Academia Journal of Medicinal Plants 7(11): 243-251, November 2019
DOI: 10.15413/ajmp.2019.0162
ISSN: 2315-7720

Academia Publishing

Research Paper

Antibacterial activity of plant parts selectively consumed by chimpanzees

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Accepted 7th November, 2019

ABTRACT

This study was conducted to determine the antibacterial properties of plant parts selectively eaten by chimpanzees in Kalinzu forest. Plant part samples obtained were freeze dried and dry samples were used for extraction using standard solvents. Dichloromethane, water, acetone and Methanol were first compared because of the differences in polarity of different plant compounds. Extraction was performed on rotator Labotec model 20.2 shaking apparatus with a 5 ml: 0.75 g solvent to sample weight ratio. The antibacterial activity of the plant extracts was quantified using the micro plate dilution method to determine the Minimum Inhibitory Concentration (MIC) values of each plant extract against each test bacteria species. Reference strains of *Escherichia coli* (gram negative) ATCC25922 and *Staphylococcus aureus* (Gram positive) ATCC 25923 were used. Other Bacterial strains used for antimicrobial assays were obtained from Chimpanzees faecal material. Selective media were chosen to culture an adequate variety of bacterial types. Antibiotic sensitivity patterns of the selected Gram-positive and Gram-negative bacterial strains used in the study were evaluated. Methanol solvents produced the extract that resulted into the lowest MIC against the test bacteria *S. aureus*. Plant parts from 8 out of the 10 species showed inhibition of bacterial growth at various extract concentrations. The least eaten plant parts showed more antibacterial activity than those consumed frequently and in large amounts. *Bersama abysinic*a leaf and *Aframomum angustifolium* fruit inhibited the growth of five out of the six tested bacteria strains with the former exhibiting the lowest Minimum Inhibitory Concentration of 0.05 towards coagulative negative *S. aureus*. *Diospyros abysinica* and *Prunus africana* fruit were active against four out of the six bacterial strains tested. Among these bacterial strains, *S. aureus* was more vulnerable to inhibition while *Klebsiella pneumoniae* was most resistant. The growth of *K. pneumonae* was only inhibited by *D. abysinica* extract at the highest extract concentration of 25 mg/ml and was also found to be bactericidal. The extracts from the rest of the plant parts were bacteriostatic. *B. abysinica* and *A. angustifolium* extracts had strong antibacterial components and may be important in fighting *Pseudomonas aeruginosa* that was resistant to most standard antibiotics. Six out of the eight plant extracts that showed antibacterial activity were able to control the growth of *S. aureus* an indication of new avenues of controlling the *S. aureus,* resistant to antimicrobials especially menthicilin.

Key words: Antibacterial activity, plant parts, consumption, chimpanzees.

INTRODUCTION

In Kalinzu, fruit availability influences consumption of fruit by chimpanzees. Some fruits however have been eaten in little amounts even when they are super abundant and eaten only during certain times of the year. Chimpanzees also exhibited some peculiar behaviours when ingesting some of such plant parts. A peculiar example was ingestion of *Bersama abysinica* leaf in small quantities. This plant is traditionally used for de-worming in communities bordering Kalinzu Forest Reserve. It is hypothesized that their peculiar and seasonal inclusion in the chimpanzee diet could be that they may have a role to play in the control against gastrointestinal bacteria. Recent evidence from the African great apes suggests that certain plants are ingested for their considerable medicinal value. Studies denote that it is in aid to control intestinal parasites such as *stephanostomium, Bertiela sturdy* and/or provide relief

from related gastrointestinal upsets (Wrangham, 1995; Huffman, 1997; Dupain et al., 2002). In chimpanzees, self medication was first hypothesized during feeding habits field observation. Such behaviors included the whole leaf swallowing of genus *Aspillia* (Huffman 1997; Huffman and Caton, 2001). Chimpanzees also ingested *Vernonia amygdalina Del.* (Compositae) at Mahale (Huffman et al., 1993) which is also used throughout tropical sub-Saharan Africa as medicine (Huffman et al., 2001).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficacy (Almagboul et al. 1984; Ikram et al., 1984; Izzo et al., 1995). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al., 1976-1986) as well as in tannin (Saxena et al., 1994). Many of such plants tested have been derived from the traditional plants used by human communities. This study tests antibacterial activity of plants consumed by chimpanzees that are hypothesized to be for self-medication. The main objective was to determine the Minimum Inhibitory Concentrations of such plant extracts against bacterial strains isolated from the Faecal samples of Captive chimpanzees.

