent nosocomial infections. This is because diseased patients are likely to excrete pathogenic microorganisms; some of which are likely to be antibiotic resistant as they have been exposed to different antibiotics at different concentrations.

Equally, it has been observed that more often than not, visitors or relatives coming to the hospitals environments who may be carriers of pathogenic microorganisms visit the toilets and often add or contribute to the bacterial load of such as observed by [21]. **Mitropoulou et al.** [22] were also of the opinion that the laboratories established for diagnosis and investigations in these basic health centers should carry out routine bacterial isolation, bacterial load assessment and antibiotic sensitivity assays of their toilets as well as assessment of the sterilizing and sanitizing agents, so as to affirm their present status; whether they are effective against the pathogens found in such environment (toilets and other materials) or not. Another suggestion is that there should be constant analysis of the water for flushing and washing of the hospital environments such as toilet, bathtubs, and floor surfaces. This will help to know if the water source serves as reservoirs of these bacteria or not.

Another important point to consider and put into consideration is the isolation of bacteria from the door handles of these basic health centers. This result has shown that there is need for constant as well as adequate hand washing and washing hand basins before entrance into the different wards of the basic health centers and when leaving the wards. This research has shown that the door handle is a major way nosocomial infection can spread like wide fire, especially in this era of corona virus infection. **Emmerson** [23] in his recent work of survey of infection in hospitals and hospital environments showed that 40% of nosocomial infections may be obtained from door handles. Therefore, it is right to conclude that the need for constant swabbing of the door handle to reduce the microbial load per time cannot be overemphasized.

The bacterial load results obtained from the floor of the wards are similar to the result obtained by [24]. When he isolated microorganisms of different types from the floor of the maternity wards of selected hospitals in central part of Greece. This result is also in accordance and concord with the result obtained by [25] when they isolated bacteria from the Pediatric wards (where injured children receiving treatment are admitted) of selected hospitals in Greece. They concluded that since the wards are not restricted to certain people of high hygienic status, many of them would be a source of the bacteria coming from either hands or bodies as well as waste products.

Many factors that have contributed in one way or the other to the frequency of nosocomial infections are not far-fetched. Among these is a

paramount fact that the hospitalized patients are often immuno-compromised (having low or reduced immunity against pathogens). Most of them are even subjected to invasive examinations and treatments that cause them to be weak or break down completely. Other reasons include the sort of patient care practices, and the hospital environment may facilitate the transmission of microorganisms among patients. There is also the selective pressure of intense antibiotic use presently that is known to promote antibiotic resistance in our communities today. While progress in the prevention of nosocomial infections has been made tremendously [36, 37], some changes in medical practice has continually present us and is presenting us with new opportunities for development of infection.

According to the possibility outlined by [1], there is correlation between the number of patients visiting a hospital and the number of visitors trooping into such hospital to visit. **Benenson's** research [18] ended up by stating that this point has and is being well abused in developing countries, Nigeria inclusive, where as a patient may have two or more and, in some cases, multiple visitors coming daily, depending on how popular the patient or his family is in the community. In this scenario, they all want to see the patient at all costs to register their presence and appear nice showing maximum concern about the patient on hospital admission. Due to this reason, [26], concluded that the floor, door handle as well as the chairs that all the visitors to the patient and staff of the hospitals would come directly in contact with cannot and will not be devoid of pathogenic microorganisms, especially pathogenic bacteria. Hence, the need arises for adequate ward or hygienic cleaning of the ward's toilets in these health centers. This will in turn help to reduce the risk of nosocomial infections invariably for both the patients who are already admitted for different infection. It will equally be valuable for the visitors who previously may be free of certain infection before coming to the health centers for visitation and the hospital staff attending to the patient on a regular basis daily in the wards.

The fungi isolated from these hospital environments are fungi that can survive in adverse environmental conditions. Research has shown that fungi do not require much water for their survival in different environments. According to [3], most of these fungi are dispersed in air via their spores and find their ways to different environments where they settle down, grow, and reproduce. All the fungi isolated from these environments are spore formers and their spores are ubiquitous and highly infective when inhaled by humans and animals. Although, there was no measurement of the quantity of spores present in these hospital environments, their presence in these environments poses potential hazards in the hospital environment. It also speaks of the hygienic level of these environments and calls for examination of the potency of the disinfectant(s) used for the cleaning.

