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Full Length Research Paper

Evaluation of the antimicrobial properties of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) on pathogens

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The investigation on the potency of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) was carried out against pathogens. The formulations were in the powder form as used locally. The antimicrobial activity of these plants was examined using different solvents and efficacy was compared. The solvents were ethanol (70%, v/v) and water. Antimicrobial activity was carried out by the agar well diffusion method. The clinical isolates include aerobic, facultative bacteria namely: *Stapyhlococcus aureus* ATCC 25921, *S. aureus*, *Salmonella paratyphi*, *Shigella flexnerii*, *Escherichia coli* ATCC 25922, *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Crude extracts of the solvents varied in zones of inhibition. All the Gram-positive bacteria (*S. aureus*, *S. aureus* ATCC 25921 and *B. subtilis)* and all Gram-negative bacteria (*E. coli* ATCC 25922, *E. coli*, *P. aeruginosa*, *S. paratyphi*, *S. flexneri* and *K. pneumonia)* were susceptible to ethanolic extracts of unripe banana, lemon grass and turmeric while some namely *E. coli* ATCC 25922, *E. coli*, *P. aeruginosa* and *S. flexneri* were not susceptible to aqueous extracts of the three medicinal plants. The minimum inhibition concentration (MIC) ranged from 4 – 512 mg/ml while the minimum bactericidal concentration (MBC) ranged from 32 – 512 mg/ml depending on isolates and extracting solvent. Ethanolic extracts showed greater antimicrobial activity than aqueous extracts. The killing rate of the extracts varied. Unripe banana had less than 2 h killing time for *S. aureus* ATCC 25921, turmeric less than 3 h for *E. coli* while lemon grass had more than 3 h killing time for *S. paratyphi*.

Key words: Antimicrobial activity, unripe banana, lemon grass, turmeric, death rate.

INTRODUCTION

The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 spp. of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006). Some plant decoctions are of great value in the treatment of diarrhoea or gastrointestinal disorder, urinary tract infections, skin infections, infertility, wound and cutaneous abscesses (Meyer et al., 1996; Dimayuga and Gracia, 1991). Plant parts used for medicinal purposes are bulb, gel, leaves, roots, barks, peels etc. The use of plants to treat illness is found throughout human culture. Among the most ancient recorded uses of medicinal plants are those found in China and India, where historic approach to the treatment of human diseases is still practised (Anne-Catherine, 2007). Medicinal plants are

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important sources for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents (Ushimaru et al., 2007).

Turmeric plant is more commonly found in Asia than in other parts of the world (Eco India, 2008; Sharma et al., 2006). It is found in Africa but in little quantity. Several pharmacological activities and medicinal applications of turmeric are known (Araujo and Leon, 2001; Eigner and Scholz, 1999; Ammon and Whal, 1991). Turmeric powder when applied as capsules to patients with respiratory diseases, gives relief from symptoms like dysponea and cough (Jain et al., 1991). Lemon grass is a perennial grass that is native to tropical Asia but is now widely cultivated in other tropical regions. It is a popular plant that finds its use in traditional medicine of many cultures. In Nigeria, lemon grass is used for stomach discomfort and for malaria therapy. It is used in combination with two more plants for effective malaria therapy (Aibinu et al., 2007). Studies have shown that the lemon grass has antibacterial and antifungal properties (Manolito, 2006). Banana is one of the most familiar of the tropical fruits (Krishnamurthy, 2002). The medicinal parts used are fruits mainly as well as peels, leaves and the juice. The root is anthelmintic and for reducing bronchocele. The fruit has been used as part of anti-ulcer diet in combination with pineapple, blueberries, cloves, ginger and cinnamon. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe banana (Gurumaa, 2008). Although the antimicrobial activity of some medicinal plants is documented, their antimicrobial activities vary widely, depending on the type of spice or herb, test medium and micro-organism (Synder, 1997).

