



EFFECT OF HONEY AND ALOEVERA ON PATHOGENS ISOLATED FROM CUTS AND WOUND

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Abstract: - Ten samples of cuts and wounds were taken randomly from Students around the school premises (Nnamdi Azikiwe University, Awka). These samples were serially diluted, cultured at a temperature of 37° C. These isolates were characterized based on their morphological appearance, gram staining, Catalase test, Oxidase test, Methyl red test, Voges Proskauer test, Iodine test, Glucose test, Sucrose test, and Maltose test. the Organisms were identified as *Candida albicans* Spp, *Klebsiella* Spp, *Enterobacter* Spp, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* Spp. These isolates were further sub cultured into mueller-hinton agar specifically disk diffusion test. The antimicrobial susceptibility or resistance of the organism to honey and aloe vera were tested. The zones of inhibition displayed shows that some of the isolates were resistant or sensitive to Honey and Aloe vera. Honey shows great sensitivity towards inhibiting the growth of *Klebsiella* spp and *Escherichia coli* spp respectively while aloe vera shows no sensitivity towards *Candida albicans* spp and *Enterobacter* spp.

Keywords: Honey, Aloe vera, Wound pathogens, Antimicrobial activity.



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Introduction

Infectious diseases caused by bacteria and fungi remain among the leading causes of morbidity and mortality worldwide. The situation has been exacerbated by the rapid emergence and spread of antimicrobial resistance (AMR), which threatens the effectiveness of currently available therapeutic agents. Antimicrobial resistance has become a global public health concern because resistant microorganisms are no longer confined to specific geographical locations but are distributed worldwide, affecting both developed and developing countries (Srinivasan et al., 2014; McNeil et al., 2011). Studies have demonstrated the widespread occurrence of pathogenic microorganisms in diverse environments, foods, and

healthcare settings, highlighting their significance to public health (Awari et al., 2023; Victor-Aduju et al., 2023).

Several bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Neisseria gonorrhoeae*, have developed varying levels of resistance to commonly used antibiotics (McNeil et al., 2011). Recent studies have also reported increasing resistance among environmental and clinical isolates of *Pseudomonas aeruginosa* and other pathogenic bacteria, emphasizing the need for alternative antimicrobial agents (Awari et al., 2024; Obasi et al., 2024; Umeoduagu et al., 2023).

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Fungal pathogens likewise contribute significantly to global disease burden. Species belonging to the genera *Candida*, *Cryptococcus*, *Pneumocystis*, and *Aspergillus* are responsible for a wide range of infections, particularly among immunocompromised individuals (Pfaller et al., 2011). *Candida albicans* remains one of the most common causes of oral and systemic candidiasis, although infections caused by non-*albicans* *Candida* species are increasing worldwide (Perlin, 2015). The pathogenic potential of fungi has also been demonstrated in food spoilage and post-harvest deterioration studies, where fungal species have been implicated in significant economic losses and health hazards (Agu et al., 2014; Agu et al., 2016a; Agu et al., 2016b; Ogbo and Agu, 2015).

Although numerous antibacterial and antifungal drugs derived from microbial and synthetic sources are currently available, the emergence of antimicrobial resistance has reduced their effectiveness (Cortes et al., 2014). This challenge has renewed interest in medicinal plants and natural products as potential sources of novel antimicrobial compounds. Plant-derived bioactive substances possess diverse phytochemical constituents that can serve as templates for the development of new antimicrobial agents (Sanglard and Coste, 2015). Several studies have demonstrated the antimicrobial efficacy of plant extracts against a variety of pathogenic microorganisms. For example, extracts of *Cola gigantea*, *Carica papaya*, *Ginkgo biloba*, and *Dioscorea alata* have shown significant inhibitory activities against bacterial and fungal pathogens (Adindu et al., 2016; Awah et al., 2017; Ubaoji et al., 2020; Okigbo et al., 2015). Similarly, recent investigations have reported the antifungal activities of *Allium cepa*, *Allium sativum*, and *Ocimum gratissimum* against clinically important fungal species (Anazodo et al., 2024a; Orji et al., 2025).

