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Antimicrobial Activities of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya

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Abstract: Plants are a source of phytomedicines. Amongst the traditional herbs used as phytomedicines in Kisii region, southwest Kenya are *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica.* A study was carried out on these herbal plants in the year 2011 to 2012. The objective was to determine the antimicrobial activity of the herbs against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*. In the study, the leaf samples of the herbs were obtained from Kisii region, washed, air-dried and milled. The samples were extracted with four solvents namely hexane, dichloromethane, ethyl acetate and ethanol. Portions of the crude extracts were screened against *Staphylococcus aureus* and *Escherichia coli* by the well diffusion method. Results obtained show that the solvents of hexane, dichloromethane, ethyl acetate and ethanol used as the control in the experiment have no effect on the *Staphylococcus aureus* and *Escherichia coli.* The antibiotics minocycline, chloramphenicol and cotrimoazol with zones of inhibition measuring (mm) 30, 33 and 18 respectively and used as references, inhibit microbial growth. The crude extracts of hexane, dichloromethane, ethyl acetate and ethanol of all the eight herbs have the best activity against gram-positive bacteria strain *Staphylococcus aureus* with zones of inhibition ranging between 16 mm and 27 mm. The best activity against gram-negative bacteria strain *Escherichia coli* was observed in the dichloromethane and ethanol extracts of *Leonotis nepetifolia* with zones of inhibition of 19 mm and 17 mm, in that order. It was concluded that extracts of all the eight selected herbs control gram-positive bacteria *Staphylococcus aureus* while dichloromethane and ethanol extracts of *Leonotis nepetifolia* control gram-negative bacteria *Escherichia coli*.

Key words: Herbs · Antimicrobial activity · *Staphylococcus aureus* · *Escherichia coli*

INTRODUCTION

An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria. Such a compound is said to have antibacterial activity [1]. Medicinal herbs are a rich source of antimicrobial agents [2-4]. A wide range of medicinal plant parts are used for extract as raw drugs and possess varied medicinal properties [5,6]. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active and also which combinations or processing methods had to be used to gain consistent and optimal results [7]. This reliance on herbal medicine has proven to be effective in the treatment of long term illness namely diabetes, malaria and pneumonia where it is seen to have lesser side effects and a cheaper form of medicine and preventive measure against diseases [8]. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care [9]. In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases and the occurrence of undesirable side effects seems to be less frequent [8]. Several factors have contributed to the growth of the use of traditional herbs worldwide, among which are: preference of consumers for natural therapies, concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free

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from side effects, great interest in alternative medicines; preference of populations for preventive medicine due to increasing population age, the belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate; tendency towards self-medication, improvement in quality, proof of efficacy and safety of herbal medicines and high cost of synthetic medicines [9-12]. In the Kisii region, southwest Kenya, the leave decoctions of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* are locally used for the treatment of diabetes, malaria and pneumonia [13]. Therefore, phytoconstituents of extracts of these herbs possibly have *antimicrobial* activity [14]. Consequently, it is important to find out the particular micro-organisms for which the herbal extracts are active [15]. The objective of this study was to determine the antibacterial effect of extracts from the eight herbs against *Escherichia coli* and *staphylococcus aureus*.

MATERIALS AND METHODS

Sample Collection and Preparation: The sample leaves of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* were used in the study. The verification of the herbal species from Kisii region, southwest Kenya was done by the Botanist at Egerton University. The leaves of the authenticated herbal plants were then collected and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and ground into fine particles with a grinder at the Department of Food Science and Technology, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology (JKUAT). The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Extraction: A sample of the powdered leaves weighing 30 g was extracted with 160 ml of hexane, dichloromethane, ethyl acetate and 96% ethanol. The extraction was carried out in a ¼ litre flask. Four extractions were done in each solvent used and the extracts were concentrated to about one-sixth of the original volume at 60^{oC} under reduced pressure using a rotary evaporator. The extracts were air-dried for three weeks to a constant weight and kept in air-tight containers for further work.

Determination of Antimicrobial Activity: Making up extract solution: Approximately 0.02g of dried hexane, dichloromethane, ethyl acetate and ethanol crude extract of each of the eight herbs was weighed and transferred to a 10 ml volumetric flask. The respective solvent was added to make up the 10 ml solution (0.02g in 0.01L).

Microorganisms: Micro-organisms namely *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Food and Science Technology, microbiology laboratory and stored in a refrigerator of the same laboratory, in JKUAT.

Nutrient Agar: Nutrient agar was purchased from the Pharmacy in Nairobi. About 7g of nutrient agar was suspended in 250ml of distilled water in a 1L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121^{0C}. The pH range for the nutrient agar was between 7.0 and 8.0.