METHODS

To assess the potential for influence of plant chemistry on enteric bacteria of chimpanzees, extracts of purposively selected plants were evaluated using Minimum Inhibitory Concentrations (MIC) bioassays for antibacterial activity, the following methods were sequentially used:

Plant part collection and preparation

The plant parts hypothesized for self-medication were identified during the two year dietary field observational study of the M-group of chimpanzees in Kalinzu Forest Reserve. Daily observation for 6 to 10 h per day of the M group was made (Kagoro-Rugunda et al., 2015). Kalinzu forest reserve is located in South western Uganda (0^0 17′ S and 30^0 07′ E) at an altitude ranging from 700 to 1,840 m above sea level (Howard, 1991; Hashimoto, 1995). The Kalinzu Forest Reserve forms a pocket of forest remnants on the western rift valley escarpment and is classified as a medium altitude moist evergreen forest with a chimpanzee population of 230 individuals (Plumptre, 2003).

Plant samples were kept in good aeration (Dilika et al, 1996; Basri, 2005) but further drying was done at IZW by the freeze drying method for 72 h until constant weight was attained. Dried plant material was used as a source of extract because: 1) the location of the study/collection site was at a long distance from the laboratory; 2) difficulty in obtaining different fruits at the same time due to differences in phenology; and 3) differences in water content of fresh samples would affect the solubility of subsequent separation by liquid-liquid extraction. Differences in solubility would affect the concentration of secondary metabolic plant components which should be relatively stable, especially if they are to be used as antimicrobial agents. Only 10 purposively collected plant species were tested. Samples were collected from plants that were eaten by chimpanzees after they left or from a patch within the same transect similar in phenophase. All dried samples were finely ground prior to analysis with an IKA A11 basic mill (IKA, Staufen, Germany).

Choice of plant extraction solvent

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include:

- Low toxicity, since the end product will contain traces of residual solvent, and should not interfere with the bioassay.
- Ease of evaporation at low heat
- Promotion of rapid physiologic absorption of the extract
- Preservative action and inability to cause the extract to complex or dissociate (Hughes, 2002).
- Extraction of targeted compounds.

Acetone has been recorded to extract total phenolics better than aqueous methanol (Cork and Krochenberger, 1991). In a study by Harmala et al. (1992), twenty different solvents were evaluated and chloroform was found to be the best solvent for the extraction of non-polar, biologically active compounds from the roots of *Angelica archangelica.* If the extraction is for general phytochemical analysis or screening, then the larger the variety of compounds the extractant would extract, the better, because there would be a better chance that biologically active compounds would be present (Eloff, 1998a).

Water has also been primarily used e.g. using sterile distilled water by autoclaving at 121°C for 15 min, and the filtrate used as a test extract. Nostro et al. (2000) used distilled water that was adjusted to pH 2.0 with HCl and neutralized with NaOH in a water bath at 37°C for 30 min (acidified aqueous environment). However, plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extracts (Parekh et al., 2005). Most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent (Parekh et al., 2006).

The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol, and

water (Parekh et al., 2005; Bisigninoet al., 1999; Lourenset al., 2004; Salieet al., 1996; Rojas et al., 2006). Dichloromethane has also been used by a number of researchers (Dilika and Meyer, 1996; Freixa et al., 1996).