The aim of this study was to investigate the potency of unripe banana, lemon grass and turmeric on some pathogens.

MATERIALS AND METHODS

Collection and preparation of plant extracts

Turmeric plant and unripe banana fruits were purchased at Jakande Market, Ketu while lemon grass leaves were obtained from Mile 12 Market, all in the Lagos metropolis, Nigeria. They were cut into small pieces with a surface-sterilized scalpel before oven-drying at 45°C for 5 days. They were each milled with a milling machine (Norris and Poole, England) into fine powder. Sterile bottles were labelled for unripe banana as 'A' and 'B', for turmeric rhizomes, 'C' and 'D' and for lemon grass 'E' and 'F'. Into each of the labelled bottles, 500 g of their respective powder were introduced and kept at 30°C.

Concentration and preparation of stock solutions

Two different solvents namely 70% (v/v) ethanol and distilled water were used for extraction of the samples. Bottles A, D and F contained ethanolic extracts while B, C and E were aqueous extracts. Each powder (500 g) was placed in sterile bottles A, B for unripe banana; C, D for turmeric and E, F for lemon grass. Exactly 500 ml of 70% ethanol was dispensed into each sterile bottle containing 500 g of the samples in Bottles A, D and F, while 500 ml of distilled water was dispensed into sterile bottles containing 500 g of powder in B, C and E. All the bottles were shaken well to obtain the extracts in their respective solvents. The samples were left to soak at room temperature for 3 days with agitation at intervals. The ethanol extraction was done using soxholt extraction machine, while the aqueous extraction was carried out by the following steps: each extract was decanted, passed through muslin cloth, filtered through Whatman No. 1 filter paper and then lyophilized. Each solid/paste obtained after lyophilization was reconstituted in its respective solvent to obtain a stock solution of 512 mg/ml according to the National Committee for Clinical Laboratory Standards (NCCLS/CLS1, 2000). The stock solutions were stored in sterile capped bottles and kept at 4 - 8°C.

Test organisms

The test organisms used were five Gram-negative organisms (*Salmonella paratyphi*, *Shigella flexnerii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli)* and two Gram-positive organisms (*Staphylococcus aureus* and *Bacillus subtilis*). Control strains of *S. aureus* ATCC 25921 and *E. coli* ATCC 25922 were used with the other test organisms. They were obtained from the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Idi-araba, Lagos, where they were stored initially at 4°C in Mueller-Hinton agar.

Standardization of inoculum

Test organisms were sub-cultured onto fresh plates of MacConkey agar and incubated aerobically at 37°C for 24 h. Colonies from these plates were suspended in Mueller-Hinton broth (Oxoid, UK) to a turbidity matching 0.5 mc McFarland standard (10^8 m cfu/ml). Mueller-Hinton agar was then used for antimicrobial assay. All the broth cultures were incubated at 37°C (Aibinu et al., 2007).

Antimicrobial assay

Suspensions of the bacteria obtained contained approximately 1 x 10^8 cfu/ml. Each labelled plate was uniformly seeded with a test organism by means of sterile swab stick rolled in the culture medium. Aliquots ranging from 2 to 512 mg/ml were dropped in each well to fullness (Shahidi, 2004). Each plate was kept in the refrigerator for 1 h to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place. These plates were then incubated at 37°C for 24 h. The zones of inhibition around the wells were measured in millimeter. Ciprofloxacin and ethanol solvent with test organisms were placed in a well on each plate along with the test extracts as control.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates (Shahidi, 2004; Prescott et al., 1999). This was determined from readings on the culture plates after incubation.

