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Original Research Article

Antibacterial Effects of Aqueous Extracts of Musa Paradisiaca, Ziziphus Mucronata and Senna Singueana Plants of Malawi

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ABSTRACT

Introduction and Background: In Malawi, plants are used to treat bacterial infections and other common diseases. Some of the plants used are *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana*. Medical herbalism is widely practiced in Malawi such that most people in rural areas of Malawi have knowledge of a wide variety of herbs that can be used to treat common ailments but they lack some scientific backing.
Methods and Materials: The roots and leaves of *Senna singueana* were collected whereas roots of *Musa paradisiacal* and *Ziziphus mucronata* were collected on Zomba plateau and Blantyre in Southern Malawi.
Extraction and antimicrobial assays of freshly collected plant materials were done according to the study protocol. The antibacterial effects of the aqueous extracts of *Musa paradisiaca* root, *Ziziphus mucronata* root bark and *Senna singueana* roots and leaves were assessed via the disc diffusion and Time-kill log reduction methods.
Results: The herbal extracts were tested against *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* strains. The findings showed that the microorganisms were less susceptible to the concentrations of herbal extracts tested. The extracts of *Ziziphus mucronata* roots, *Senna singueana* leaves, *Senna singueana* roots had a microbial effect (ME) of zero (0) for all the herbal concentrations of both micro-organisms. *Musa paradisiaca* extracts showed ME of 1 for the concentrations of 1000ug and 500ug and zero (0) for 200ug for both micro-organisms. This means that an ME of 1 equals to a killing of 90% of the initial number of bacteria, an ME of 0 had no lethal effect.
Conclusion: The findings suggest that *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* strains used in this study were less susceptible to the tested plants extracts. The microbial strains used may be resistant to the extracts but the results could better represent the real susceptibility pattern when tested against different microorganisms in various concentrations. Further research toward the formulation of an effective antimicrobial remedy of *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana* plants needs to be done in order to validate the use of these plants as antimicrobial alternatives by herbalists.

Key words: Antimicrobial, Malawi, *Musa paradisiaca, Ziziphus mucronata*, *Senna singueana*, herbal, plants, medicine.

INTRODUCTION AND BACKGROUND

In Africa plants are usually used as medicinal agents. Roots, leaves and barks are extracted and processed to produce chemical compounds that work for different diseases and infections. Some of the plants used are *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana.*

Traditional herbal medicine have good efficacy and are less costly as opposed to western medicine (Dahiru et al. 2006).These plants are commonly available in Africa and specifically in Malawi. They are used to treat different ailments. Some of the commonly isolated pathogens in Malawi are *Staphylococcus aureus, Escherichia coli, Salmonella species, Streptococcus pneumoniae*, enteric gram negative species, *Neisseria meningitides* and many others. The present therapeutic options against bacterial infections are limited due to emerging drug resistance in human pathogenic microorganisms partially due to the uncontrolled use of antimicrobial agents by the community (Makoka et al. 2012).

It is established that medicinal plants contain phytochemical compounds which may be potential traditional remedies and therefore serve as alternative, less expensive and safe antimicrobial agents for the treatment of common infectious ailments. Malawi has a rich floral biodiversity with a variety of plants most of which are used in traditional management of a host of diseases some which were reported by (Hussain et al. 2011; Green et al. 2010) include malaria, urinary tract infections, tuberculosis, diarrhoea and others in their studies outside the country. Like elsewhere in the developing world, medicinal plant usage in the management of infections is widespread in Malawi. Medical herbalism is widely practiced in Malawi such that most people in rural areas of Malawi have knowledge of a wide variety of herbs that can be used to treat common ailments (Morris 2011).

Despite the usage of medicinal plants in traditional management of infectious diseases and other ailments, there have been very few studies that have assessed their effectiveness and safety in Malawi. Contemporary use of medicinal plants is not fully supported by scientific understanding, therefore this study attempted to explain such existing discrepancy. Despite lack of enough scientific backing, it is clear that herbal medicine is a dominant form of medicine in Malawi but this enormous indigenous knowledge is not properly documented by traditional healers. The cost of western medicine, emergence of growing resistant microbial strains, adverse side effects and traditional beliefs could be some of the factors that contribute to heavy reliance on herbal medicine in our communities.