Acetone, although not a very commonly used solvent, has been used by a number of scientists (Basri and Fan, 2005; Dilika et al., 1996; Lourens et al., 2004; Mathkega et al., 2006). Masoko and Eloff (2006) investigated the antifungal activity of *Combretum* species, from the extracting solvents used, which included hexane, dichloromethane, acetone and methanol, and discovered that acetone and methanol extracted more chemical compounds from the leaves than the other solvents. Both acetone and methanol were found to extract saponins which have antimicrobial activity.

Methanol and acetone are sometimes chosen as solvents because, in addition to dissolving the extracts completely, they show no inhibition of the microorganisms even at 2% final concentration (Meyer and Afolayan, 1995; Afolayan and Meyer, 1997; Mathekga et al., 2000). Methanol followed by acetone was reported as the most effective solvents for extracting antibacterial compounds from plants (Vlachos et al., 1996). Studies revealed that besides the efficacy of methanol as an antibacterial extractant, its extracts gave the most consistent antibacterial activity whereas acetone extracted a complex mixture of different components (Lin et al., 1999; Martini and Eloff, 1998).

Since the aim was to extract the aromatic or saturated organic compounds due to the fact that all of such identified components from plants are active against microorganisms and since antibacterial activity varies with the extraction solvent, it was therefore essential to select the most appropriate solvent for extracting antibacterial compounds (Lin et al., 1999; Vlachos et al., 1996) because a specific extracting solvent may extract different antimicrobial compounds from plants with different chemical profiles. In view of the above, extraction was done using four different extraction solvents namely Dichloromethane, water, acetone and Methanol to compare and determine the best solvent to use because of the differences in polarity of different plant compounds. Extraction was performed on rotator Labotec model 20.2 shaking apparatus with a 5 ml: 0.75 g solvent to sample weight ratio. Each solvent was allowed to extract for one hour while shaking. Extracts were decanted into pre-weighed glass vials. The same amount of fresh solvent (5 ml) was added again and the process was repeated six times for all solvents. The solvent was removed by placing the extracts in front of a stream of air in a fume cupboard at room temperature till dry residues were formed. The extract residues from each solvent was weighed and re-dissolved in 5 ml of 10% solvent for antibacterial activity testing.

Bacteria reference strains used for antimicrobial assays

Escherichia coli (gram negative) ATCC25922 and *Staphylococcus aureus* (Gram positive) ATCC 25923 were the reference strains used.

Bacterial strains from chimpanzees used for antimicrobial assays

Extra four bacterial strains were obtained from chimpanzee faecal samples. Each collection of faecal sample was split open to reveal the interior unexposed portion, and sampled with a culture swab (Difco laboratories, Dextroit, MI). Selective media were chosen to isolate an adequate variety of bacterial types.

To isolate anaerobes, prepared plates were inoculated by smearing with culture swabs from the interior of the faecal samples. Every sample was cultured on an identical set of agar-plates containing 5% sheep blood (1x aerobic incubation, 1x anaerobic incubation) 1x Gassner-plate (Lactose utilization), 1 Chrom-UTI-plate (chromogenic medium). All smears of inocular were serially diluted, by streaking twice with a flame-sterlized inoculating loop, and incubated for 24 h at 37°C giving adequate growth for colony isolation. After the first reading, they were incubated for another 24 h at 37°C. Anaerobic plates were incubated in a GasPak™ anaerobic chamber equipped with a GasPakPlus™ H-2 and CO_2 generating envelope with palladium catalyst (BBL, BecktonDickison) to maintain oxygen-free conditions.

Subcultures were prepared on Columbia plates with 5% sheep blood and incubated for 24 h. From each plate, one or two colonies were chosen for sub-culturing and testing. Further characterization and differentiation were based on the phenotype of the colonies, Gram-stain and different biochemical reactions (either home-made tubes or commercial ID-Systems such as API and rapid ID system of biochemical tests).