Determination of minimum bactericidal concentration

Doubling diffusion containing different concentrations as used in MIC determination was carried out thus: to a 0.5 ml extract, 0.5 ml of sterile distilled water was dispensed, from this test tube labelled '1', 0.5 ml of the mixture was taken and dispensed to a test tube labelled '2' containing 0.5 ml sterile distilled water, this was done

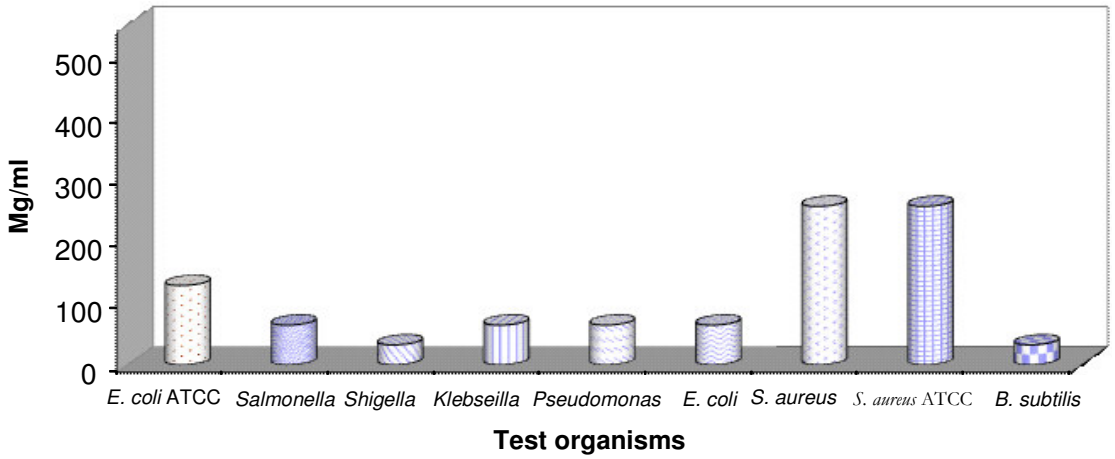

Figure 1. Minimum inhibitory concentration (MIC) of isolates to unripe banana (ethanolic extract).

twice and from the last test tube labelled '4', 0.5 ml of the mixture was taken so that the mixture remained as 0.5 ml. The stock solution (512 mg/ml) is 0.5 ml (without any dilution) and to this was added 0.5 ml of test organism. To the other tubes containing different concentrations of the extracts (256 to 32 mg/ml) 0.5 ml of each test organism was added. Samples were streaked from the tubes onto Nutrient agar plates to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by failure of the extract to kill the organisms. The lowest concentration that prevented bacterial growth after two days of incubation was recorded as minimum bactericidal concentration (Aibinu et al., 2007).

Determination of death rate of isolates

Death rate of the most susceptible and least susceptible organisms to banana, lemon grass and turmeric extracts were carried out. The organisms include *E. coli*, *S. aureus*, *B. subtilis* and *S. paratyphi*. This was done by mixing 0.1 ml of 10^8 cfu/ml of each test organism with 0.9 ml of stock (512 mg/ml) of each extract. Aliquots of each (0.1 ml) of the mixture was plated out on Nutrient agar duplicate plates at 30 min intervals for 30 – 150 min. They were incubated aerobically at 37°C for 24 h. The number of colonies on each plate at the time intervals was noted.

RESULTS

All ethanolic extracts of unripe banana, lemon grass and turmeric at the stock concentration had antimicrobial activity. Under the conditions employed, all the test samples had potent inhibitory effects on the group of bacteria tested. Unripe banana (ethanolic extract) showed a high antimicrobial activity against all test organisms with zone diameters ranging from 8 mm (*E. coli* ATTC 25922) to 31 mm (*S. aureus)*. Lemon grass (ethanolic extract) showed antimicrobial activity against all test organisms ranging 9 mm (*B. subtilis*) to 26 mm (*S. paratyphi*) while turmeric (ethanolic extract) had activity from 14 mm (*E. coli*) to 26 mm (*B. subtilis*). For the aqueous extracts, only unripe banana had good antimicrobial activity against five organisms with zones of inhibition ranging from 15 mm *(S. aureus* ATCC 25921) to 36 mm (*S. paratyphi)*. *S. flexnerii* was the least susceptible.

