Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (3) : 2020 : 541-547

ISSN-0972-3005

ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS FROM NANDI COUNTY, KENYA

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(Received 28 March, 2020; accepted 13 August, 2020)

Key words : *Medicinal plant, Antimicrobial, Infectious diseases and Bioactive constituent*

Abstract – Medicinal plants are used by the local people to treat different human diseases since time immemorial. The efficacy of most of these plants has not been determined. The present study was therefore conducted at Nandi County to determine antimicrobial activities of seventeen selected medicinal plants that are commonly used to treat infectious diseases caused by bacteria. The plants were collected from the field and dried in a room at 25 °C. The samples were ground into powder and the components extracted using methanol. Plant extracts were tested against ten standard and clinical isolates of human pathogenic bacteria, using disc diffusion and broth dilution methods. The solvents used for extraction were used as negative control while Gentamycin was used as positive controls. *In vitro* antimicrobial assays indicated that four plant extracts exhibited antimicrobial activity in which the highest activity was observed from root bark extracts of *Albicia coriaria, Acacia lahai, Olinia rochetiana, Leucas calostachys* and stem bark of *Sygium cordatum* against *S. aureus* among others. This study demonstrated support for the claimed antimicrobial uses of the plants in the traditional medicine probably due to the phytochemicals present. Isolation and purification of bioactive chemical constituents from the active crude plant extracts can be obtained to supplement conventional drugs.

INTRODUCTION

The use of traditional medicine plays a critical role in healthcare particularly in developing countries. In the last decade, more intensive studies for natural therapies against microbial organisms have been happening because of emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria. This has become a significant public health threat because there are fewer effective antimicrobial agents available for the infection caused by pathogenic bacteria. Therefore, traditional medicine can serve as a source for both traditional and conventional medicine (Akinyemi *et al.,* 2007).

According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developing countries including Kenya use traditional medicine, which has compounds derived from medicinal plants (Gude, 2013). The emergence of the antibiotic resistant bacteria to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Marasini *et al.,* 2015). With the rapid global spread of resistant clinical isolates, determination of antibacterial activities of different medicinal plants of special interest is important. It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs, against Tuberculosis and HIV/AIDS pandemic (Kisangau *et al.*, 2007). Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi (Olila *et al.*, 2001; Kisangau *et al.*, 2007). Therefore, it is highly imperative to determine compounds that can be used to develop novel medicines with higher antimicrobial properties. The screening of plant products for antimicrobial activity has shown that the higher plants represent a

potential source of novel antibiotic prototypes (Afolayan, 2003). The plant produces a wide variety of secondary metabolites that includes alkaloids, anthraquinones, flavonoids, and phenolic compounds (Edeoga *et al.,* 2005). They are used either directly as precursors or as lead compounds in the pharmaceutical industry. This has forced scientists to search for new therapeutic substances from various sources like the medicinal plants. A number of researchers all over the world have studied the actions of phytochemicals on bacteria, (Doughari *et al*., 2006).

Some medicinal plants have been found to be used ethno botanically for the treatment of respiratory disease, tuberculosis, pneumonia, and dysentery (Kisangau *et al*., 2007), intestinal pathogens (Oyewole *et al*., 2012). Furthermore, some plants such as, *Ekerbergia capensis* and *Kigelia africana* have antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, (Kimutai *et al*., 2015). However, several other medicinal plants in the county have not been investigated for their antibacterial properties. For instance, *Cythula schimperiana, Lactuca glandulifera, Plantago palmate, Senecio discifolius, Zehneria minutiflora, Albizia coriaria, Erythrinia abyssinica, Leucas calostachys, Ficus cycomorus, Szigium cordatum, Urtica mossaica and Cleodendrum myricoides* (Table 1). Among these plants *Cythula schimperiana*, *Plantago palmate*, *Zehneria minutiflora* and *Albicia coriaria* are traditionally used to treat diarrhea, among other infections, (Kokwaro, 1973). The choice of these plants was based on the ethnobatanical studies carried out in the county, (Kimutai *et al*., 2019). Therefore, the present study investigated the antimicrobial activity of seventeen medicinal plants used against human pathogenic bacteria.