The bacterial growth in form of colonies was quantified in the bacteriology Laboratory at IZW thus:

+ = small amount (5-10 colony forming units (CFU));
++ = medium amount (11-~30 CFU);
+++ = high amount (>30 CFU)

Determination of the minimal inhibitory concentration (MIC) of bacteria to plant extracts via broth microdilution (considering CLSI M31-A3)

Determination of the Minimal Inhibitory Concentration (MIC) via broth microdilution as the gold standard in susceptibility testing of bacteria to antimicrobial substances was used. In this case, this method was used for the quantification of antimicrobial effects of plant extracts on the strains of bacteria mentioned above. This protocol was created under consideration of guideline M31-A3 of the Clinical Laboratory and Standards Institute (CLSI). On the

first day, frozen bacteria was streaked with a burnt out loop on a Columbia agar plate and incubated for 24 h at 37°C (revitalization). On second day, fresh bacterial cultures were prepared on Columbia agar (fractioned, streak with bunt out loop) and incubated at 37°C overnight. On the third day, the inoculum was prepared by taking single colonies from the overnight culture (not older than 24 h) with a sterile cotton swab or loop, and rubbing it in 5 ml sterile Sodium Chloride suspension (0.9%) solution and vortexing thoroughly for one minute.

The turbidity of the suspension was adjusted to McFarland 0.5 standard using a nephelometer. 50 µl of the suspension was put into 10 ml of cation adjusted Müller-Hinton broth (recommended: Müller-Hinton-II broth; cation adjusted; Becton Dickinson) and vortexed thoroughly for 20-30 s. The inoculum then contained ~5x105cfu/ml and was used within the first 15 min.

Preparation of the test plates

In this study, the antibacterial activity of the plant extracts was quantified using the micro plate dilution method to determine the Minimum Inhibitory Concentration (MIC) values of each plant extract against each test bacteria species (Elloff, 1998). This was determined by two-fold serial dilutions of the extract beyond the level where no inhibition of growth was observed. Plant extracts were reconstituted to 50 mg/ml in 10% acetone and 200 µl of the plant extract were put in the first column of the plate. 100 µl of the inoculated Müller-Hinton-II broth culture was put in each well other than the first column containing the plant extract. 100 µl of the plant extract was picked serially diluted with the inoculated Müller-Hinton-II (MH) broth. Two wells were used as sterility and growth controls respectively with the sterility control containing only the Müller-Hinton-II broth and the growth control containing the Müller-Hinton-II broth inoculated with the test organism followed by two-fold dilutions. The micro plates were sealed and incubated at 37°C for at least 18 h.

On the fourth Day, plates were taken from the incubator without hard vibrations or shaking. They were placed over a mirror and the bacterial growth noted. As an indicator of Bacterial growth, bacterial colonies could be visibly seen as buttons at the bottom of each well where inhibition did not occur. The MIC was recorded as the lowest concentration of the plant extract that completely inhibits growth of the bacteria in microdilution wells as detected by the unaided eye (CLSI M31-A3). In the wells of the positive control, growth was visible (Button ≥2 mm) and no growth was visible in the wells of the negative control. After reading, the microtitre plates were stored at 4°C in the refrigerator for future reference.

The culture was checked for quality and quantity control. One loop of each tube of inoculum was streaked on Columbia agar (fractionated streak). This was repeated for every prepared tube of inoculum and the agar plates incubated for 18-24 h at 37°C. Lack of growth of bacterial colonies showed that the culture was pure. To test for quantity control, 10 µl of the broth was plated and colonies on the plates of the quantity control were counted. The number had to range between 20 and 80 colonies per plate (2x105 – 8x105 CFU/ml in the inoculum, optimum 5x105 CFU/ml). If one of the quality or quantity controls was out of range, all values of the test day were disposed and the testing repeated after trouble-shooting.

The microtitre plate assay was performed in duplicate in the microtitre plates, with all the plant extracts for determination of the minimal inhibitory concentrations (MIC's). The MIC's for plants were expressed in mg/ml.