The minimum inhibitory concentration (MIC) of unripe banana ranged between 2 and 512 mg/ml depending on the isolate and extracting solvent (Figures 1 and 2). The minimum inhibitory concentration for unripe banana (ethanolic extract) was 32 mg/ml, while for lemon grass and turmeric it was 2 mg/ml. The minimum bactericidal concentration (MBC) ranged from 32 to 512 mg/ml. The minimum bactericidal concentration for unripe banana was 32 mg/ml (Figure 3), while for lemon grass it was 128 mg/ml (Figure 4) and for turmeric it was 512 mg/ml.

The MIC of lemon grass (aqueous extract) showed that *K. pneumoniae*, *S. aureus*, *S. aureus* ATTC 25921 and *B. subtilis* were only inhibited at the high concentrations of 512 and 256 mg/ml while other test organisms were resistant (Figure 5). The MBC of lemon grass (ethanolic extract) had good bacteriocidal effect on *E. coli* ATTC 25922, *S. paratyphi*, *S. flexnerii*, *K. pneumoniae*, *P. aeruginosa* and *B. subtilis*. At 128 mg/ml, *P. aeruginosa* and *B. subtilis* were killed, while at 512 mg/ml, *E. coli* ATTC 25922, *S. paratyphi*, *S. flexnerii* and *E. coli* were killed (Figure 6).

Turmeric (ethanolic extract) was found to be bacteriostatic on *E. coli* ATTC 25922, *S. paratyphi*, *S. flexnerii*, *K. pneumonia*, *S. aureus* ATTC 25921 and *B. subtilis* (Figure 7). The aqueous extract of turmeric had bactericidal effect only on *S. aureus* ATTC 25921, indicating that it showed only bacteriostatic effect on the other eight test organisms (Figure 8). Minimum bactericidal concentration of turmeric was only effective on some of the Gram-negative organisms (Figure 9).

The killing rate of the unripe banana (ethanolic extract) on *S. aureus* was 1 h 30 min, while turmeric (ethanolic extract) had a killing rate of 2 h 30 min on *E. coli*. Time course study showed that some of the extracts were able

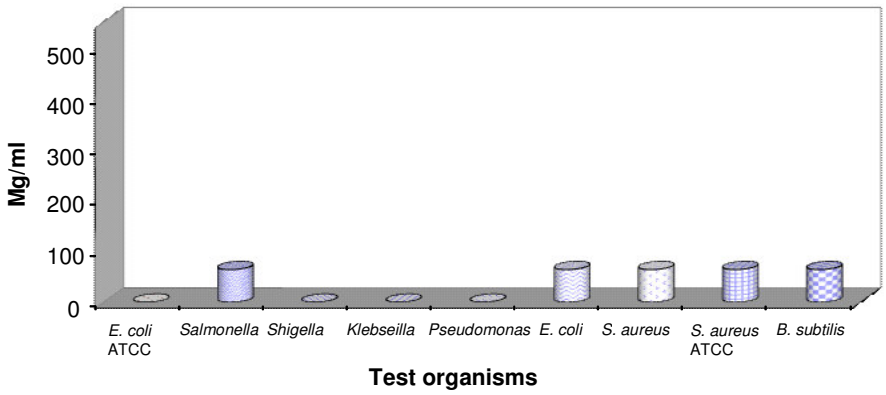

Figure 2. Minimum inhibitory concentration (MIC) of isolates to unripe banana (aqueous extract).

