Antimicrobial Activities of Moringa, Neem and Ginger Plant Extracts against Bacteria Associated with the Spoilage of Fruit Juice

Chibuzo V. Nwokafor1*, Chukwuma G. Udensi1, Henry N. Ogbonna2, Chinedu E. Udekwu2, Ugonna D. Nwankpa2, Emmanuel K. Amanze1, Wisdom N. Chibuzor and Kenechukwu C. Okeke1

1Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
2Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

ABSTRACT

Aim: This study aims to evaluate the antibacterial activity of Moringa, Neem, and Ginger plant extracts on the bacteria species isolated from fruit juice samples.

Place and Duration of Study: Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, between October 2019 and November 2019.

Methods: The fruit juice sample was prepared and cultured on Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB), Salmonella Shigella Agar (SSA), and Blood Agar using streak plate techniques. Four (4) bacteria species were isolated and identified from the fruit juice sample. These organisms served as the test isolates. Two (2) solvents (methanol and water) were used to get a comparative result. Disc diffusion method was used to determine the antibacterial effects of the Moringa, Neem, and Ginger on the test organisms.

Results: The methanolic extract of Moringa, Neem and Ginger was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition.
produced by the methanolic extract on the test microorganisms. The highest in-vitro antibacterial activity is 16 mm, which was exhibited by the methanolic extract of Moringa at the highest concentration of 200 mg/ml against Staphylococcus aureus. In comparison, the Methanolic extract exhibited no antibacterial activity (0.0 mm) at the lowest concentration of 50 mg/ml against all the test organisms. The minimum bactercidial concentration from this study revealed that methanolic and aqueous extract was active against Staphylococcus aureus, Shigella species, Bacillus species, and Escherichia coli. However, the water extract of Moringa demonstrated more significant antibacterial activity on Shigella species, Bacillus species, and Escherichia coli with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on Shigella species alone, with the range of 200 mg/ml each.

**Conclusion:** Moringa, Neem, and Ginger extract had both a bacteriostatic and bactericidal activity when tested in vitro using methanolic and aqueous preparation of Moringa, Neem, and Ginger extract. Therefore, these plants may be used successfully for treating illness caused by Staphylococcus aureus.

**Keywords:** Antimicrobial; Moringa oleifera; neem; ginger; plant extract; fruit juice.

**1. INTRODUCTION**

Fresh fruits are a regular part of the daily diets of Nigerians and are known for their high nutritional and health values [1]. Some components of fruits (phytochemicals) are potent antioxidants and function to modify the metabolic activation and detoxification/disposition of carcinogens, or even influence processes that alter the course of the tumor cell [2]. Spoilage of fruit may occur by the mechanical fracture of fruits; by autolysis of chemical and mineral contents or by the microbial flora of fruit itself and contaminating microbes, which is the primary concern to study. Foodborne illnesses have been reportedly associated with the consumption of fruit juices in several places [3]. However, the contamination of fresh produce is a primary public concern, as foodborne diseases are increasingly becoming a global public health problem [4], resulting in a substantial amount of worldwide annual morbidity and mortality [5].

Various pathogens are associated with the contamination of fruits, and with different outbreaks of gastroenteritis, associated with the consumption of contaminated fruits that have been recorded at various times [6]. Fruit juice spoilage bacteria include acid-tolerant bacteria such as acetic acid bacteria, lactic acid bacteria, Clostridium, Bacillus, members of the Enterobacteriaceae family (Klebsiella sp., Citrobacter spp., and Serratia sp.), and some heat-resistant bacteria such as Alicyclobacillus acidoterrestris and Propionibacterium cyclohexanicum. Some yeasts, like Pichia candida, Saccharomyces, and Rhodotorula are commonly encountered and generally responsible for spoilage of processed foods [7].

Plant extracts or their active components have been recognized for their antiviral, antimycotic, antiparasitic, insecticidal, antioxidative, and antibacterial properties [8]. They even have promising activity against several antibiotic-resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) and Salmonella enterica [9].

The antimicrobial activity of a compound can be influenced not only by its composition and extraction method but also by the volume of inoculum present. The concentration of the extract, type of pH media used, and the growth phase of the organism can also influence the antimicrobial activity. Also, the use of an emulsifier or solvent to aid in suspension, as well as incubation times and temperatures, have also been reported [8]. However, several studies have shown various plant extracts to be effective in Gram-negative bacteria. *Pseudomonas aeruginosa*, for example, is more resistant to the extracts [10].