Literature Review: A number of laboratory based studies have demonstrated that medicinal plants used in folkloric treatment of infectious diseases possess potent antimicrobial activity (Perianayagam et al. 2012). Antibacterial compounds identified in medicinal plants include; flavonoids, alkaloids, tannins (Erasto et al. 2004; Banso, A. and Adeyemo 2007), triterpenes, steroids and phenolic compounds (Ccahuana-vasquez 2007). Although medicinal plants could be considered safe, there have been many reports of side effects attributed to active ingredients, contaminants, or interactions with other drugs (Bent 2008; Fennell et al. 2004).

***Senna Singueana* (**English Name: Scrambled egg). (Local name: Mpatsachokolo)

It is used for skin cancer, fresh bark chewed for stomach spasm (Moellering et al. 2007). *Senna singueana* has many medicinal uses throughout Africa that include treatment of skin cancer (Gebrelibanos 2012). A hot water infusion of the leaves is drunk and the warm leaves are applied as a compress to treat fever. The leaf sap is drunk to cure malaria. The leaves in decoction or infusion or as dried powder are applied to wounds caused by leprosy and syphilis. An infusion of the leaves is applied as eye drops to cure conjunctivitis. Extracts of the stem bark are taken to cure stomach complaints (Moellering et al. 2007). Like the leaves, the stem bark is used to treat skin disorders and malaria, as well as reducing fever (Adzu et al. 2003). An infusion of the flowers is used as an eye lotion. The fruit pulp soaked in water and cooked with a staple food is eaten by lactating women as it is considered lactogenic. The roots are used to treat venereal diseases, stomach complaints and as a purgative (Gebrelibanos 2012). The roots are also used to cure impotence caused by diabetes. The ash of burnt roots is eaten mixed with porridge to cure abdominal pain. Leaves, stem and root bark are used as an anthelmintic and to treat bilharzias. Leaves are used for worms and stomach pains (Kareru P. G., Gachanja A. N., Keriko J M. 2008).

Musa Paradisiaca (English Name: Banana). (Local name: Nthochi)

Musa paradisiaca is a monoherbacious plant belonging to family; Musaceae, commonly known as plantain. Banana is the common name for herbaceous plants of the genus *Musa* and for the fruit them produce (Sanjeev Kumar, Chanchal Kumar Mishra, Anil Ahuja 2012). Traditionally the plant *Musa paradisiaca* was used for different purposes such as to treat abscess, alopecia in females, cancer, diabetes, diarrhoea, snake bite, hypertension, migraine headaches, bacterial, fungal and viral infections, and wounds (Sahu et al. 2014). It is also used to treat different general illnesses in pregnant women (Maliwichi-nyirenda & Maliwichi 2010).

Ziziphus Mucronata (English Name: Buffalo thorn). (Local name: Kankhambe)

The fruits, bark, roots and leaves of *Ziziphus Mucronata* are used as traditional medicine in Africa, India and South America (Mokgolodi et al. 2011). Roots extracts are drunk as abdominal pains, infertility in women medicine whereas the root powder is applied on wounds (Olajuyigbe & Afolayan 2011; Olivier & van Wyk 2013).

The plant fresh fruits are sucked by children, dried ones can be pounded and prepare porridge, while in some parts they are fermented to make traditional beer. The seeds can be used as coffee, leaves are edible. In many parts of Africa, the plant is used to treat ulcers, chest pains, diarrhoea, abscesses, sexually transmitted infections (STI) for instance Syphilis and gonorrhoea, snake bites, Measles, *Mycobacterium tuberculosis*, Helminths, and many bacterial infections (Mokgolodi et al. 2011; Olajuyigbe & Afolayan 2011; Kwape & Chaturvedi 2012). Besides having medicinal importance, it could be used as laxatives, sedatives, anti-oxidants and anti-nausea among others (Hussain et al. 2011; Kwape & Chaturvedi 2012). The investigators were informed by the local herbalists that they use the plant to treat different ailments that include dysentery, swellings, chest pains, toothache, eye diseases, swollen and open wounds in Malawi.