The potency of the extracts tested on the bacteria isolates may be from the various phytochemicals named to have been detected in them by various authors. These active phytochemicals include alkaloid, terpenoid, flavonoids, etc. Although, the quantitative phytochemicals were not carried out, [27] listed some of the phytochemicals detected in these extracts as having antibacterial properties. Among these phytochemicals with antibacterial activities are terpenoid and flavonoid. Though the mechanism of action of the extract was not studied, the presence of biologically active chemicals such as saponin, tannin, phenol, cyanogenic glycoside, and flavonoids could be responsible. According to [28] phenol is a hydroxyl benzene, an antiseptic, anesthetics, and a disinfectant that has been found to be escharotic in concentrated form and neurolytic in 3-4% solution that makes it an escharotic poison internally.

Saponin on the other hand is said to have foaming properties in water and capable of lysing cells (as in haemolysis of erythrocytes). It has powerful surfactant property and is antibiotic in nature. Flavonoid contains flavone in various combinations (anthoxanthins, apigenins, flavone, quercitins) that gives it varying biological activities. Alkaloid is a heterocyclic nitrogen containing substances such as morphine, atropine, codeine sulfate/phosphate and colchicines that makes it possess pharmacological activity and constitute the active principle of the crude drug nature. Cyanogen on the other hand consists of cyano radicals that make it highly toxic, especially to microorganisms and cells. The presence of these biologically active components, especially phenol and cyanogens also explain the reason for the low lethal dose of the extract on laboratory animals, as well as the reason for giving the animals 0.5ml of the extract during the bioassay [29]. The compounds containing phenolic groups are usually most effective.

The results of the susceptibility of the extracts on the isolates showed that the garlic extract exhibited the highest diameter of zones of inhibition against all the Gram-negative and Gram-positive bacteria respectively; but generally, more on the Gram-positive bacteria. The garlic extract exerted the highest diameter of zone of inhibition on the *Bacillus* genera with the highest diameter of zone of inhibition on *Bacillus licheniformis*, while it also exerted the highest diameter of zone of inhibition on *E. coli*. According to [30], most of these Gram-negative pathogens in hospital environment have become highly resistant to antimicrobial agents. However, the high susceptibility of garlic extracts against most of these bacterial pathogens have been attributed to the presence of allicin compounds in it which have high antibacterial properties [31,32].

The bitter cola extract exerted its highest diameter of zones of inhibition against *Staphylococcus aureus*, while the *Pseudomonas* species were not susceptible to it at all. This result is similar to the results obtained by [33] in which the extract of bitter cola was not effective against Gram-negative bacteria isolates tested on. Generally, all the Gram-negative Enterobacteriaceae were not too susceptible to it. Sesame extract on the other hand exerted its highest diameter of zone of inhibition on Gram-positive *Bacillus* species. Although, none of the bacteria isolates was completely resistant to the sesame extract, the Gram-negative bacteria were more susceptible to the extract.

The castor oil also exerted a higher effect on the Gram-positive bacteria than the Gram-negative bacteria, especially on *Staphylococcus aureus*. According to [34], this is why the folklore medicine employ castor oil in treatment of infectious diseases such as impetigo, carbuncle and other infections caused by *Staphylococcus aureus* bacteria.

The result of the sensitivity of commercial antibiotic discs on the bacterial isolates from the health centers in which most of the bacteria were not susceptible to the antibiotics comparatively showed the extracts to be more effective than the antibiotics. In a related study carried out by [35] on some pathogenic bacteria reported that it may be due to accumulation of resistant genes due to constant exposure to these antibiotics in hospital environment.

Conclusion

One solution to resistant bacteria responsible for nosocomial infections in hospital environments may the either the incorporation of natural highly effective antibacterial extracts into existing antibacterial agents and disinfectants. Some of these natural products or extracts include the one that have been tested in this work that comparatively exceeded the potency of antibiotics in this research. However, the dosage and the amount to be used as semi-synthetic antibiotics have not been studied. Also limiting the use as seen in this research is the fact that the Gram-positive bacteria are not very susceptible to the extracts.

Abbreviations

PHC: Primary health center; SD: Standard deviation; CLSI: Clinical and laboratory standard institute; SPSS: Statistical Package for Social Science; ANOVA: Analysis of variance; WHO: World Health Organization.

Ethics approval and consent to participate

The study was approved by the Local Ethical Committee of Elizade University. Written consent was obtained from every health center prior to the procedures. This study has been carried out in accordance with the code of Ethics of the Ondo State Hospitals' Management Board, Nigeria.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Authors' contributions

Both authors one and two designed the study, analyzed and made the interpretation of data; and developed the first draft of the manuscript. Authors 3 and 4 were deeply involved in the laboratory analysis and extractions. All authors read, edited, and approved the final manuscript.

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Competing interests

No conflict of interest nor competing interest were observed.

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