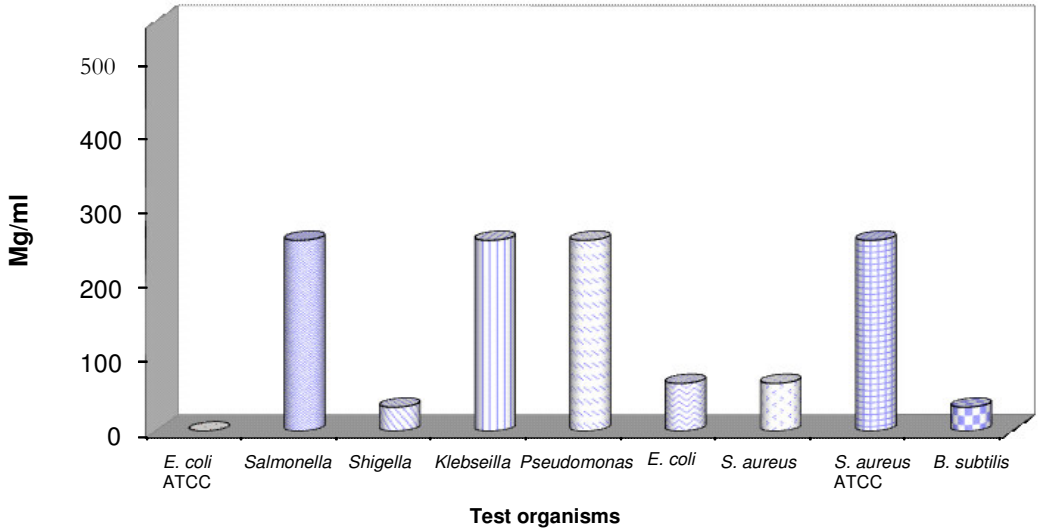

Figure 3. Minimum bactericidal concentration (MBC) of isolates to unripe banana (ethanolic extract).

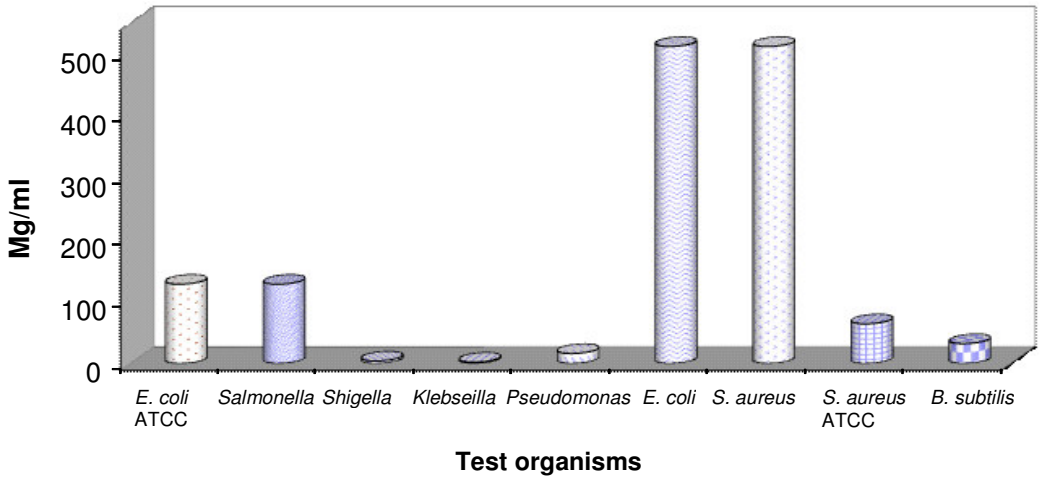

Figure 4. Minimum inhibitory concentration (MIC) of isolates to lemon grass (ethanolic extract).