Among medicinal plants, *Aloe vera* has received considerable attention due to its broad spectrum of pharmacological properties. Since ancient times, the gel and leaf exudates of *Aloe* species have been used in traditional medicine for the treatment of wounds, infections, inflammation, and other ailments (Chassot et al., 2016). The therapeutic effects of *Aloe* species have been attributed to the presence of anthraquinones, flavonoids, saponins, tannins, and other bioactive compounds with antimicrobial properties. In Ethiopia and several other African countries, species such as *Aloe megalacantha* are widely used for managing malaria, wounds, diabetes, gastrointestinal disorders, and microbial infections (Calvo et al., 2016). Experimental studies have further confirmed the antimalarial, wound-healing, anti-inflammatory, antidiabetic, and antimicrobial activities of *Aloe* species (Araujo et al., 2017).

Another natural product that has attracted considerable scientific interest is honey. Honey produced by *Apis mellifera* has been used for centuries as both a food and a therapeutic agent. Its medicinal value is largely attributed to its antibacterial, antifungal, anti-inflammatory, and wound-healing properties (Blum et al., 2013). Honey exhibits antimicrobial activity through multiple mechanisms, including osmotic effects, acidity, hydrogen peroxide production, and the presence of phytochemical compounds. Different varieties of honey, particularly *Leptospermum scoparium* (Manuka honey), have demonstrated inhibitory effects against a broad spectrum of Gram-positive and Gram-negative bacteria, as well as fungal pathogens (Blum et al., 2013; Akazar and Mellado, 2014). The increasing interest in apitherapy reflects the growing recognition of honey as a potential alternative or complementary treatment for infectious diseases.

Several studies have reported the successful use of natural products in controlling pathogenic microorganisms associated with food, oral infections, and environmental contamination. Herbal extracts and naturally derived products have shown inhibitory effects against *Streptococcus mutans*, pathogenic bacteria isolated from food products, and various spoilage fungi (Awah et al., 2016; Agu et al., 2013; Umeoduagu et al., 2023; Obianom et al., 2023). These findings support the continued exploration of medicinal plants and natural substances as sources of effective antimicrobial agents.

Despite the extensive traditional use and reported medicinal benefits of *Aloe vera* and honey, information regarding their comparative antibacterial and antifungal activities against selected pathogenic microorganisms remains limited. Therefore, this study was undertaken to evaluate the antimicrobial activities of *Aloe vera* and honey extracts against selected disease-causing microorganisms.

The aim of this study is to evaluate the antimicrobial activity of *Aloe vera* and honey extracts against selected disease-causing microorganisms. Specifically, the study seeks to investigate the antifungal and antibacterial properties of *Aloe vera* leaf extracts and to assess the antifungal and antibacterial activities of honey extracts against selected pathogenic microorganisms. Through this evaluation, the study aims to determine the potential of these natural products as alternative sources of antimicrobial agents for the control of microbial infections.

Materials and Methods

3.1 Sample collection

Ten (10) different samples of cuts and wounds were taken randomly from students in Nnamdi Azikiwe university, Awka. These samples were taken using a sterile sealed swab stick. The samples were corked and immediately taken to the laboratory for immediate analysis to avoid 222 drying up.

3.2 Analysis of sample

The collected wound samples were serially diluted, 0.1ml of 10^{-3} and 0.1ml of 10^{-4} were inoculated into sterile nutrient agar and Sabouraud dextrose agar plates and incubated for about 24 hours at 37°C. Different colonies based on shape, size, colour was observed. Macroscopic and microscopic examination and some biochemical test on each of the different colonies were done to identify the various organism present in the wound samples.

Antibiotic sensitivity testing: This was carried out using disc diffusion method. In this test, an aliquot (0.2mL) of the test organism was transferred from nutrient medium into sterile petri dish. The antibiotic discs were placed aseptically on the surface of the inoculated plates using sterile forceps.

Determination of Antimicrobial Activity of *Aloe vera* Gel and Honey against the Isolates: These were carried out using 5 ml each of *Aloe vera* gel, honey and a combination of *Aloe vera* gel and honey respectively. Aseptically prepared disc was impregnated with the dilutions that were dried using hot air oven. The plates were then incubated at 37 °C for 24 h. The zones of inhibition diameter were measured and recorded.

3.3 Identification of Isolates

Identification of isolates were based on morphological and biochemical characteristics following standard methods.