MATERIAL AND METHODS

Collection and processing of plant material

The information on the medicinal plants was gathered, from the traditional practitioners using structured questionnaires in order to obtain information on the medicinal plants that are traditionally used for the management of infectious diseases in Nandi county. The information that was gathered include; the common/local name of plants, the source, the part(s) used, methods of preparation, mode of administration, the disease it cures, dosage, frequency of use, their effectiveness and duration of treatments. Selection of the plants was based on available ethnobotanical information from traditional health practitioners consulted during the study, as well as the available literature. Plant specimens - leaves, roots, fruits, and flowers were collected from various parts of Nandi county for identification. Authentication of the plants species was done by a botanist from the University of Eldoret, where samples were assigned voucher specimen numbers and deposited at the same university herbarium, (Kimutai *et al.,* 2019). These plant samples were washed with tap water in order to remove the dust. Collected plant materials were then dried in a room at 25 ^{o}C for three weeks, ground using a grinder and stored for extraction.

Extraction procedure

Fifty grams of powdered plant materials was soaked in 200 mL methanol using 250 mL conical flasks, corked and allowed to stay for 24 hours. The macerate obtained was filtered with whatman No. 1 filter paper and the filtrate (methanol) concentrated in a round bottomed flask using a rotary evaporator and air dried for three days, put in sterile airtight vials weighed and kept in a desiccator at 4^{o} C in readiness for use (Ana *et al*., 2005).

Microorganisms

Microorganisms used in this study were both clinical isolates and standard reference strains sourced from Centre for Microbiology Research (CMR) of Kenya Medical Research Institute, (KEMRI). The standard and clinical isolates used include: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, Methicillin resistant *Staphylococcus aureus* (MRSA), ESBL *Escherichia coli, Klebsiella pneumonia*, *Shigella sonnei, Salmonella typhi, Citrobacter freudii* and *Enterococcus faecalis* (Clinical isolates).

Antimicrobial activity

Disk Diffusion Assay

Five millimeters of sterile distilled water were used to make suspension. From an overnight growth of the test organism, 4-6 colonies were emulsified and the suspension adjusted to match the 0.5 McFarland's standard. Respective plates were inoculated using a sterile cotton wool swab. Antimicrobial susceptibility test was done using disk diffusion methods. Briefly, 100 mg of each

extract was dissolved in 1ml of dimethyl sulfoxide(DMSO) and 10 µL of the mixture were impregnated onto 6 mm sterile filter paper disk and air dried. The disk was placed aseptically onto the inoculated plates and incubated at 37 °C for 24 hours. After incubation, inhibition zone diameter was measured in millimeters using 15cm ruler and recorded against the corresponding concentrations as described by Elgayyar *et al.,* 2000. Positive controls were set against standard antibiotic gentamycin while negative controls were set using disk impregnated with solvent (DMSO).

Minimum inhibitory concentration (MIC)

MIC, of the active plant extracts was determined using broth micro dilution method against the test microorganisms. The method is recommended by the National Committee for Clinical Laboratory Standards now Clinical Laboratory Standard Institute (CLSI) (NCCLS, 2002). The tests were performed in 96 wellmicro-titer plates. Plant extracts were dissolved in respective solvents and transferred into micro-titer plates to make serial dilutions ranging from 10^1, 10^2, 10^3……..$10.^{10}$ The final volume in each well was 100 µL. The wells were inoculated with 5 µL of microbial suspension and incubated at 37 ^{0}C for 24 hours. The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth turbidity (Michael *et al.,* 2003). Wells that were not inoculated with microbial suspensions served as controls. All the assays were done in triplicates and average values computed.

Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting a loop full of broth from those wells which did not show any growth in MIC assay, two wells above and two wells below the MIC value and inoculated on sterile Muller-Hinton agar by streaking and incubated at 37°C for 24 hours. The highest dilution that did not yield a colony fraction on a solid medium was considered as MBC (Motamedi, 2009).