Heat resistant fungi and other spore-forming bacteria such as Clostridium pasteurianum and Bacillus coagulants have been used as targets for industrial processes such as fruit juice pasteurization [11]. Convention thermal pasteurization in tropical fruit juices is thought to reduce the overall product quality since tropical fruits are heat sensitive [12]. Alternative technologies to reduce microbial loads include irradiation, high pressure, biocontrol, pulsed electric field, pulsed light, oscillating magnetic fields, ultrasound, and U.V. treatment [13].

A variety of plant and spice based anti-microbials are used for reducing or eliminating pathogenic microorganisms and increasing the shelf life of
food. Natural herbs and spices are used for several purposes including food or medicine, maintaining proper sanitation, health, and personal hygiene and to boost longevity [14]. Plant extracts have also shown great potential in the food industry and approved by various regulatory agencies such as the U.S. Food and Drug Act (USFDA), the European Union standards, and Codex Alimentarius and Food Standard Safety of India (FSSAI) [15]. There have been many studies published on the activities of plant extracts and essential oils against different microbes, including foodborne pathogens [16]. Hence, the study of the antimicrobial activities of selected plant extracts against bacteria associated with the spoilage of fruit juice.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Isolation of Microorganisms

2.1.1 Sample inoculation

At the laboratory, 50 ml of juice samples were measured and then transferred into various conical flasks. Ten-fold dilutions were prepared under aseptic conditions from each sample using 10 ml of distilled water as diluents. This resulted in a dilution of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ was then prepared by serial dilution. Diluted suspensions of 0.1 ml samples were plated over Nutrient agar Medium, Eosin Methylen Blue agar, Mannitol Salt Agar, and Salmonella Shigella agar using a pour plate method. The plates were incubated at 37°C for 24 to 48 hours. Colonies that appeared on the plates were further purified for identification using the techniques of Srinivasan et al [17].

2.1.2 Identification of bacterial isolates

Isolates were analysed based on morphological features, Gram staining [18], and biochemical characterization, which includes; catalase, oxidase, coagulase, citrate, motility, indole, and urease tests of the isolates were carried out to verify the identity of the organisms [19]. The bacterial isolates were identified, and confirmatory identities of bacteria were made [20].

2.1.3 Preparation of Moringa, Neem and Ginger extracts

The freshly collected Moringa, Neem, and Ginger in bulbs and leaf foams were purchased from a local market in Ahiaieke, and 500 g of that was cleared of dirt, washed in running tap water three (3) times, to remove dirt. It was rewash with sterile distilled water. Then it was blended and crushed using mortar and pestle into a paste form. Two (2) solvents were used to get comparative results. Five (5) grams of moringa, neem, and ginger paste were measured into a conical flask, mixed with 100 ml each of cold distilled water and methanol respectively, stirred for 10 minutes and filtered using a filter paper. The extracts generated are classified as: Methanol/Moringa (M/M), Aqueous/Moringa (A/M), Methanol/Neem (M/N), Aqueous/Neem (A/N), Methanol/Ginger (M/G), Aqueous/Ginger (A/G).

2.1.4 Preparation of different concentration of the extracts

The aqueous extract was reconstituted by weighing 0.8 g quantity of each extract into a sterile test tube, and made up to 2 ml using distilled water to give a concentration of 400 mg/ml. Also, the methanolic extract was also reconstituted by the dissolution of 0.8 g of each crude methanolic extract weighed into a sterile test tube made up to 2 ml with dimethyl sulfoxide (DMSO) 50% respectively to get a concentration of 400 mg/ml [21]. This 400 mg/ml concentration of the extracts was then, doubly diluted in sterile water to obtain concentration 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml.

2.2 Media Preparation

Four different types of media were used for the isolation of each organism from the fresh fruit juice, respectively. The media include Mannitol Salt Agar (MSA) and Blood Agar (B.A.), for isolation of Staphylococcus aureus, Eosin Methylen Blue (EMB), for isolation of Escherichia coli and Salmonella Shigella Agar (SSA), for isolation of Salmonella and Shigella spp., Nutrient Agar for general purpose. The media used for sensitivity test and MIC was Nutrient Broth and Mueller-Hinton Agar, as previously done by Srinivasan et al [17].

