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Assaying for the effectiveness and efficiency of some local plant extracts in the control of dairy farm-associated bacteria pathogens using *in vitro* well diffusion technique

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Abstract

Though antimicrobial agents have been used for the effective control of infectious agents, there is a growing concern about the emergence and re-emergence of multidrug-resistant bacteria pathogens against commonly prescribed antibiotics. Consequently, there is gross reduction in the effectiveness of the control mechanism thereby increasing health care costs and its associated consequences. Plant parts and extracts have been used in crude forms for the local control of several ailments including those of animals. Two-phase research was set-up. One, to isolate and identify multi-drug bacteria pathogens from some small-scale farms in Kwali, FCT, Abuja and their drug resistance status. The second part was to screen and evaluate the antimicrobial effects of common phytochemicals from some local plants, viz; guava (*Psidium guajava*), ginger (*Zingiber officinale*), neem (*Azadirachta indica*), and moringa (*Moringa oleifera*) against the previously isolated MDR bacteria (*Salmonella spp. Clostridium sp. Staphylococcus aureus*, *Streptococcus sp.* and *E. coli*.). The aqueous, ethanolic and methanolic extracts of the plants were used for susceptibility test, minimum inhibitory concentration and minimum bactericidal concentrations using *in vitro* agar disc diffusion protocol based on the CLSI methods. The results showed significant Inhibitory zones range of 23-32mm. This is in agreement with several research previously done using plant extracts. The type of solvent used also influenced the quality of the extract and the output. Based on the results, we concluded that the plant extracts have some antimicrobial activities comparable to the currently prescribed modern drugs tested. It is justifiable that the therapeutic agents derived from these plants would be effective in the control of emerging diseases and also contribute to the growth of scientific knowledge about herbal medicines as important alternatives or complementary treatment of animal diseases. It is hereby recommended that further research (The third phase) carry out studies on the clinical efficacy trial, safety, toxicity and affordability analyses as well as optimization and upscaling parameters so as to proceed to the final step for the mass production of the tested products.

Keywords: Antimicrobial; Extracts; Pathogens; Susceptibility; Resistance; Assay

1. Introduction

Diseases and health challenges caused by the enterobacterial such as Typhoid fever is a global health concern particularly for the developing world, including Nigeria. The reported cases are on the increase resulting in several death and morbidity (Mirza and Khan, (2008). Its annual incidence is around 16.5 million cases and the disease led to

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190 000 deaths worldwide in 2010. A very high annual incidence of cases in Africa was reported in 2020 and some multi-drug resistant (MDR) bacteria pathogens such as Salmonella Typhi exhibiting resistance to some first line drugs (Ampicillin, Chloramphenicol and Co-trimoxazole). This have been reported to be of high health significance. Although the problem of multi-drug resistant (MDR) Typhi was solved by fluoroquinolones and these drugs became the treatment of choice for Typhi. However, the efficacy of fluoroquinolones was questioned due to emergence of Typhi strains resistant to nalidixic acid and with decreased susceptibility to ciprofloxacin. The rising antimicrobial resistance is because of cases of under-use and indiscriminate use of the antibiotics concerned (Barah and Gonçalves, 2010). The alternative search for antimicrobial agent from plant extracts has been on for several years. The local folks have used these products at several occasions with amazing results (Parvin *et al*., 2015). The critical issue of antibiotic resistance and treatment failures led to the need to explore for new antimicrobial agents from various sources like plants. Hence a valuable approach to address the issue of antibiotic resistance among pathogenic bacteria is to formulate new antimicrobial agents using the plant extract singly or the combination of natural plant extracts having antimicrobial properties. This method would be cost effective and the treatment would be easily available to several people in Africa. (Gupta *et al.,* 2009).

2. Material and Methodology

2.1. Bacterial Isolates

This experimental study was conducted by first isolating some drug resistant bacteria; Salmonella spp. Clostridium sp. Staphylococcus aureus, *Streptococcus sp*. and *E. coli*. The respective isolates were identified by gram staining, biochemical profile using API and were maintained in microbanks at -70˚C, where the working stock were thawed and then sub-cultured on nutrient agar plates when needed for the experiment (Wasfy, 2002).