Gram staining

A Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope ($\times 100$). Purple colour indicated Gram-positive organisms while red or pink colour indicated Gram-negative organisms.

Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24-hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Oxidase Test

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Whatman No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observed the area around the inoculated paper for a colour change to deep blue or purple within 10-30 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). **Positive** was indicated by development of dark purple colour (indophenols) within 10 seconds. **Negative**: Absence of colour.

Methyl Red Test (MR Test):

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red-Voges Proskauer (MR-VP) broth. The production of a bright red colour

indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

VP Test (Voges Proskauer)

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5% α -naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that these reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

Indole Test:

This test was done as described by (Cheesbrough 2000), (Seenivasan, 2006) and (Oyeleke). Peptone water of 1.5g weight was dispensed into 250ml capacity conical flask. 100ml of distilled water was gradually added and shaken. It was then enriched with 1g of Tryptophan and heated on hot plate to homogenize and finally dispensed in to test tubes and corked for sterilization in the autoclave at $121^\circ C$ for 15minutes. A speck of each isolate was inoculated into 5ml of sterile peptone water enriched with 1% Tryptophan in test and was incubated at $37^\circ C$ for 48hours to the culture, 0.5ml Kovac's indole reagent was added and gently shaken. In a positive test, indole (present in the culture) dissolves in the reagent which then becomes pink or red, and forms a layer at the surface of the medium. A yellow layer at the surface of the medium was observed. Control test was read and recorded.

Sugar Fermentation:

Each of the isolates was tested or its ability to ferment a specific sugar, 1g of the sugar each (glucose, sucrose, maltose, lactose) and 1g of peptone water were dissolves in 100ml of water. Bromothymol blue which serves as an indicator was added until the color changes. 5ml of the solution were poured into clean test-tubes containing Durham's tube in inverted position. These were sterilized for 10mins and allowed to cool before inoculating the isolate. The test-Tubes were incubated for 48hrs. The production of acid only indicates utilization of sugars. Acid production was indicated by change in color of the medium from green to yellow will gas production was observed by gas in the Durham's tubes.

Chapter Four

Results

Table 1 Shows the Biochemical Properties of Isolates

Microorganism	G S	Cat	oxi	MR	VP	Iod	Glu	Suc	Mal
<i>Escherichia coli</i> spp	-ve	-ve	-ve	+ve	-ve	+ve	+ve	Variable	-ve
<i>Klebsiella</i> spp	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
<i>Pseudomonas</i> spp	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Enterobacter</i> spp	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
<i>Staphylococcus</i>	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
<i>Aureus</i> spp									

Keys;

G S = Gram Staining Test

Cat = Catalase Test

Oxi = Oxidase Test

MR = Methyl Red Test

VP = Voges Proskauer Test

Iod = Iodine Test

Glu = Glucose

Suc = Sucrose

Mal = Maltose

+ve = Positive

-Ve = Negative

Table 2 shows the colonial morphology and microscopy of *Candida albicans*

Microorganism	Colonial Morphology	Microscopy
<i>Candida albicans</i>	Opaque	Oval shaped

Table 3 shows the minimum inhibitory concentration

Microorganisms	Aloe Vera	Honey	Control
<i>Candida albicans</i> spp	Negative	15mm	10mm
<i>Escherichia coli</i> spp	9mm	20mm	7mm
<i>Klebsiella</i> spp	6.5mm	24mm	Negative
<i>Pseudomonas</i> spp	10mm	18mm	8mm
<i>Enterobacter</i> spp	Negative	14mm	8mm
<i>Staphylococcus aureus</i> spp	10mm	18mm	Negative

Chapter Five

Discussion

A total of Ten samples of cuts and wounds were taken from different Students in Nnamdi Azikiwe university, Awka. These samples were taken to the laboratory for analysis, the samples were serially diluted and cultured in a nutrient agar at 37°C for 24hrs, they were observed microscopically and macroscopically. Seven isolates were identified, These isolates undergo series of biochemical tests

Table 1 shows the list of biochemical tests carried out on different microorganism. Biochemical tests are used to identify bacterial species by differentiating them on the basis of biochemical activities. The difference in protein and fat metabolism, carbohydrate metabolism, enzyme production, compound utilization ability, etc. are some factors that aid in bacterial identification.