RESULTS AND DISCUSSION

Medicinal plants used in the treatment of bacterial infections

Seventeen (17) medicinal plants from 13 botanical families were documented as shown in Table 1 below. The plants' families, scientific names, parts used, and medicinal use, are shown.

The roots were the most used parts 9 (53 %), followed by barks 5 (29 %) while whole plant was 3 (18 %). The roots and barks were frequently used because they have high concentration of exudates stored in them being an excretory organ. They are also easy to prepare (Abera *et al.,* 2014) (Table 1). The family with the highest number of species that are used by the Nandi community was Fabaceae with 3 species, (17.65%), while each of the remaining 14 families had single species representing, (5.88.0%).

The main methods of preparation were decoction and infusion. However, some plants were prepared by more than one method. The most commonly use method of administration was oral especially for the internal ailments, dosages involved taking half or quarter of a cup twice a day or thrice a day depending on the efficacy of the drug and the nature of the ailment (Table 1). Most plants were used to treat more than one infection or symptom except *Szigium cardatum* that was used to cure diarrhoea only. Pneumonia was reported to be treated by highest number of plants followed by typhoid and STD's with three plants each, (Table 1). Pneumonia is one of the killer diseases in the county being in the highland, which is cold; and tuberculosis which is due to the HIV/AIDS, (Jeruto *et al.,* 2015). It is, therefore, necessary to preserve this indigenous knowledge on traditional medicines by proper documentation, identification of plant species used, herbal preparation, and dosage. This will assist future studies on the selection of medicinal plants to evaluate for phytochemical safety and efficacy.

Antibacterial activities

Crude extracts of 17 selected medicinal plants were screened for antibacterial activity against 10 pathogenic bacteria using disk diffusion methods. The activities of the test plants were expressed in inhibition zone diameters.

Antimicrobial potential of the plant extracts

As shown in Figure 1, all microorganisms were susceptible to the positive control (Gentamicin) and all the plants tested had antimicrobial activity on at least one of the test microorganisms. The extract from *Vangueria volkensii* was active against the highest number of the test microorganisms (60%). *Olinia rochetiana, Acacia lahai, Leucas calastachys, Urtica mossaica, Solanum micranthum* and *Senecio discifolius* were active against half (50%) of the test microorganisms. *Cythula schimperiana* and *Zehneria minutiflora* showed antimicrobial activity agaist 20%

Table 1. Ethnobonical values of selected medicinal plants

Scientific name	Family	Infections treated	Parts used
Acacia lahai	Fabaceae	Diarrhea and coughs, pneumonia	Bark
Cythula schimperiana	Amaranthaceae	Diarrhoea and Wounds	Root
Adenia gummifera	Passifloraceae	TB and leprosy	Root
Lactuca glandulifera	Asteraceae	Skin diseases and wounds	Whole
Plantago palmate Hoof.	Campanulaceae	Tonsils, Pneumonia, diarrhoea, eyeinfections	Root
Senecio discifolius Oliv.	Compositae	Stomachache, syphilis and eye infections	Whole
Zehneria minutiflora (cogn) c. Jeffrey	Cucurbitaceae	Diarrhoea, Skin diseases Pneumonia and STDI's	Bark
Albizia coriaria Welw. ex Oliv.	Fabacea	Diarrhoea and tonsils	Root
Erythrinia abyssinica DC.	Fabacea	Wounds and skin infections	Root
Leucas calostachys Oliv.	Labiataceae	STD'Is and wounds	Root
Ficus cycomorus L.	Moraceae	Diarrhoea, Tuberculosis and STD'Is	Bark
Szigium cordatum Hochst. ex sond	Myrtaceae	Typhoid, Tonsils, Rheumatism, Gonorrhoea	Root
Solanum micranthum Dunal	Solanaceae	STD'Is and wounds	Root
Urtica mossaica Mildbr	Urticaceae	Coughs, wounds	Whole
Cleodendrum myricoides (Hochst.) Vatke	Verbanaceae	Typhoid, Tonsils, Rheumatism, Gonorrhoea	Root
Olinia rochetiana	Oliniace	Rheumatism, bronchitis and Skin diseases	Bark
Vangueria volkensii	Rubiaceae	Wounds and skin infections	Bark