2.2. Preparation of Plant Extracts

Indigenous plants; guava (*Psidium guajava*), ginger (Z*ingiber officinale*), neem (*Azadirachta indica*), and moringa (*Moringa oleifera*) were procured from local market of Lugbe, Abuja, Nigeria and the aqueous, ethanolic and methanolic extracts were prepared by the method used by Hannan *et al*. (2012). A hundred grams of each sample were homogenized in 300ml of the respective clean solvent using a blender. The homogenate was filtered by passing it first through sterile cotton mesh and then through Whattman filter paper 1. Thereby producing crude extract.

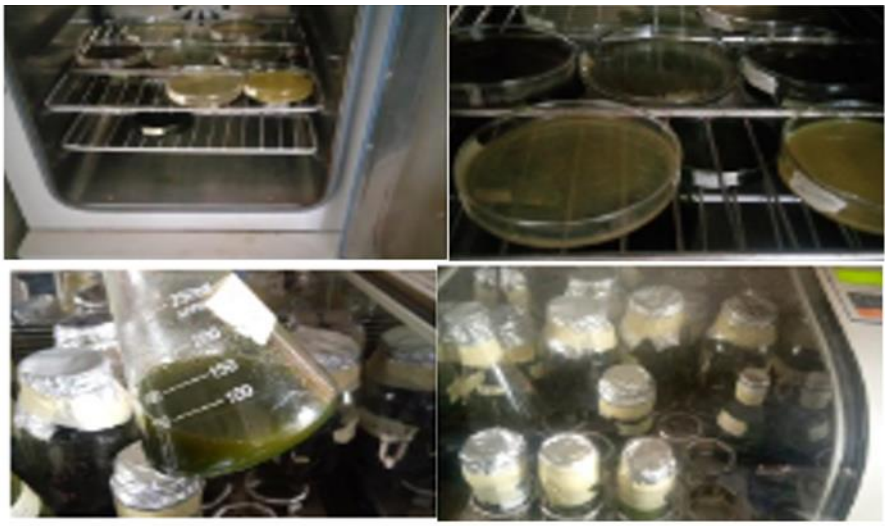

Figure 1 Processing of plant extracts

Figure 2 Antimicrobial Susceptibility Tests

2.3. Screening for Inhibitory Effect of the Extract

The respective extract was tested for their potencies by Agar well diffusion method. The pure cultures of the Isolates were screened for the inhibitory effect of the extracts. In the process, the Bacteria suspension equivalent to 0.5 Mc Farland turbidity standard was lawned over the surface of Mueller-Hinton agar (Oxoid). Afterwards, four wells were cut into the agar with sterilized cork borer. The extracts and controls were tested in triplicate by adding 120µl of each in individual wells. 100% extract, 50%, 25%, distilled water (negative control), and 6% Streptomycin (positive control) were run. The diameters of the clear zones were measured in mm with a piece of ruler after incubation for 24 hours at 37˚C (Zhou and Pollard, 2010).

3. Results and Discussions

The current study was conducted to address the problem of multi-drug resistance among some bacteria with a view to providing alternative antimicrobial sources from plants. The 100% concentration of all the extracts produced an inhibition zone ranges from 12.32 to 16.23 mm. At 50% concentration, it produced inhibition zone of 21.43 to 24.53 mm and 25.2 to 27.5mm at 25% concentration of the Methanol, Ethanol and aqueous extraction agents respectively. Thus, a decline in antibacterial activity was observed with decreasing concentration. The antibiotic control produced an inhibition zone of 35.32mm. All the extract tested showed significant inhibition zone by agar well diffusion method. The results are consistent with some other studies which showed that several plant extracts can be utilized and healing therapy for several bacteria pathogens either singly or in combination (Al-Blooshi *et al*., 2021). Therefore, the individual fractions of the respective extracts need to be done in order to identify the actual portions that are active for further developmental purposes.

4. Conclusion

The synergies of the antimicrobial agents could also be considered in the in vivo trial system. In conclusion all the plant extracts used in this work showed significant antibacterial activity individually against all the bacteria tested.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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