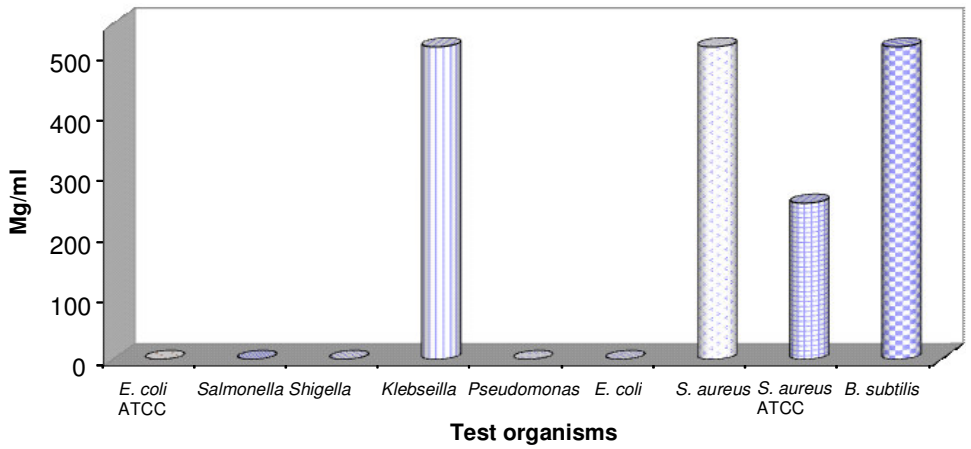

Figure 5. Minimum inhibitory concentration (MIC) of isolates to lemon grass (aqueous extract).

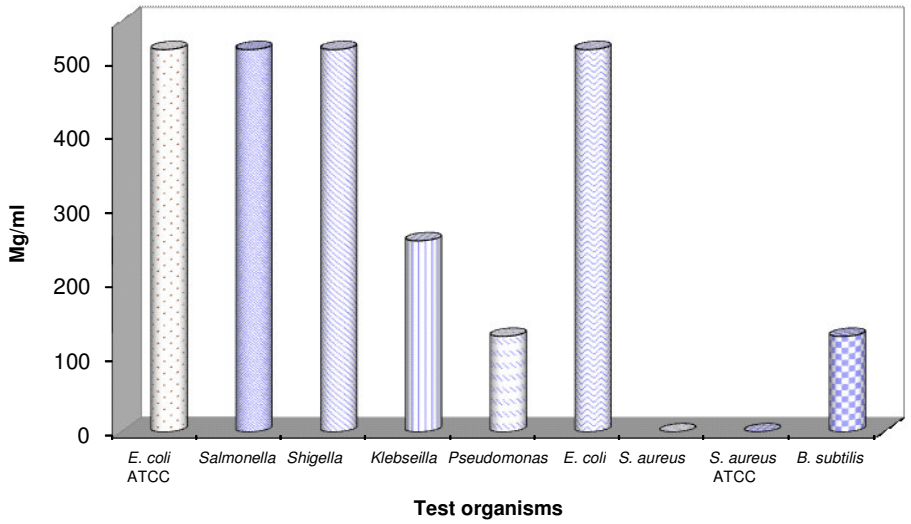

Figure 6. Minimum bactericidal concentration (MBC) of isolates to lemon grass (ethanolic extract).

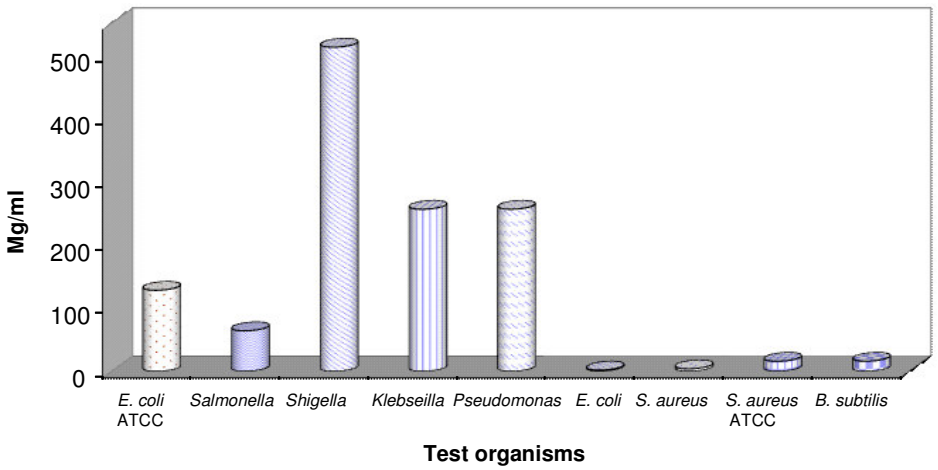

Figure 7. Minimum inhibitory concentration (MIC) of isolates to turmeric (ethanol ic extract).