Table 2 shows the fungi microscopy observation and colonial morphology which is a method that is used to describe the characteristics of an individual colony of fungi growing on agar in a Petri dish. *Candida albicans* which was the only fungi isolated in the seven isolates is an opportunistic pathogenic yeast that is a common member of the human gut flora. It can also survive outside the human body.

After proper isolation and identification of the organisms, antimicrobial susceptibility testing was carried out to determine the effect of Aloe vera and Honey on each isolate. Therefore, observing their zones of inhibitions which can be seen in Table 3.

The zones of inhibitions on the agar plates gives us explicit information on the effectiveness of the antimicrobial properties of Aloe vera and Honey on different organisms isolated from the samples.

The zones of inhibition displayed shows that some of the isolates were resistant or sensitive to Honey and Aloe vera. Honey shows great sensitivity towards inhibiting the growth of *Klebsiella spp* and *Escherichia coli spp* respectively while aloe vera shows no sensitivity towards *Candida albicans spp* and *Enterobacter spp*. From the results it can be deduced that Aloe vera and Honey is capable of inhibiting the growth of certain microorganisms found in wounds.

Conclusion

The application of Aloe Vera and Honey to wounds surfaces tends to reduce the microbial load of microorganisms present in the wound, It also has high potential of Antimicrobial properties thereby inhibiting the growth of microorganisms.

Due to the properties of Aloe vera and Honey, it can be used to retain skin moisture and integrity. It also prevents skin ulcers as it contains mucopolysaccharides, amino acids, zinc, and water. In terms of quality and speed of wound healing, Aloe vera and Honey are much more effective and less costly compared to the currently available alternative treatments. Considering the tendency to promote traditional medicine as well as rare side effects. After the isolation of the organisms, culturing in their respective medium then microscopic and macroscopic examination of the growth of the isolates within 24-48hours, Honey showed more anti-microbial

properties than aloe vera based on the result from their zones of inhibition.

References

1. Adindu, C.S., Odili, L.C., Nwagu, E.E., Agu, K.C., Awah, N.S., Okeke, B.C. and Orjionwe, R.N. (2016). Phytochemical and Antimicrobial Screening of *Cola gigantea* Leaves, Stem and Bark. *Universal Journal of Microbiology Research*, **4**(2): 49–54.
2. Agu, K.C., Awah, N.S., Nnadozie, A.C., Okeke, B.C., Orji, M.U., Iloanusi, C.A., Anaukwu, C.G., Eneite, H.C., Ifediegwu, M.C., Umeoduagu, N.D. and Udoh, E.E. (2016). Isolation, Identification and Pathogenicity of Fungi Associated with Cocoyam (*Colocasia esculenta*) Spoilage. *Food Science and Technology*, **4**(5): 103–106. DOI: 10.13189/fst.2016.040503.
3. Agu, K.C., Awah, N.S., Sampson, P.G., Ikele, M.O., Mbachu, A.E., Ojiagu, K.D., Okeke, C.B., Okoro, N.C.N. and Okafor, O.I. (2014). Identification and Pathogenicity of Rot-Causing Fungal Pathogens Associated with *Xanthosoma sagittifolium* Spoilage in South Eastern Nigeria. *International Journal of Agriculture Innovations and Research*, **2**(6): 1155–1159.
4. Agu, K.C., Igweoha, C.A. and Umeh, C.N. (2013). Antimicrobial Activity of the Ethanolic and Petroleum Ether Extracts of Tangerine Seeds on Selected Bacteria. *International Journal of Agriculture and Bioscience*, **2**(1): 22–24.
5. Ahmad, S., Benjakul, P., Sumpavapol, N.P. and Nirmal, M. (2012). Quality Changes of Sea Bass Slices Wrapped with Gelatin Film Incorporated with Lemongrass Essential Oil. *International Journal of Food Microbiology*, **155**(3): 171–178.
6. Alcazar-Fuoli, L. and Mellado, E. (2014). Current Status of Antifungal Resistance and Its Impact on Clinical Practice. *British Journal of Haematology*, **166**(4): 471–484.
7. Amirdivani, A.S. and Baba, S. (2011). Changes in Yogurt Fermentation Characteristics, Antioxidant Potential and In Vitro Inhibition of Angiotensin-I Converting Enzyme upon the Inclusion of Peppermint, Dill and Basil. *LWT-Food Science and Technology*, **44**(6): 1458–1464.
8. Anazodo, C.A., Abana, C.C., Agu, K.C., Victor-Aduloju, A.T., Okoli, F.A., Ifediegwu, M.C., Awari, V.G. and Chidozie, C.P. (2024). In Vitro Antifungal Efficacy of *Allium cepa* and *Allium sativum*: A Comparative Study with Commercial Drugs.
9. Anazodo, C.A., Adepeju, D.M., Okoli, F.A., Obasi, C.J., Abana, C.C., Agu, K.C., Ezenwelu, C.O., Awari, V.G. and Umeoduagu, N.D. (2024). Investigating the Susceptibility of Otomycosis-Causing Microorganisms to Different Ear Drops. *International Research Journal of Modernization in Engineering Technology and Science*, **6**(6): 1958–1965.
10. Araujo, R., Pina-Vaz, C. and Rodrigues, A.G. (2007). Susceptibility of Environmental versus Clinical Strains of Pathogenic *Aspergillus*. *International Journal of Antimicrobial Agents*, **29**(1): 108–111.