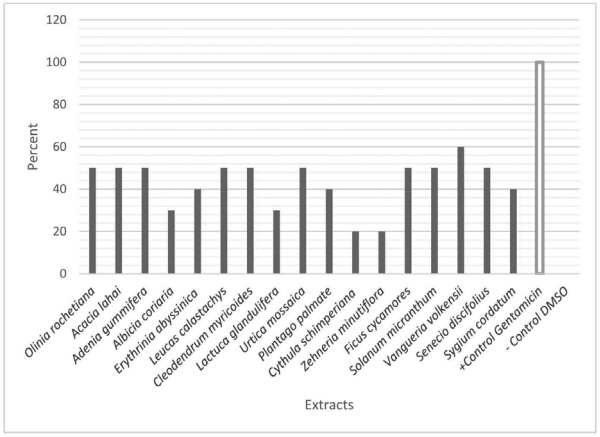

Fig. 1. Percentage activity of plant extracts against test microorganisms.

of the test microorganism.

All the plants extracts had low mean inhibitory zones when compared with Gentamicin (Table 2). Gentamicin had a mean of 19.10 (sd=2.119) whereas all the plant extracts apart from *Acacia lahai* had mean inhibitory zones of less than 10, (Table 2).

Except ESBL *Escherichia coli*, all the test microorganisms were susceptible to at least one of the plants extracts studied. As depicted by figure 2, *Klebsiella pneumonia*, Methicillin resistant *Staphylococcus aureus* and *Enterococcus faecalis* with 88.24%; n=15, 76.47; n=13 and 70.59; n=12 respectively were suscetible to the highest number of the plant extracts. However, *Escherichia coli* and *Citrobacter freudii* were susceptible to only 3 plant extracts with (17.65%) and 2 (11.76%) of the plant extracts respectively. In addition, ESBL *Escherichia coli* was not susceptible to any of the plant extracts.

Minimum inhibitory concentration and Minimum bactericidal concentration.

Six medicinal plants namely; *Acacia lahai, Albicia coriaria, Adenia gummifera, Cleodendrum myricoides, Leucas calostachys* and *Olinia rochetiana* were selected for further tests for MIC and MBC due to their significant activities against varoius microorganisms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against each sensitive bacterial strain were determined.

The MIC of *Olinia rochetiana* showed potent antibacterial effect of 0.78mg/ml against Methicillin resistant *Staphylococcus aureus*. (Table 3). Similar result was also obtained by Hailu *et al.* (2005). Suggesting that the methanolic extract of *Olinia rochetiana* could possibly act as a bactericidal agent to this microorganism. In addition, *Acacia lahai* extracts also showed good antibacterial activity against MR*S.aureus* and *S. aureus* with a MIC of 4.17 mg/mL and MBC of 25.00 mg/mL respectively. However, *C. myricoides* had MIC and MBC of 50.0 mg/mL for MR*S. aureus* and 66.67 mg/mL for *S. aureus* respectively. *P. aeruginosa* and *S. aureus* were among the most susceptible bacteria, (Table 3).

Majority of the selected plant extracts were bactericidal against *S. aureus*, and other selected bacteria with different MBC values.