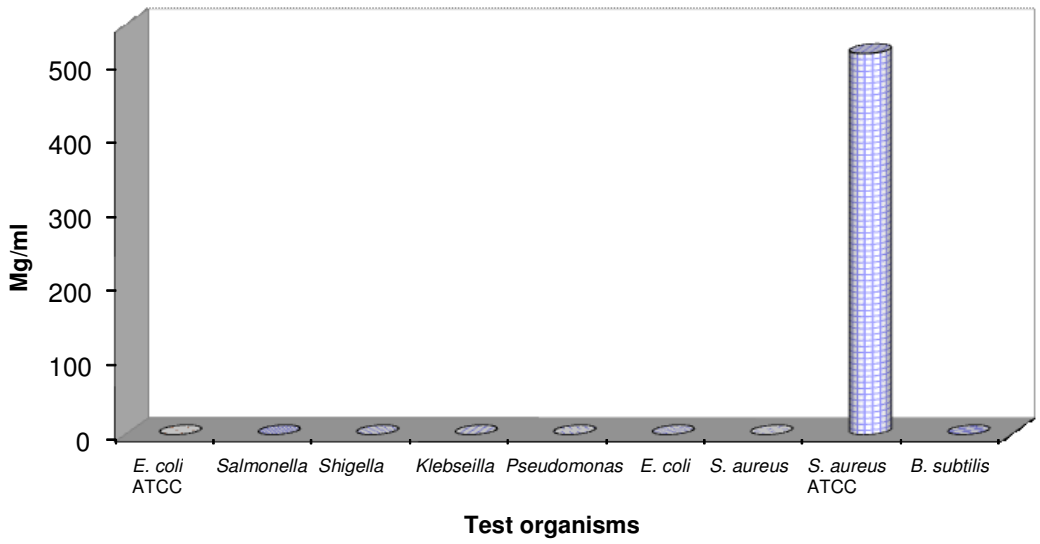

Figure 8. Minimum inhibitory concentration (MIC) of isolates to turmeric (aqueous extract).