11. Arshad, M.A., Hanif, R.W.K., Qadri, M.M. and Khan, Z. (2014). Role of Essential Oils in Plant Disease Protection: A Review. *International Journal of Chemical and Biochemical Sciences*, **6**: 11–17.
12. Awah, N.S., Agu, K.C., Ikedinma, J.C., Uzoechi, A.N., Eneite, H.C., Victor-Aduloju, A.T., Umeoduagu, N.D., Onwuatuogwu, J.T.C. and Ilikannu, S.O. (2017). Antibacterial Activities of the Aqueous and Ethanollic Extracts of the Male and Female *Carica papaya* Leaves on Some Pathogenic Bacteria. *Bioengineering and Bioscience*, **5**(2): 25–29. DOI: 10.13189/bb.2017.050201.
13. Awah, N.S., Agu, K.C., Okorie, C.C., Okeke, C.B., Iloanusi, C.A., Irondi, C.R., Udemezue, O.I., Kyrian-Ogbonna, A.E., Anaukwu, C.G., Eneite, H.C., Ifediegwu, M.C., Umeoduagu, N.D., Abah, N.H. and Ekong, U.S. (2016). In Vitro Assessment of the Antibacterial Quality of Some Commonly Used Herbal and Non-Herbal Toothpastes on *Streptococcus mutans*. *Open Journal of Dentistry and Oral Medicine*, **4**(2): 21–25. DOI: 10.13189/ojdom.2016.040201.
14. Awari, V.G., Umeoduagu, N.D., Agu, K.C., Obasi, C.J., Okonkwo, N.N. and Chidozie, C.P. (2024). Antibigram of Pathogenic *Pseudomonas aeruginosa* Isolated from Hospital Environment. *International Refereed Journal of Engineering and Science*, **13**(2): 01–10.
15. Awari, V.G., Umeoduagu, N.D., Agu, K.C., Okonkwo, N.N., Ozuah, C.L. and Victor-Aduloju, A.T. (2023). The Ubiquity, Importance and Harmful Effects of Microorganisms: An Environmental and Public Health Perspective. *International Journal of Progressive Research in Engineering Management and Science*, **3**(12): 1–10.
16. Berkow, E. and Lockhart, S. (2017). Fluconazole Resistance in *Candida* Species: A Current Perspective. *Infectious Drug Resistance*, **10**: 237–245.
17. Blum, G., Hörtnagl, C., Jukic, E., Erbeznic, T. and Pümpel, T. (2013). New Insight into Amphotericin B Resistance in *Aspergillus terreus*. *Antimicrobial Agents and Chemotherapy*, **57**(4): 1583–1588.
18. Bonhomme, J. and D'Enfert, C. (2013). *Candida albicans* Biofilms: Building a Heterogeneous, Drug-Tolerant Environment. *Current Opinion in Microbiology*, **16**(4): 398–403.
19. Brown, G.D., Denning, D.W., Gow, N.A.R., Levitz, S.M. and Netea, M.G. (2012). Hidden Killers: Human Fungal Infections. *Science Translational Medicine*, **4**(165): 165rv13.
20. Calvo, B., Melo, A.S.A., Perozo-Mena, A., Hernandez, M. and Francisco, E.C. (2016). First Report of *Candida auris* in America: Clinical and Microbiological Aspects of 18 Episodes of Candidemia. *Journal of Infection*, **73**(4): 369–374.
21. Chassot, F., Venturini, T.P., Piasentin, F.B., Rossato, L. and Fiorini, A. (2016). Exploring the In Vitro Resistance of *Candida parapsilosis* to Echinocandins. *Mycopathologia*, **181**(9–10): 663–670.