2.3 Determination of Antimicrobial Activity

The different bacteria (Escherichia coli, Staphylococcus aureus, Salmonella species, and Bacillus species) isolates were inoculated respectively onto the solid, sterile Muller Hinton Agar. This was done using the spread plate
method [22]. After the inoculation of the isolates, the prepared disc was dipped into the concentrated extract and allowed to absorb it. Carefully, with the aid of a flame pair of forceps, the disc bearing extract was transferred to the inoculated plate. Four extract discs were used for each plate, and they were placed about the same distance from one another and not less than 1 cm from the edge of the Petri dish. The plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for the zone of inhibition [23]. The presence of a clear zone around any disc gave a positive result. The diameters of the zone of inhibition were measured with a transparent rule and recorded in millimeter (mm). The test was carried out in duplicates, and the mean values were calculated and recorded. Ciprofloxacin and Rifampicin served as the control [24].

2.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Using sterile forceps, the paper disc of different concentration from (200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml) were placed at different portions of the inoculated labelled plates. The plates were incubated at 37°C for 24 hours [25]. After incubation, the plates were examined for the presence of inhibition zones and the concentration that caused inhibition was recorded, the lowest diluents which caused inhibition was recorded as the MIC [26]. The Minimum Bactericidal Concentration (MBC) was determined by streaking the content of the tubes used for minimum inhibitory concentration determination, which were showing reduced turbidity on freshly prepared nutrient agar plates. The Minimum Bactericidal Concentration was then identified as the concentration that completely inhibited the growth of the bacteria [26].

3. RESULTS

Table 1 shows the bacterial isolates from the fruit juice samples, which were identified by their cultural morphology, Gram's reaction, and biochemical reaction. The bacteria identified are Staphylococcus aureus, Bacillus spp, Shigella spp, and Escherichia coli.

Table 2 shows the antibacterial activity of the methanolic and aqueous extract of Moringa, Neem, and Ginger. The methanolic extract of Moringa, Neem, and Ginger was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition produced by the methanolic extract on the test microorganisms. The highest in-vitro antibacterial activity is 16 mm, which was exhibited by the methanolic extract of Moringa at the highest concentration of 200 mg/ml against Staphylococcus aureus, followed by Shigella species 15 mm (200 mg/ml). In comparison, the methanolic extract exhibited no antibacterial activity (0.0 mm) at the lowest concentration of 50 mg/ml against all the test organisms. The aqueous extract of neem exhibited the highest antibacterial activity, 14 mm at a concentration of 200 mg/ml against Escherichia coli, followed by aqueous ginger extract at 12 mm against Staphylococcus aureus.

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Moringa, Neem, and Ginger extracts against the test organisms. Methanolic and aqueous soluble extracts of the three plants were active against Staphylococcus aureus, Shigella spp, Bacillus spp, and Escherichia coli. However, the aqueous extract of Moringa demonstrated more significant antibacterial activity on Shigella spp, Bacillus spp, and Escherichia coli with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on Shigella species alone, with the range of 200 mg/ml each.

4. DISCUSSION

The antibacterial activity of Moringa, neem, and Ginger on some pathogens was investigated with different solvents. Plant extracts or their active components have been recognized for their antiviral, antimycotic, antiparasitic, insecticidal, antioxidogenic, and antibacterial properties. In this work, it was observed that the methanolic extract had a more significant inhibitory effect than aqueous extracts. Shigella spp, and Staphylococcus aureus exhibited the highest sensitivity (15 mm, and 16 mm), respectively, against the methanol extract of Moringa, followed by Escherichia coli (14 mm) against aqueous extract of neem. The need for this study is to compare the differences in antimicrobial effects as a result of the extraction solvent. The solvent used in the preparation of the spice (Moringa, Neem, and Ginger) extract plays a major role in the inhibitory effect of the spice (Moringa, Neem, and Ginger) as described by Ekwenye et al. [24].
Table 1. Identification of bacterial species from the fruit juice sample

| Colonial features | Gram reaction | Cell arrangement | Catalase | Oxidase | Coagulase | Indole | Citrate | Motility | Methyl Red | Voges-P | Suspected bacteria |
|-------------------|---------------|------------------|----------|---------|-----------|--------|---------|----------|------------|---------|-----------------|
| Pink Pigment      | –             | Short Rod        | +        | –       | –         | +      | +       | +        | +          | –       | Escherichia coli  |
| Golden Yellow     | +             | Cocci Group      | +        | –       | +         | –      | +       | –        | –          | –       | Staphylococcus aureus |
| Black Spot        | –             | Short Rod        | +        | –       | N/A       | –      | –       | +        | –          | –       | Shigella species  |
| Creamy Mucoid     | +             | Short Rod        | –        | +       | –         | N/A    | N/A     | –        | N/A        | N/A     | Bacillus species |

Key: – = Absent, + = Present, N/A = Not Applicable
This study also revealed that the methanolic extract of Moringa was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition produced by the methanolic extract, specifically against *Staphylococcus aureus* at 16 mm (200 mg/ml). This could be as a result of some antimicrobial or phytochemical components which were not extracted by the aqueous extract, but were extracted by the methanol extract. This result is in conformation with a study by Onyeagba et al. [28] who reported the crude extracts of garlic and Ginger applied singly and in combination did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp at low of concentrations (60 mg/ml) of the extracts.