It is clear that the plant is used to treat different ailments in different places. (Olivier & van Wyk 2013) reported that some traditional healers use it to treat respiratory infections, scarlet fever, malaria, asthma and infertility/sterility. *Ziziphus mucronata* subsp. *mucronata* has strong antioxidant property and free radical scavenging capability (Olajuyigbe & Afolayan 2011).

This study investigated the phytochemical compounds in this plant and tested them against common microbial isolates in Malawi.

There are few studies in Malawi that have been conducted to assess the efficacy and safety of medicinal plants. This study was aimed at assessing the antibacterial potential of some indigenous Malawian plants, *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana* commonly used in traditional management of abdominal pains, diarrhoea and sexually transmitted diseases in rural areas of Malawi as reported by local herbalists. The study also evaluated the toxicity properties of the medicinal plant extracts using brine shrimp toxicity assay. The potential benefits of such assessment included identification and isolation of the therapeutic principles which may lead to drug development, in addition to validating its traditional usage.

MATERIALS AND METHODS

The methods used in this study were adopted from (Kareru P. G., Gachanja A. N., Keriko J M. 2008; Sahu et al. 2014). The necessary adjustments were made to suit our study conditions

The plant materials were identified and authenticated by botanists of the National Herbarium and Botanical Gardens at Zomba, Malawi. The roots and leaves of *Senna singueana* were collected whereas roots of *Musa paradisiacal* and *Ziziphus mucronata* were collected on Zomba plateau and Blantyre in Southern Malawi.

Freshly collected plant materials were thoroughly cleaned using water and air-dried at room temperature. Dried plant roots or leaves were pulverized in a blender. The coarsely ground plant materials were macerated in distilled water for 72 hours at room temperature and collected in a clean beaker. The materials were filtered using whatman filter paper No. 2. The filtrate was left to dry through evaporation in an open space for between 72 hours. The evaporation process was enhanced by the use of an electrical fan which was left to blow over the plant extracts. The dry crude extracts were scrapped from the beakers and the powder was immediately used for antimicrobial assays according to the study protocol.

The study used *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* strains which were obtained from the University Of Malawi College Of Medicine, Microbiology Unit. The antibacterial effects of the aqueous extracts of *Musa paradisiaca* root, *Ziziphus mucronata* root bark and *Senna singueana* roots and leaves were assessed via the disc diffusion and Time-kill log reduction methods. The neat extracts were serially diluted to 1000µg, 500µg and 200µg and tested against both *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662*organisms.

Quantitative suspension test was performed using both *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* bacterial species. Pure isolated colony of bacteria from *Mueller Hinton* (MH) agar plate was grown separately in 10ml of MH broth for 24 hours at 37°C. After incubation, the tubes were centrifuged for 20 minutes at 2,000 rpm. The cell pellets were washed with 10ml of MH broth. The bacterial suspension in MH broth was adjusted to the McFarland 0.5 standard.1µl of bacterial suspension was added to 9µl of the herbal solutions at room temperature for a contact time of 3hrs and then 1µl was removed into 9µl of the neutralizer system and serially diluted to 10^{-1} to 10^{-8}. Three drops of 10µl of each dilution was placed onto MH agar plates by the spread-plate technique (Miles and Misra method) and incubated at 37°C for 18 to 24 hours. The reduction rate was calculated as the expression of the herbal efficacy, according to the following formula: log10 reduction = log10 pre-treatment count – log10 post treatment count. The microbicidal effect (ME) was obtained by subtracting the logarithm of the former from the logarithm of the latter, the decimal log reduction. An ME of 1 equals to a killing of 90% of the initial number of bacteria, an ME of 2 means 99% killed. A generally accepted requirement is an ME that equals or is greater than 5: at least 99.999% of the germs are killed. Log10 reductions of 5 or more should be taken as an indication of satisfactory microbicidal activity.