22. Chau, A.S., Gurnani, M., Hawkinson, R., Laverdiere, M. and Cacciapuoti, A. (2005). Inactivation of Sterol 5,6-Desaturase Attenuates Virulence in *Candida albicans*. *Antimicrobial Agents and Chemotherapy*, **49**(9): 3646–3651.
23. Chowdhary, A., Anil Kumar, V., Sharma, C., Prakash, A. and Agarwal, K. (2014). Multidrug-Resistant Endemic Clonal Strain of *Candida auris* in India. *European Journal of Clinical Microbiology and Infectious Diseases*, **33**(6): 919–926.
24. Cortés, J.A., Reyes, P., Gómez, C.H., Cuervo, S.I. and Rivas, P. (2014). Clinical and Epidemiological Characteristics and Risk Factors for Mortality in Patients with Candidemia in Hospitals from Bogotá, Colombia. *Brazilian Journal of Infectious Diseases*, **18**(6): 631–637.
25. Cowen, L.E., Sanglard, D., Howard, S.J., Rogers, P.D. and Perlin, D.S. (2015). Mechanisms of Antifungal Drug Resistance. *Cold Spring Harbor Perspectives in Medicine*, **5**(7): a019752.
26. Davood, H., Fatemeh, M., Kobra, R. and Atefeh, A. (2019). The Effect of Aloe Vera Clinical Trials on Prevention and Healing of Skin Wound. *Iranian Journal of Medical Sciences*, **44**(1): 1–9.
27. Denning, D.W. (2003). Echinocandin Antifungal Drugs. *The Lancet*, **362**(9390): 1142–1151.
28. Eddouzi, J., Parker, J.E., Vale-Silva, L.A., Coste, A. and Ischer, F. (2013). Molecular Mechanisms of Drug Resistance in Clinical *Candida* Species Isolated from Tunisian Hospitals. *Antimicrobial Agents and Chemotherapy*, **57**(7): 3182–3193.
29. Kanafani, Z.A. and Perfect, J.R. (2008). Resistance to Antifungal Agents: Mechanisms and Clinical Impact. *Clinical Infectious Diseases*, **46**(1): 120–128.
30. Kelly, S.L., Lamb, D.C., Corran, A.J., Baldwin, B.C. and Kelly, D.E. (1995). Mode of Action and Resistance to Azole Antifungals. *Biochemical and Biophysical Research Communications*, **207**(3): 910–915.
31. Lamb, D.C., Kelly, D.E., White, T.C. and Kelly, S.L. (2000). The R467K Amino Acid Substitution in *Candida albicans* Sterol 14 α -Demethylase Causes Drug Resistance. *Antimicrobial Agents and Chemotherapy*, **44**(1): 63–67.
32. McNeil, M.M., Nash, S.L., Hajjeh, R.A., Phelan, M.A. and Conn, L.A. (2001). Trends in Mortality Due to Invasive Mycotic Diseases in the United States, 1980–1997. *Clinical Infectious Diseases*, **33**(5): 641–647.
33. Noori, S., Al-Waili, K., Salom, G.B. and Ahmad, A.G. (2011). Honey and Microbial Infections: A Review Supporting the Use of Honey for Microbial Control. *Journal of Medicinal Food*, **14**(10): 1.
34. Obasi, C.J., Agu, K.C., Anazodo, C.A., Aniekwu, C.J., Okeke, C.B., Adepeju, D.M., Okoli, F.A. and Umeoduagu, N.D. (2024). In Vitro Activity of β -Lactams and Other Antimicrobials against Multidrug-Resistant *Pseudomonas aeruginosa*. *International Journal of Scientific Research and Engineering Trends*, **10**(3): 426–432.
35. Obianom, O.A., Ogbonna, U.S.A., Agu, K.C., Ozuah, C.L., Okonkwo, N.N., Victor-Aduloju, A.T. and Umeoduagu, N.D. (2023). Microbiological and Phytochemical Evaluation of