The aqueous extract of neem exhibited the highest antibacterial activity, 14 mm at a concentration of 200 mg/ml against *Escherichia coli*, followed by aqueous ginger extract at 12 mm against *Staphylococcus aureus*. In a similar study, the results of the antibacterial activity of *Azadirachta indica* (Neem) can be well said to be in accordance with several earlier studies. Bohora et al [22] have observed the significant antibacterial activity of Neem leaf extract against *E. faecalis* and mixed bacterial cultures. [29] reported its anti-*Staphylococcal* activity.

### Table 2. Antibacterial activity of Moringa, Neem, and Ginger extract against test organisms

| Test bacteria       | Concentration (mg/ml) zone of inhibition (mm) | Extract       |
|---------------------|-----------------------------------------------|---------------|
|                     | 50mg/ml | 100mg/ml | 150mg/ml | 200mg/ml | Control |
| *Staphylococcus aureus* |          |          |          |          |         |
|                     | –       | –        | 10       | 16       | 16      | M/M     |
|                     | –       | –        | 8        | 14       | –       | A/M     |
|                     | –       | –        | 11       | 13       | 20      | M/N     |
|                     | –       | –        | 8        | 10       | 17      | A/N     |
|                     | –       | –        | 7        | –        | 16      | M/G     |
|                     | –       | –        | –        | 12       | 17      | A/G     |
| *Escherichia coli*  | –       | 8        | 11       | 14       | 20      | M/M     |
|                     | –       | –        | –        | 8        | 9       | A/M     |
|                     | –       | –        | 12       | 14       | 20      | M/N     |
|                     | –       | 6        | 11       | 14       | 20      | A/N     |
|                     | –       | –        | 6        | 11       | 18      | M/G     |
|                     | –       | –        | 7        | –        | 8       | 17      | A/G     |
| *Shigella* spp      | –       | 8        | 13       | 15       | 18      | M/M     |
|                     | –       | –        | 6        | 8        | 12      | A/M     |
|                     | –       | –        | –        | –        | 11      | M/N     |
|                     | –       | 8        | –        | –        | 11      | A/N     |
|                     | –       | –        | 6        | –        | 15      | M/G     |
|                     | –       | –        | 8        | –        | 18      | A/G     |
| *Bacillus* spp      | –       | 6        | 8        | 10       | 11      | M/M     |
|                     | –       | –        | –        | 10       | 12      | A/M     |
|                     | –       | 6        | 7        | –        | 20      | M/N     |
|                     | –       | 6        | –        | –        | 21      | A/N     |
|                     | –       | –        | –        | 10       | 16      | M/G     |
|                     | –       | –        | –        | 10       | 15      | A/G     |

Key: Antibiotic positive control on gram-positive isolate = Ciprofloxacin 20mg/ml, Antibiotic positive control on gram Negative isolate = Rifampicin 10mg/ml, Methanol/Moringa (M/M), Aqueous/Moringa (A/M), Methanol/Neem (M/N), Aqueous/Neem (A/N), Methanol/Ginger (M/G), Aqueous/Ginger (A/G)
Sinaga et al [30] reported that Gram-positive bacterial strains were more sensitive than the Gram-negative ones. Owolabi et al [31] reported the antibacterial activity of Neem leaf water extract against the same test organisms with a clear zone of inhibition from 10±0 mm to 15.5±0.71 mm.

The minimum bactericidal concentration from this study revealed that methanolic and aqueous soluble extract was active against Staphylococcus aureus, Shigella species, Bacillus species, and Escherichia coli. However, the aqueous extract of Moringa demonstrated greater antibacterial activity on Shigella species, Bacillus species, and Escherichia coli with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on Shigella species alone, with the range of 200 mg/ml each. Similarly, Sivan et al [32], in a study to determine the minimum bactericidal activity of Ginger, neem and garlic extract on Helicobacter pylori, observed that at the same protocol ginger, neem and garlic extracts had the bactericidal effect at a high concentration of 160 mg/ml.