The bactericidal activity of the crude extracts were found against gram positive bacteria. This is because all plant extracts except four (Table 2) were active against the two bacteria with the highest zone of inhibition of 18.00 mm and 15.30 mm shown by *Acacia lahai.* The probable reason for this, apart from being gram positive, is because of them being associated with secondary bacterial infections, (Tong *et al.*, 2015). In addition, *S. aureus* and MR*S. aureus* also had an inhibition zone of 12.00 mm shown by both *A. coriaria* and *S. cordatum* against *S. aureus*. *L. calastachys* and *S. cordatum* also indicated an inhibition zone of 12.00 mm against MR*S. aureus* and *S. aureus.* This is because these bacteria apart from being gram positive are more often associated with

Table 2. Inhibition zones in millimeters of Methanol extracts from the selected medicinal plants

Plant extracts	Average inhibition			Zone diameters in millimeter for each *Test microorganism*						
	MR*S.a*	*S.a*	*P. a*	*E.c*	E. *f*	*S.s*	*K.p*	*C.f*	*S.t*	*E.Ec.*
Olinia rochetiana	16	14	20	6	10	6	8	7	6	6
Acacia lahai	18	15	17	6	12	6	12	9	6	6
Adenia gummifera	14	13	19	6	10	6	13	6	6	6
Albicia coriaria	12	10	6	6	7	6	6	6	6	6
Erythrinia abyssinica	8	11	6	6	7	6	7	6	6	6
Leucas calostachys	12	12	6	6	8	6	8	6	8	6
Cleodendrum myricoides	10	10	6	6	7	6	8	6	9	6
Lactuca glandulifera	10	7	6	6	Nd	6	7	6	6	6
Urtica mossaica	9	8	6	6	7	6	8	6	7	6
Plantago palmate	8	8	6	6	8	6	6	6	7	6
Cythula schimperiana	6	6	6	6	7	6	7	6	6	6
Zehneria minutiflora	6	6	6	6	7	6	7	6	6	6
Ficus cycamores	8	8	6	9	6	7	9	6	7	6
Solanum micranthum	9	9	6	6	6	7	8	6	7	6
Vangueria volkensii	6	6	8	8	8	9	8	6	8	6
Senecio discifolius	6	6	6	8	6	8	9	7	9	6
Sygium cordatum	12	12	6	6	6	6	11	7	6	6
+Control Gentamicin	21	23	23	21	21	20	19	21	22	22
- Control DMSO	6	6	6	6	6	6	6	6	6	6

Values represent means of triplicate test

Key: Diameter of disk was 6.0mm, +ve -positive control (Gentamicin) **-ve-** negative control(DMSO) - -No activity, Nd-not done, MR.*S. a-* Methicillin resistant *Staphylococcus aureus, S.a-Staphylococcus aureus, P.a-Pseudomonas aeruginosa, E.c-Escherichia coli, E.f- Enterococcus faecalis, S.s-Shigella sonnei, K.p-Klebsiella pneumonia, C.f-Citrobacter freudii, S. t- Salmonella typhi* and E.*E.c*-ESBL *Escherichia coli*

Table 3. Minimum inhibitory concentration and Minimum bactericidal concentration of the active crude extracts against sensitive bacteria (in mg/mL)

Plant extracts	*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Klebsiella pneumonia*		Methicillin resistant *Staphylococcus aureus*		*Enterococcus faecalis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A. lahai	25.00	16.67	12.50	25.00	Nd	Nd	4.17	33.30	12.5	41.60
A. gummifera	12.50	-	5.21	-	25.00	-	16.67	-	12.50	-
A. coriaria	12.50	25.00	6.25	-	Nd	Nd	2.08	-	Nd	Nd
O. rochetiana	12.50	-	12.25	25.00	Nd	Nd	0.78	41.67	20.83	50.0
L. calastachys	3.15	66.67	Nd	Nd	Nd	Nd	12.50	33.33	Nd	Nd
C. myricoides	66.67	100.00	Nd	Nd	Nd	Nd	50.00	100.00	Nd	Nd
+ ve cont.GEN.	10.40	25.00	3.13	16.67	6.25	12.50	0.78	8.30	3.13	6.25
-ve,DMSO	-	-	-	-	-	-	-	-	-	-

Values represent means of triplicate test

Key: Nd- not done, - No activity (static), -ve-negative control (DMSO), + ve control (Gentamicin).

secondary bacterial infections, (Nissanka *et al*., 2001). MR*S. aureus* is resistant to methicillin but majority of the plant extracts tested were active against the bacterium. This is in agreement with the studies of Venkatadri who found ethanol extracts of *T. asiatica* being active against multidrug resistant bacteria (Venkatadri *et al*., 2015).