- Jackfruit and Sourp. *International Journal of Science Academic Research*, **4**(11): 6662–6674.
36. Ogbo, F.C. and Agu, K.C. (2015). Evaluation of a New Method for Testing the Pathogenicity of Molds to Yam Tubers. *Edorium Journal of Microbiology*, **1**: 9–17.
 37. Okafor, A.C., Igwesi, S.N., David, E.E., Okolo, V.K. and Agu, K. (2016). Presence of Bacteria with Pathogenic Potential among Already-Used Toothbrushes from University Students. *American Journal of Life Science Researches*, **4**(3): 16–20. DOI: 10.20286/ajlsr-040302.
 38. Okigbo, R.N., Enweremadu, C.E., Agu, K.C., Ironi, R.C., Okeke, B.C., Awah, N.S., Anaukwu, C.G., Okafor, I.O., Ezenwa, C.U. and Iloanusi, A.C. (2015). Control of White Yam (*Dioscorea rotundata*) Rot Pathogen Using Peel Extract of Water Yam (*Dioscorea alata*). *Advances in Applied Science Research*, **6**(10): 7–13.
 39. Orji, M.U., Agu, K.C., Nwafor, H.C., Ogbonna, U.S.A., Egurefa, S.O., Umeoduagu, N.D., Igwilo, C.Q., Awari, V.G. and Uwanta, L.I. (2025). Harnessing the Antifungal Properties of *Ocimum gratissimum* for Sustainable Water-Based Paint Formulations. *International Journal of Innovative and Applied Research*, **13**(1): 12–29. DOI: <http://dx.doi.org/10.58538/IJIAR/2119>.
 40. Perlin, D.S. (2015). Echinocandin Resistance in *Candida*. *Clinical Infectious Diseases*, **61**(Suppl 6): S612–S617.
 41. Pfaller, M.A. and Diekema, D.J. (2010). Epidemiology of Invasive Mycoses in North America. *Critical Reviews in Microbiology*, **36**(1): 1–53.
 42. Pfaller, M.A., Messer, S.A., Moet, G.J., Jones, R.N. and Castanheira, M. (2011). *Candida* Bloodstream Infections. *International Journal of Antimicrobial Agents*, **38**(1): 65–69.
 43. Sanglard, D. and Coste, A.T. (2015). Activity of Isavuconazole and Other Azoles against *Candida* Clinical Isolates. *Antimicrobial Agents and Chemotherapy*, **60**(1): 229–238.
 44. Sanglard, D. (2016). Emerging Threats in Antifungal-Resistant Fungal Pathogens. *Frontiers in Medicine*, **3**: 11.
 45. Scorzoni, L., de Paula e Silva, A.C.A., Marcos, C.M., Assato, P.A. and de Melo, W.C.M.A. (2017). Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis. *Frontiers in Microbiology*, **8**: 36.
 46. Srinivasan, A., Lopez-Ribot, J.L. and Ramasubramanian, A.K. (2014). Overcoming Antifungal Resistance. *Drug Discovery Today: Technologies*, 65–71.
 47. Ubaoji, K.I., Nwosu, O.K., Agu, K.C., Nwzor, K.O., Ifedilichukwu, N.H. and Okaka, A.N.C. (2020). Gas Chromatographic Analysis of the Phyto-Constituents and Assessment of the Antimicrobial Properties of the Leaf Extracts of Nigeria-Grown *Ginkgo biloba*. *Journal of Scientific Research in Medical and Biological Sciences*, **1**(2): 45–56.
 48. Umeoduagu, N.D., Chidozie, C.P., Ifemeje, E.M., Anazodo, C.A., Okoli, F.A., Okonkwo, N.N., Uwanta, L.I., Agu, K.C. and Azuka, G.E. (2023). Halitosis: The Antimicrobial Susceptibility Pattern of Some Commercially Sold Toothpaste on Selected Isolates. *International Journal of Scientific Research and Engineering Trends*, **9**(5): 1309–1315.
 49. Umeoduagu, N.D., Egurefa, S.O., Awari, V.G., Agu, K.C., Udenweze, E.C. and Uwanta, L.I. (2023). Bacteriological and Antibiotic Resistance Evaluation of Bacteria Isolated from Fresh Meat Sold at Afor Oba Market, Anambra State. *International Journal of Research Publication and Reviews*, **4**(8): 554–560.
 50. Wiederhold, N.P. (2017). Antifungal Resistance: Current Trends and Future Strategies to Combat. *Infectious Drug Resistance*, **10**: 249–259.