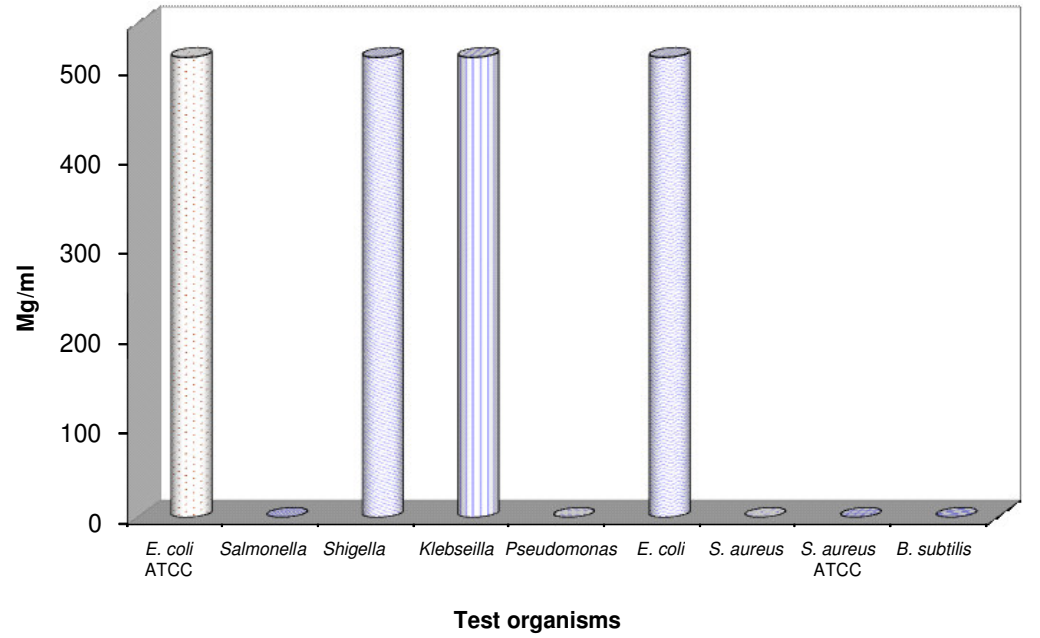

Figure 9. Minimum bactericidal concentration (MBC) of isolates to turmeric (ethanolic extract).

to kill all the cells after 1 h 30 min of exposure and 3 h 30 min of exposure for each plant extract respectively. Lemon grass (ethanolic extract) required more than 3 h of contact with the organisms before killing was affected thereby bringing about bactericidal effect (Figure 10).

DISCUSSION

The results obtained from the test analysis carried out reveal that unripe banana, lemon grass and turmeric plant have antimicrobial activity and therefore are medicinal plants (Manolito, 2006). In this study, it was observed that the potency of unripe banana, lemon grass and turmeric plant was enhanced by the type of solvent used, indicating that some of the active materials in these medicinal plants dissolve well in ethanol than in water. Unripe banana had more antibacterial activity when used with the two different solvents (ethanol and water) than lemon grass and turmeric that had good antibacterial activity with only ethanolic extract. However this study corresponds with the results of Gurumaa (2008), Aibinu

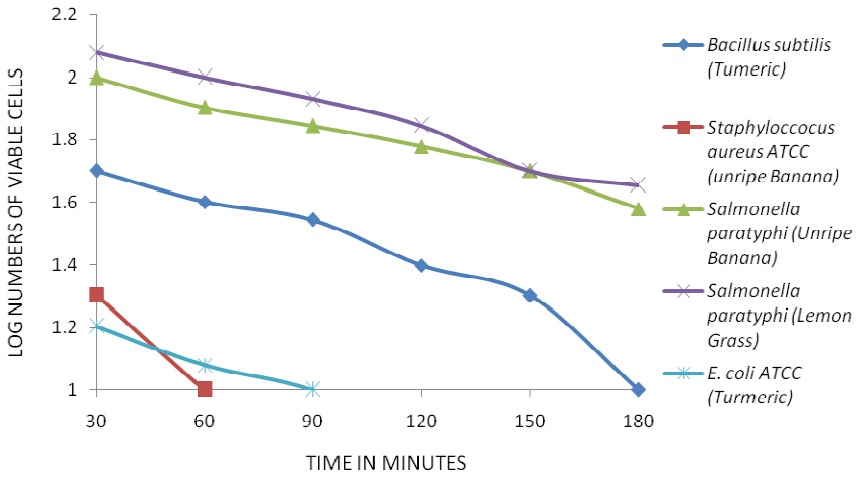

Figure 10. Determination of death rate.

et al. (2007), Araujo and Leon (2001), Eigner and Scholaz (1999) and Ammon and Whal (1991) who observed that unripe banana, lemon grass and turmeric have antimicrobial activities. This implies that there is still a lot to gain from these medicinal plants as an antimicrobial pointer to new sources of novel drugs, which need further investigations. Many plants are used in Nigeria in the form of crude extracts, infusions and plasters to treat common diseases without specific evidence of efficacy. In this study, the microbiological investigations done on unripe banana, lemon grass and turmeric obtained in Nigeria have shown activity coherent with the use of these plants in folk medicine.

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