RESULTS

The plant extraction was successful and yielded enough powder for testing. The herbal extracts were tested against *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* strains. The findings suggest that the tested concentrations of all herbal extracts had no activity against the microorganisms used in this study. The zone of inhibition for all the measurements was 6mm which was the actual size of the disk, indicating resistance. Table 1 below shows the sizes of zones of inhibition obtained from each disk.

Table 1: Antimicrobial susceptibility testing

Chemical Substance	Tests	Zone of Inhibition measured in mm						
Senna singueana leaves		1000ug	500ug	200ug	1000ug	500ug	200ug	Mean
		Staphylococcus aureus NCTC 6571			PsuedomonasaeuroginosaNCTC 10662			
	1	6	6	6	6	6	6	6
	2	6	6	6	6	6	6	6
	3	6	6	6	6	6	6	6
	NC	6	6	6	6	6	6	6
Mean		6	6	6	6	6	6	6
Chemical Substance	Tests	Zone of Inhibition measured in mm						
*Senna singueana*roots		1000ug	500ug	200ug	1000ug	500ug	200ug	Mean
		Staphylococcus aureus NCTC 6571			Psuedomonasaeuroginosa NCTC 10662			
	1	6	6	6	6	6	6	6
	2	6	6	6	6	6	6	6
	3	6	6	6	6	6	6	6
	NC	6	6	6	6	6	6	6
Mean		6	6	6	6	6	6	6
Chemical Substance	Tests	Zone of Inhibition measured in mm						
*Ziziphusmucronata*roots		1000ug	500ug	200ug	1000ug	500ug	200ug	Mean
		Staphylococcus aureus NCTC 6571			Psuedomonasaeuroginosa NCTC 10662			
	1	6	6	6	6	6	6	6
	2	6	6	6	6	6	6	6
	3	6	6	6	6	6	6	6
	NC	6	6	6	6	6	6	6
Mean		6	6	6	6	6	6	6
Chemical Substance	Tests	Zone of Inhibition measured in mm						
Mussaparadisiaca		1000ug	500ug	200ug	1000ug	500ug	200ug	Mean
		Staphylococcus aureus NCTC 6571			PsuedomonasaeuroginosaNCTC 10662			
	1	6	6	6	6	6	6	6
	2	6	6	6	6	6	6	6
	3	6	6	6	6	6	6	6
	NC	6	6	6	6	6	6	6
Mean		6	6	6	6	6	6	6

Table2: log reduction results

Chemical Substance	**[CFU %] Before 3hrs**	**Microbial Effect After 3hrs Herbal Suspension**					
		1000ug	500ug	200ug	1000ug	500ug	200ug
		Staphylococcus aureus NCTC 6571			***Psuedomonasaeuroginosa NCTC 10662***		
*Ziziphusmucronata*roots	1.5E8	0	0	0	0	0	0
Senna singueana leaves	1.5E8	0	0	0	0	0	0
*Senna singueana*roots	1.5E8	0	0	0	0	0	0
Mussaparadisiacal roots	1.5E8	1	1	0	1	1	0

Table 3: Log reduction reference table

Conversion Table (assuming an initial pathogen concentration of log 7 CFUs)

CFU Concentration (%)	% Reduction	CFU/ml	Log Reduction
100	0	10,000,000	0
10	90	1,000,000	1
1	99	100,000	2
0.1	99.9	10,000	3
0.01	99.99	1,000	4
0.001	99.999	100	5
0.0001	99.9999	10	6
0.00001	99.99999	<1>	7

Adopted from http://www.smith-nephew.com/new-zealand/healthcare2/products/product-types/antimicrobial-dressings/silver-dressings/acticoat--product-range/acticoat--faqs/acticoat--log-reduction/

Table 2 below shows the antimicrobial assay (log reduction) results whereas table 3 is a Log reduction reference table.