If there was an inhibitory effect of the plant extract on the bacteria, it could also be checked whether this effect was irreversible, meaning the substance was bactericidal and killed the bacteria, or if it was reversible, meaning the bacteria were only inhibited by the substance (bacteriostatic effect). The seal of the first well of a row without bacterial growth (MIC) was melted with a hot loop. 10 µl of the content of the well was picked with a pipette and spread it on a Columbia agar plate with a sterile spatula. The plate was incubated overnight at 37°C overnight and investigated for bacterial growth after 24 h. If visible bacterial colonies were observed, the plant substance was regarded bacteriostatic. If no visible bacterial colonies were observed (may be 1 or 2 sporadic colonies), the plant material was regarded to have a bactericidal effect.

Standard antimicrobial sensitivity patterns of bacteria

Antibiotic sensitivity patterns of the selected Gram-positive and Gram-negative bacterial strains from the bacterial strains used in the study were evaluated. Ten antibiotics namely Ampicilin, Amoxicilin, penicillin (P10), Cefotaxin, Chloramphenical, Gentamycin, Tetracyclin (TE30), clindamycin (DA2), Tulathromycin, Trimeto/sulf, and Enrofloxacin were used for sensitivity testing of the bacteria.

RESULTS

Extraction solvent

A difference was observed in the amount of plant material extracted with the different extraction solvents when tested using only four species of different plant parts. The characteristics of extraction solvents and the specific chemical composition of medicinal plants influenced the amount of plant material extracted. The dried and ground plant material of *Aframomum angustifolium, Prunus africana, Piper guinense* and *Phytolacca dodecandra* was extracted six times with each extraction solvent namely

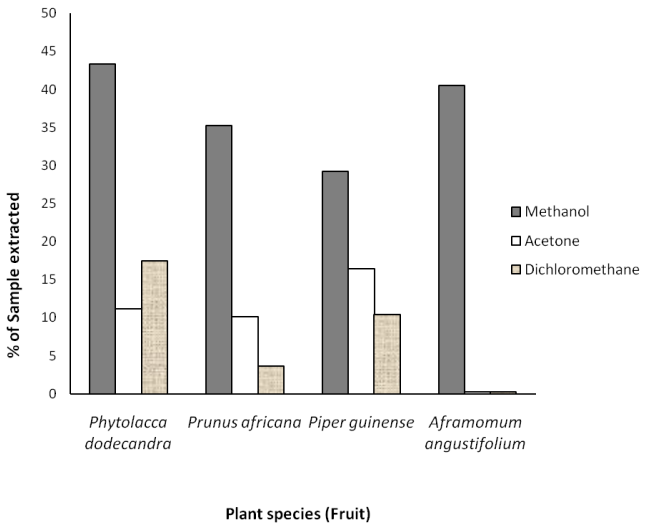

Figure 1: Amount of extract obtained using different solvents.

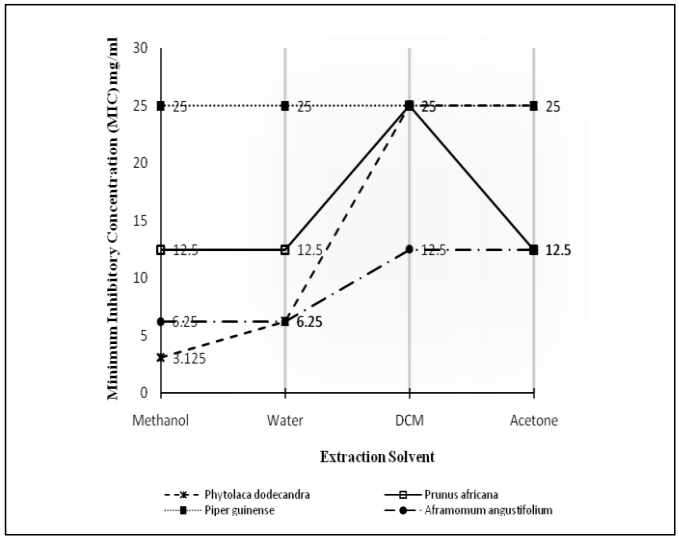

Figure 2: Minimum inhibitory concentration for different solvents using *Staphylococcus aureus* ATCC 29213test bacteria.