It was also observed in this study that the Moringa, neem, and Ginger extract had both a bacteriostatic and bactericidal activity when tested in vitro using methanolic and aqueous preparation of Moringa, neem and Ginger extract. Therefore, these plants may be used successfully for treating food poisoning causative agents like Staphylococcus aureus. It may be useful on other microbes on Gram-positive and gram-negative bacteria, so that further in vivo and in vitro studies are necessary. The standard antibiotics (Ciprofloxacin and Rifampicin) [33] used as control inhibited the test bacteria; it had higher inhibitory activity compared to the Moringa, neem, and Ginger extracts.

The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as Staphylococcus aureus, Shigella species,
Bacillus species, and Escherichia coli, is encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause foodborne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically synthesized sanitizers/preservatives with "naturally derived" ones [34]. Medicinal plants having antimicrobial compounds in comparison with antibiotics are usually with no side effects, better patient tolerance, relatively less expensive, and acceptable due to the long history of use, and it is renewable [35].

Therefore, the results of this study support the use of Moringa, Neem, and Ginger in health products and herbal remedies in Nigeria as described by [36] and in good agreement with the reports of earlier investigators.

5. CONCLUSION

The results of this finding indicate that methanolic and aqueous extracts of Moringa, Neem, and Ginger have broad-spectrum antimicrobial activity. Hence, they can serve as a natural therapeutic agent against some enteric pathogens. Based on the findings of this research, aqueous extracts and to a lesser extent, methanolic extract of the Moringa, Neem, and Ginger possess antibacterial activity. Also, methanolic extract was found to be more potent than the aqueous extract against four pathogens tested. It was observed that Shigella spp. and Staphylococcus aureus had the highest sensitivity against the extracts.

CONSENT AND ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee. Consent and approval was also given by the Head of Department, Microbiology, Michael Okpara University of Agriculture, Umudike. We sincerely appreciate the input of love and assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Senjuti JD, Feroz F, Tahera J, Das KK, Noor R. Assessment of microbiological contamination and the in vitro demonstration of the antibacterial traits of the commonly available local fruit blend within Dhaka Metropolis. J Pharmacogn Phytochem. 2014;3(1):73-77.
2. Wargovich MJ. Anticancer properties of fruits and vegetables. Horticulture Science. 2010;35:573-575.
3. Chumber SK, Kaushik K, Sav S. Bacteriological analysis of street foods in Pune. Indian J Pub Health. 2007;51(2):114-116.
4. Hannan A, Rehman R, Saleem S, Khan MU, Qamar MU, Azhar H. Microbiological analysis of ready-to-eat salads available at different outlets in Lahore, Pakistan. Inter Food Res J. 2014;21(5):1797-1800.
5. Hanson LA, Zahn EA, Wild SR, Döpfer D, Scott J, Stein S. Estimating global mortality from potentially foodborne diseases: An analysis using vital registration data. Pop Health Metr. 2012;10(5):33-67.
6. Jedd MZ, Yunessian M, Gorji ME, Noori N, Pourmand MR, Khaniki GRJ. Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. J Health Popu Nutr. 2014;32(3):100-210.
7. Aneja, Kamal & Dhiman, Romika & Aggarwal, Neeraj & Aneja, Ashish. Emerging preservation techniques for controlling spoilage and pathogenic microorganisms in fruit juices. Inter J Micro. 2014;758942.
8. Swerdlow DL, Mintz ED, Rodriguez M, Tejado E, Ocampo C, Espejo L, et al. Waterborne transmission of epidemic cholera in Trujillo, Peru: lessons for a continent at risk. Lanc. 2012;340:28-33.
9. Ries AA, Vugia DJ, Beigoloea L, Palacios AM, Vasquez E, Wells JD, et al. Serotype Newport infection linked to mango consumption: Impact of water-dip disinfection technology. Clin Inf Dis. 2012;37:1585–1590.
10. Chomvarin C, Kotimanusvanij D, Rhotomrak T. Study on the correlation between the enterotoxin producing *Staphylococcus aureus* isolated from prepared food and cooks. Srinagarind Hospital Medical Journal. 2013;6:231-242.

11. Atribst AAL, Sant’Ana A, Massaguer P. Review: Microbiological quality and safety of fruit juices past, present and future perspectives microbiology of fruit juices. Crit Rev Micro. 2009;35:310-39.