Although, gram-negative bacteria were quite resistant to majority of plant extracts *Olinia rochetiana*, *Adenia gummifera* and *A. lahai* extracts recorded the highest zone of 20, 19, and 17 mm against *P. aeruginosa*. This is because *P. aeruginosa* is an opportunistic bacteria that infect burns and wounds with low level of resistance in comparison with the intestinal pathogenic bacterial species such as *S. sonnei* and *E. coli* that develop resistance, (Farman *et al*., 2019). In addition, plant extracts of *A. gummifera, A. lahai* and *S. cordatum* showed significant activities of 13, 12 and 11mm against *K. pneumoniae* respectively. (Table 2). This is in agreement with the findings of dichloromethane extracts of *S. didymobotrya* (Tabuti, 2007).

In conclusion the antibacterial activity of the plant extracts tested was mainly active against gram-positive bacteria. The activities against other gram-negative bacteria were quite low in many plant extracts with none of the plant extracts showing significant activity (Zone of inhibition of 10mm and above) against ESBL *E. coli, E. coli*, *S. sonnei*, *S. typhi* and *C. freudii*. This could be because gram negative bacteria are rich in a molecule called lipopolysaccharide found in the outer membrane which exclude certain drugs and antimicrobials from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than are gram positive bacteria, (Kaplan, 2012).

It is interesting to note that some plants, e.g

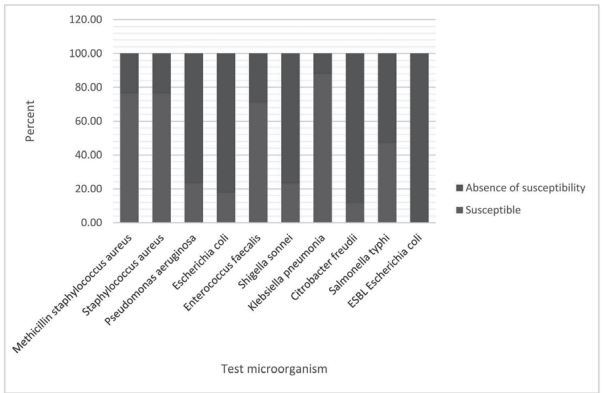

Fig. 2. Percentage susceptibility of bacteria to the extracts

Cythula schimperiana and *Zehneria minutiflora* had little activity against *E. faecalis* and no activity against other selected bacteria, yet the traditional practitioners use these plants to treat bacterial infections. This implies that perhaps some medicinal plants could be treating just the symptoms associated with the disease rather than the disease itself (analgesic). Moreover, all the plant extracts had no antibacterial activity against ESBL *E*. coli. This may be because the bacterium contain ESBL resistant genes, that resist ESBL drugs and possibly plant extracts. Furthermore, the absence of antibacterial activity in some plant extracts may suggest that these plant extracts may be acting in an indirect way; where the active ingredient may exist as a precursor form, which requires activation in the body by some yet unknown mechanism (pro-drugs), (Bruno *et al*., 2014). It is possible that the extract would achieve effect via an immunopharmacological mechanism. This, however, requires further investigation. The medicinal plants studied also showed variations in antibacterial activities. This could be attributed to the variation of the active constituents of various plant species, (Doughari *et al*., 2008).

Conflicts of interest

There are no conflicts of interest
The results/data/figures in this manuscript have not been published, nor are they under consideration for publication elsewhere.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Biological Sciences, Masinde Muliro University of Science and Technology, for providing the facilities for this study and Kenya Medical Research Institute-Centre for Microbiology Research,(KEMRI-CMR) for providing the microorganisms used in this study.

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