absolute methanol, Dichloromethane and acetone. Among the undiluted plant extract concentrations of plant material extracted, methanol solvent yielded the highest amount of plant material extracted from all the different medicinal plants. Extraction with Dichlomethane produced the lowest extract concentrations for the different plant parts, except *P. dodecandra* fruit (Figure 1)

Trials for finding out the best possible extracts that would produce the lowest MICS were done with the plant extracts extracted using four solvents: methanol, acetone, Dichloromethane and water. Four representative fruits from *P. dodecantra, P. Africana, P. guinense,* and *A. angustifolium* were used for extraction. Methanol extracts of *P. dodecandra* produced the lowest MIC. Methanol and water extracts of *A. angustifolium* had the same MIC. For *P. africana* extracts of methanol, water, and acetone had the same effect on bacteria producing the same MIC save for the extract from Dichloromethane. *P. guinsense* extracts produced the same minimum ihnihibitory concentrations with the four extraction solvents (Figure 2). All in all, methanol solvents produced the extract that resulted into the lowest MIC against the test bacteria *S. aureus.*

Bacterial strains isolated

Characterization of bacteria from five chimpanzee faecal samples showed different species of aerobic and anaerobic

Table 1: Test bacteria obtained from chimpanzee faecal samples.

Sub Culture	Chimpanzee faecal samples				
	I.	**II.**	**III.**	**IV.**	**V.**
1	*Klebsiellapneumonae*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
2	*Aerobic sporeformers*	*Yeast*	*Enterococcus sp*	*Escherichia coli*	*Escherichia coli*
3	*Escherichia coli*	*Coagulase-negative Staphylococcus* sp. *of Epidermidis group*	*Clostridium sporogenes*	Coagulase-negatve *Staphylococcus* sp. of Epidermidis group	*Enterococcus* sp.
4	*Klebsiella pnuemoniae*	*1) Prevotella* sp. *2)Clostridium sporogenes*	-	*Alpha-haemolytic Strepotococcus* sp.	*Pseudomonas aeruginosa*
5	Yeast	*Pseudomonas aeruginosa*	-	*Escherichia coli*	*Bacteriodessp*
6	*Clostridium septicum (anaerobic)*	*Aerobic spore formers*	-	-	-
7	*Pseudomonas aeruginosa*	*Aerobic spore formers*	-	-	-

Table 2: MIC values of plant parts eaten by chimpanzees.

Extracts from different plants	***Escherichia coli ATCC 25922***	***Escherichi a coli***	***Staphylococcus aureus ATCC 29213***	***Coagulative Negative Staphylococcus sp***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***
*Diospyros abysinica (*fruit)	-	-	3.125	3.125	25	25
*Phytolacca dodecandra (*fruit)	-	-	25	-	-	-
*Prunus africana (*fruit)	25	25	25	25	-	-
*Piper guinense (*fruit)	-	25	-	25	-	-
*Aframomum angustifolium (*fruit)	25	25	25	12.25	25	-
*Diospyros abysinica (*leaf)	-	-	12.25	12.25	25	-
*Bersama abysinica (*leaf)	1.25	1.25	6.25	0.05	0.806	-
*Antiaris toxicaria (*fruit)	-	-	-	-	-	-
*Uvariospsis congensis (*fruit)	-	-	-	-	-	-
*Warburghia ugandensis (*fruit)	25	-	25	12.25	-	-

bacteria (Table 1). Only some of the aerobic bacteria were used as test organisms. These include *S. aureus (CNS), E. coli, K. pneumoniae* and *P. aeruginosa*. *E. coli* was the most prevalent in chimpanzee faeces since it occurred in 8 out of the 35 subcultures. An *enterococcus* species was also isolated but could not visibly grow in the Muller Hinton broth so it was not used as a test organism.