The findings in table 2 show that the extracts of *Ziziphus mucronata* roots, *Senna singueana* leaves, *Senna singueana* roots had a microbial effect (ME) of zero (0) for all the herbal concentrations of both microorganisms. *Musa paradisiaca* extracts showed ME of 1 for the concentrations of 1000ug and 500ug and zero (0) for 200ug for both microorganisms. This means that an ME of 1 equals to a killing of 90% of the initial number of bacteria, an ME of 0 had no lethal effect. A generally accepted requirement is an ME that equals or is greater than 5: at least 99.999% of the microbes are killed. Log10 reductions of 5 or more should be taken as an indication of satisfactory microbicidal activity.

DISCUSSION

The study was conducted on commonly used herbal extracts (three plants extracts *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana*) in Malawi based on information supplied by the local herbalists. The findings suggest that the tested microorganisms were not susceptible to the herbal concentrations they were subjected to save *Musa paradisiaca* roots which showed lethal effects on *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662*. These organisms are usually isolated in clinical microbiology laboratories using conventionally techniques as opposed to fastidious microorganisms (Bronzan et al. 2007).In a previous study by Makoka et al, on bacterial infection in Lilongwe, *Staphylococcus aureus* was recovered from patients' blood, bone, joint and pus but was not isolated in cerebral spinal fluid (CSF) (Makoka et al. 2012).

The extraction method employed in this study could have potentially affected the susceptibility pattern of pharmacological active compounds of the extracts.

Other studies on *Musa paradisiacal* reported that this plant has significant antimicrobial activity against commonly isolated microbes. The soxhlet extracts recorded more inhibitory activity than the aqueous extracts on *Bacillus* and *Streptococcus* species (Kemka-Evans, C.I., Ngumah et al. 2013). Our findings are in line with (Ahmad & Beg 2001) who did not find any correlation between antimicrobial resistance behaviour of the microbial strains with plant extracts. They used (*Escherichia coli* UP2566, *Staphylococcus aureus* IOA-106 which was drug resistant, and Salmonella species against antibacterial and anti-fungal drugs.

Similar experiments done by (Nair & Chanda 2005) used ethanol, acetone, and propanol to prepare their extracts. Their results showed positive effects on Candida species but our study used water to prepare herbal extracts mimicking the same conditions used by herbalists. A study conducted in South Africa showed that the roots, leaves and barks of *Ziziphus mucronata* had antimicrobial effects against microorganisms such as *Staphylococcus aureus* (ATCC 12600) and *Moraxella catarrhalis*. The antimicrobial activity recorded for the plant extracts validates their traditional usage to treat various respiratory infections (Suliman 2010). However, in this study, we used different strains of microorganisms. Therefore it is not clear whether susceptibility patterns were due to the type of microbial strains used, herbal extracts concentrations, or the extraction solvents. Olajuyigbeet al. (2013) (Olajuyigbe & Afolayan 2011) observed that the ethanolic extracts of *Ziziphus mucronata*, demonstrated synergistic properties with each of the following antibiotics; Amoxicillin, Chloramphenicol, Ciprofloxacin, Tetracycline hydrochloride, Kanamycin, and Nalidixic acid to produce significant antibacterial effects at their supposed target sites. The observed antibacterial effects were experimented on the following bacterial strains: *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Shigella flexneri*.

CONCLUSION AND RECOMMENDATIONS

With various biological activities, *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana* continue to be used as traditional remedies in most parts of the country. The findings suggest that *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa NCTC 10662* strains used in this study were less susceptible to the tested plants extracts. Known resistant strains were used as a crucial step towards understanding the effect of traditional medicine on difficult to treat microorganisms.

Further research toward the formulation of an effective antimicrobial remedy of *Musa paradisiaca, Ziziphus mucronata* and *Senna singueana* plants needs to be done in order to validate the use of these plants as antimicrobial alternatives by herbalists. Therefore, we suggest repeating the susceptibility testing using different microorganisms. The microbial strains used may be resistant to the extracts but the results may better represent the real susceptibility pattern when tested against different microorganisms in various concentrations.

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