APPENDIX 1

Materials and reagents used are as follows:

-Swab sticks

-Manual weighing balance

-Concave microscope slides, Wire loop, Conical flasks, Petri dishes, Measuring cylinder, Aluminium foil

-Cotton wool, Masking tape, Spatula, Nutrient agar, Citrate agar, Oil immersion,

Reagents used;

Safranin, Ethanol, Gram's iodine, Crystal violet, peptone water, Methyl red solution, Distilled water, Bromothymol blue solution, Hydrogen peroxide, Sheep plasma

Appendix 2

Media Preparation

Eosin Methylene Blue (EMB) agar is both a selective and differential culture medium. It is a selective culture medium for Gram-negative bacteria (selects against Gram-positive bacteria) and is commonly used for the isolation and differentiation of coliforms and fecal coliforms.

Composition of EMB agar

Ingredient	Eosin methylene blue (EMB) Agar (g/L)
Peptone	10 g
Lactose	5 g
Sucrose	5 g
Dipotassium, PO ₄	2 g
Agar	13.5 g
Eosin Y	0.4 g
Methylene blue	0.065 g
Distilled water	1 L
Final pH	7.1

• Mannitol Agar

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. It is a selective medium prepared according to the recommendations of Chapman for the isolation of presumptive pathogenic staphylococci.

Composition of Mannitol Salt Agar (MSA)

- *Ingredients*
- *Gms / Litre*
- Pancreatic Digest of Casein
- 5.0 gm
- Peptic Digest of Animal Tissue
- 5.0 gm
- Beef Extract
- 1.0 gm
- Sodium Chloride
- 75.0 gm
- D-Mannitol
- 10.0 gm
- Phenol Red
- 0.025 gm
- Agar
- 15.0 gm
- Total 111.025 g
- Distilled Water = 1000 ml
- Final pH 7.4 ± 0.2 at 25°C.

Cetrimide Agar

Cetrimide Agar is a selective and differential medium used for the isolation and identification of *Pseudomonas aeruginosa* from clinical and non-clinical specimens. Cetrimide is the selective agent and inhibits most bacteria by acting as a detergent (Cetyltrimethylammonium bromide, a quaternary ammonium, cationic detergent).

It is also known as *Pseudomonas* Cetrimide Agar or Pseudogel Agar.

Composition of Cetrimide Agar

Ingredients

In gm/Litre

Pancreatic Digest of Gelatin

20.0 gm

Potassium Sulfate

10.0 gm

Magnesium Chloride

1.4 gm

Cetyltrimethylammonium Bromide

0.3 gm

Glycerine

10.0 ml

Agar

13.6 gm

Distilled Water

1000 ml

Final pH 7.2 +/- 0.2 at 25° C

Mueller Hinton Agar

Mueller-Hinton agar (MHA) is the best medium to use for routine antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method for non fastidious bacteria (both aerobe and facultative anaerobe).

Composition

Mueller Hinton Agar

Formula for Mueller-Hinton agar per liter of purified water

- Beef extract: 2.0 g
- Acid Hydrolysate of casein: 17.5 g
- Starch: 1.5 g
- Agar: 17.0 g

APPENDIX 3

Agar Used

Agar	Microorganisms	Duration
Sabourand Dextrose	<u><i>Candida albicans</i></u>	<u>48/72 hours</u>
Eosine Methylene Blue	<u><i>Klebsiella Spp, Enterobacter, Escherichia, coli</i></u>	<u>24 hours</u>
Mannitol salt	<u><i>Staphylococcus aureus</i></u>	<u>24 hours</u>
Cetrimide agar	<u><i>Pseudomonas</i></u>	<u>24 hours</u>