Antibacterial minimum inhibitory concentrations of plant extracts

Of the 10 plant species tested for antibacterial activity, only 8 showed inhibition of bacterial growth at various extract concentrations. The least eaten fruits showed more antibacterial activity than those fruits consumed in large amounts and more frequently. *B. abysinica* leaf and *A. angustifolium* fruit inhibited growth of five out of the six tested bacteria strains with the former exhibiting the lowest Minimum Inhibitory Concentration towards coagulative negative *S. aureus. D. abysinica* and *P. africana* fruit were active against four out of the six bacterial strains tested. *D. abysinica* fruit extract exhibited more inhibitory characteristics than the leaf extract (Table 2).

Six bacterial strains represented by two Gram-positive bacteria (*S. aureus* ATCC29213 and coagulase negative *S. aureus*) and four Gram-negative bacteria (*E. coli* 25922, *E. coli, P. aeruginosa,* and *K. pneumoniae*) were used for assessing the antibacterial activity of plant extracts in the microtitre plate assay. Among these bacterial strains, *S. aureus* was more vulnerable to inhibition while *K. pneumonia* was most resistant. The growth of *K.*

Table 3: Standard sensitivity patterns of disc diffusion results of bacterial strains selected for MIC determination to standard antibiotics.

Bacterial strain	Sensitivity results of standard antibiotics											
	Ampicilin	**Amoxicilin/clav**	**Penicilin**	**Cefotaxim**	**Cefovecin**	**Chloramphenical**	**Gentamicin**	**Tetracyclin**	**Clindamycin**	**Tulathromycin**	**Trimeto/Sulf**	**Enrofloxacin**
Escherichia coli ATCC 25922	I	S	**R**	S	S	S	S	S	**R**	S	S	S
Staphylococcus aureus ATCC 25923	**R**	S	**R**	-	S	S	S	S	S	S	S	S
CNS *Staphylococcus aureus*	S	S	S	-	S	S	S	S	S	S	S	S
Klebsielapneumonae	S	S	**R**	S	S	S	S	S	**R**	I	S	S
Pseudomonaaeruginosa	-	**R**	**R**	**-**	**R**	**R**	S	**R**	**R**	**R**	**R**	I
Escherichia coli	S	S	**R**	S	S	S	S	**R**	**R**	S	**R**	I

R:Resistant, **S:**Susceptible, **I:**Intermediate, **-:**Not tested

pneumonae was only inhibited by *D. abysinica* at the highest extract concentration of 25 mg/ml. When 10 µl of contents of the wells of the microtitre plate that showed no bacterial growth were plated, it showed that the *D. abysinica* extract was bactericidal. The rest of the extracts were bacteriostatic.

Standard antimicrobial sensitivity patterns of bacteria

Table 3 shows the antibiotic sensitivity patterns of the selected Gram-positive and Gram-negative bacterial strains, respectively. *S. aureus* ATCC strain was resistant to Ampicilin and Penicillin; and sensitive to the rest of the tested antibiotics, while CNS *S. aureus* was sensitive to all the antibiotics. One of the MRSA strains was resistant to Penicillin and Ampicilin but sensitive to the other antibiotics tested.

The reference strain *E. coli* ATCC 25922 was sensitive to all antibiotics except Penicilin and Clindamycin. In addition to the two above, *E. coli* from faecal sample was sensitive to two additional antibiotics of Tetracyclin and Trimeto/sulf. *K. pneuminiae* was resistant only to Penicilin and Clindamycin. The last of the strain tested for antibiotic sensitivity was *P. aeruginosa* that was resistant to all antibiotics except Gentamycin.

DISCUSSION

The different chemical composition of medicinal plants and extraction solvent characteristics influenced the amount of plant material extracted from medicinal plants. Methanol was recorded as a good extraction solvent since it produced the highest amount of extract with the lowest inhibitory concentration. The large amount of extracts by methanol meant they could easily be quantified into a uniform concentration (mg/ml) for all bacterial assays. However, methanol was not ideal for re- dissolving the dry extract in its absolute form since it interfered with the transparency of the microtitre plates.