12. Walkling-Ribeiro M, Noci F, Cronin DA, Lyng JG, Morgan DJ. Inactivation of *Escherichia coli* in a tropical fruit smoothie by a combination of heat and pulsed electric fields. J Food Sci. 2008;73:395–399.

13. Bassett J, McClure P. A risk assessment approach for fresh fruits. J Appl Micr. 2008;104:925–943.

14. Tajkarimi M, Ibrahim S, Cliver DO. Antimicrobial herb and spice compounds in food. Food Contr. 2010;21:1199-1218.

15. Negi PS, Jayaprakasha GK. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. J. Food Sci. 2003;68:1473–1477.

16. Shan B, Cai Y, Brooks J, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extract. Int J food Mic. 2007;117:112-9.

17. Srinivasan D, Nathan S, Suresh T, Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm. 2001;74: 217-20. DOI: 10.1016/S0378-8741(00)00345-7

18. Cheesbrough M. District laboratory practical in tropical countries. Cambridge University Press, Edinburg building, Trumpington Street, Cambridge CB2 1IR, United Kingdom (Antimicrobial sensitivity testing). 2006;132-148.

19. Bergey DH, Holt JG. Bergey's manual of determinative bacteriology. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.

20. Vashist H, Sharma D, Gupta A. A review on commonly used biochemical tests for bacteria. Inno J Life Sci. 2013;1:1-7.

21. Peggy. Antimicrobial activity of medicinal plants. Afr J biochem. 2006;12(3):379-399.

22. Bohora A, Hegde V, Kokate S. Comparison of antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against *E. faecalis*, *C. Albicans* and mixed culture. Endo. 2010;22:10-13.

23. Chris C, John G, Patricia L, Joseph F. Collins, and Lyne’s Microbiological Methods (8th Edition). 2004;67-89.

24. Ekwenye UN, Elegalam NN. Antibacterial activity of Ginger (*Zingiber officinale Roscoe*) and garlic (*Allium sativum L.*) extracts on *Escherichia coli* and *Salmonella typhi*. Int J Mol Med Adv Sci. 2005;1:411-416.

25. Singh G, Kapoor IPS, Singh P, De-Heluani CS, De-Lampasona MP, Catalan CAN. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. Food Chem Tox. 2008;46:3295-3302.

26. Andrews JM. Determination of minimum inhibitory concentrations. Antimicrob Chemother. 2001;48(6):5-16.

27. Aiyeogoro OA, Akinpelu DA, Afolayan AJ, Okoh AI. Antibacterial activities of crude stem bark extracts of *Distemonathus benthamianus* Baill. J Bio Sci. 2008;8(2): 356-361.

28. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O. Studies on the antimicrobial effects of garlic (*Allium sativum* linn), Ginger (*Zingiber officinale* roscoe) and lime (*Citrus aurantifolia* linn). Afri J Biotech. 2004;3(10):552-554.

29. Bezalwar PM, Gomashe AV, Gulhane PA. A quest of anti-acne potential of herbal medicines for the extermination of MDR *Staphylococcus aureus*. Inter. J Pharm. Sci. 2014;3(6):12-17.

30. Sinaga M, Ganesan K, Kumar S, Nair P, Banu SG. Preliminary phytochemical analysis and in vitro antibacterial activity of bark and seeds of Ethiopian neem (*Azadirachta indica* a. juss). World J Pharm. and Pharm. Sci. 2016;5(4):1714-1723.

31. Owolabi AO, Abah KA, Oranusi S. In vitro antimicrobial and antioxidant activity of *Carica papaya* and *Azadirachta indica* leaf and stem bark extracts on selected clinical isolates. J Ind Res Tech. 2017;6(1):209-220.

32. Sivam GP, Lampe JW, Ulness B, Swanzey SR, Potter JD. *Helicobacter pylori* in vitro susceptibility to garlic (*Allium sativum*) extract. Nut. and Can. 2007;27: 118-121.

33. Shokrzadeh M, Ebadi AG. Antibacterial effect of Garlic (*Allium sativum*) on
34. Lanciotti R, Patrignani F, Bagnolini F, Guerzoni ME, Gardini F. Evaluation of diacetyl antimicrobial activity against E. coli, L. monocytogenes and S. aureus. Food Microbiology. 2013;20:557–543.

35. Laport MS, Santos OC, Muricy G. Marine sponges: Potential sources of new antimicrobial drugs. Cur Pharm Biotech. 2009;10:86-105.

36. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. In vitro antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J Med Food. 2004;7:327-333.