The incomplete and problematic drying of the aqueous extract supernatants in the Fume-cupboard prompted alternative drying in a rotary evaporator to obtain a dried extract residue for analysis as also noted by George et al. (2001). Although many studies report on using the respective solvents for re-dissolving the dried extract residues (e.g. Pillay et al., 2001; Eloff, 1999), in this study, it was found to be problematic, especially with the use of absolute methanol. A visual inspection method was used for assessing the antibacterial activity of plant extracts. However, using high concentrations of extracts re-dissolved in absolute methanol interfered with the transparency of microtitre plates in this study, so 10% methanol was used. The concentration of extracts could only be halved, for example 12.5 to 6.25 mg/ml. It was not possible to tell whether any value in between could not have an antibacterial effect. It is therefore better in subsequent studies to try and halve these concentrations or determine the rate of kill of bacteria by these plant extracts.

Although secondary plant compounds are almost invariably viewed as antinutrients, some may have beneficial roles in animal nutrition. Tannins can reduce the infectivity of viruses or other pathogens by binding with them in the gastro-intestinal tract (Keating et al., 1988). It should be noted that *B. abysinica* and *A. angustifolium* extracts have strong antibacterial components and may be important in fighting bacteria in chimpanzees because they had an effect on *P. aeruginosa* that was resistant to most standard antibiotics. Several species from the genus *Aframommum* are major food plants for chimpanzees and gorillas in many habitats. The results of this study are in line with extensive

literature survey of the pharmacological properties of *Aframomum* e.g. by Cousins and Huffman (2002) who showed a wide variety of considerable biological activity including bactericidal activities against *E. coli, P. aeruginosa, Yersinia entercolitica, Bacillus subtilis, Proteus vulgaris* and *K. pneumoniae.* However, these study findings did not show activity against *K. pneumoniae*, although it was bacteriostatic against *S. aureus.*

Berries of *P. dodecandra L. Hent* are reported to also be frequently ingested by chimpanzees in Kalinzu and Kibale and also reported to have antiviral, antibacterial, anti-fertility and embryotoxic activities (Kloos & McCullough, 1987). This is in agreement with the current results, in that, *P. dodecandra* showed bacteriostatic effects on the reference strain *S. aureus.* Six out of the eight plant extracts that showed antibacterial activity were able to control the growth of *S. aureus*. The antimicrobial properties of plants investigated by a number of other researchers worldwide, especially in Latin America show similar results. For instance, in Argentina, a research tested 122 known plant species used for therapeutic treatments (Anesini and Perez, 1993). It was reported that among the compounds extracted from these plants, twelve inhibited the growth of *S. aureus*, ten inhibited *E. coli*, and four inhibited *Aspergillus niger*).

A more detailed study on antimicrobial compounds was also done to evaluate extracts from 120 plant species belonging to 28 different families (Santos Filho et al., 1990). In the present study, of the 20 plant species tested for antibacterial activity, only 8 showed inhibition to bacterial growth at various extract concentrations. *D. abysinica, B. abysinica* and *A. angustifolium* inhibited the growth of bacteria at lowest concentrations. *B. abysinica* and *A. angustifolium* extracts have strong antibacterial components and may be important in fighting bacteria in chimpanzees as they were shown to have an effect on *P. aeruginosa* that was resistant to most standard antibiotics. Six out of the eight plant extracts that showed antibacterial activity were able to control the growth of *S. aureus. S. aureus* is resistant to antimicrobials especially menthicilin. This methicillin resistant *S. aureus*, generally referred to as MRSA strains, is as a result of alteration of the penicillin binding protein PBP2a encoded by the mecA gene. In recent years, *S. aureus* strains with decreased susceptibility to vancomycin continue to be isolated and they pose a potential threat to effective treatment of *S. aureus* infections. An increasing problem of *S. aureus* and its resistance may in future be solved by development of antibiotics from active plant extracts' innovations.

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Cite this article as:

Kagoro-Rugunda G (2019). Antibacterial activity of plant parts selectively consumed by chimpanzees. Acad. J. Med. Plants. 7(11): 243-251.

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