Reference and Control: The references were standard antibiotic in nature. Erythromycin (15µg), Minocycline (5µg), Chloramphenicol (30µg) and Cotrimoazol (25µg) was choosen as the reference for all bacterial species used: *E.Coli* and *S.aureus*. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

Well Diffusion Method: Bioassay tests were performed on the herbal crude extracts to ascertain their activity against *Escherichia coli* and *Staphylococcus*. In the test tube, 20ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1ml inoculums were added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar. The loop was flamed before it was used each time. The content of the test tube was poured into a Petri dish and allowed to set. The Petri dishes were then labelled with the respective organism (inoculums) and date. By means of a 6 mm cork borer, four cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with the four crude extracts. Each cup was filled with its corresponding 0.00002µg/ml extract to about three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37°C and examined for any zone of inhibition after 24 hours. The same procedure was repeated with the references using the chosen

antibiotics and control using the pure solvent hexane, dichloromethane, ethyl acetate and ethanol. The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition were measured with the help of Hi Antibiotic zone scale (range 1cm to 35 cm or 10mm to 400mm) from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken. The actual zones were calculated by subtracting the diameter of the cups (6 mm) from the total zone of growth.

Data Collected: The antibacterial activity of the selected eight herbs against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* were obtained by measuring the diameters of the inhibition zones and compared them with that of the control drug erythromycin, chloramphenicol, minocyline and cotrimoazol. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the herb extracts.

Data Analysis: The null hypothesis being tested is that there is no significant biological activity displayed by the compounds present in the selected traditional herbs used in Kisii region to treat diabetes, malaria and pneumonia diseases. Results obtained in this study were expressed as mean inhibition zone (mm) ± S.D of three replicates. The mean and the S.D of each herbal extract were used to compute the calculated t-value. Differences between the critical t-value and calculated t-values of the diameter of the inhibition zones of the herbal extracts on gram-positive *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* were computed. For all the eight herbal species, the null hypothesis was retained because the calculated t-value was less than the critical t-value at $p = 0.05$.

RESULTS AND DISCUSSION

Zones of Inhibition of Hexane Extracts Against *Staphylococcus aureus*: Results obtained show that the control experiments which necessitate the use of pure distilled solvent alone, rather than pure plant extract induced no zone of inhibition. The zone of inhibition for the control measured 12 mm indicating lack of suppression of the bacteria involved and therefore negative result. The reference antibiotic compounds, erythromycin, chloramphenicol, minocyline and cotrimoazol for bacteria displayed positive results of zone of inhibition (mm) of 31.0, 33.0, 32.0 and 18.0 respectively. It is observed that the hexane extracts of *Carissa spinarum, Warburgia ugandensis, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* against *Staphylococcus aureus* exhibited maximum zone of inhibition of 24.0 mm, 23.0 mm, 20.0 mm, 24.0 mm, 24.0 mm and 25.0 mm respectively. The hexane crude extracts of *Urtica dioica* and *Senna didymobotrya*, had zone of inhibition of 16.0 mm. The hexane extracts of *Toddalia asiatica* show the highest antibacterial activity. The hexane extract of *Urtica dioca* and *Senna didymobotry* against *Staphylococcus aureus* exhibited less activity when compared with that of *Carissa spinarum, Warburgia ugandensis, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia and Toddalia asiatica.* The reference antibiotic compounds, erythromycin, chloramphenicol, minocyline and cotrimoazol showed high zone of inhibition indicating more activity than hexane extracts of the eight selected herbs (Table 1, Figure 1).

Zones of Inhibition of Hexane Extracts Against *Escherichia coli*: Results of hexane extract of all the eight herbs against gram-negative bacteria *Escherichia coli*

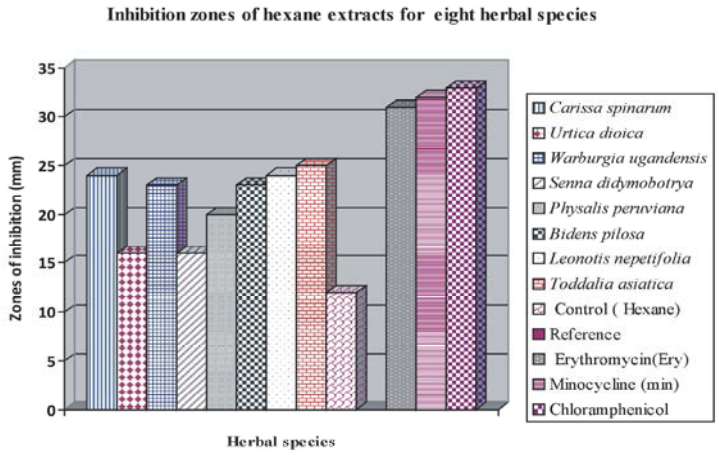

Fig. 1: Zones of inhibition of hexane extracts against *Staphylococcus aureus*.

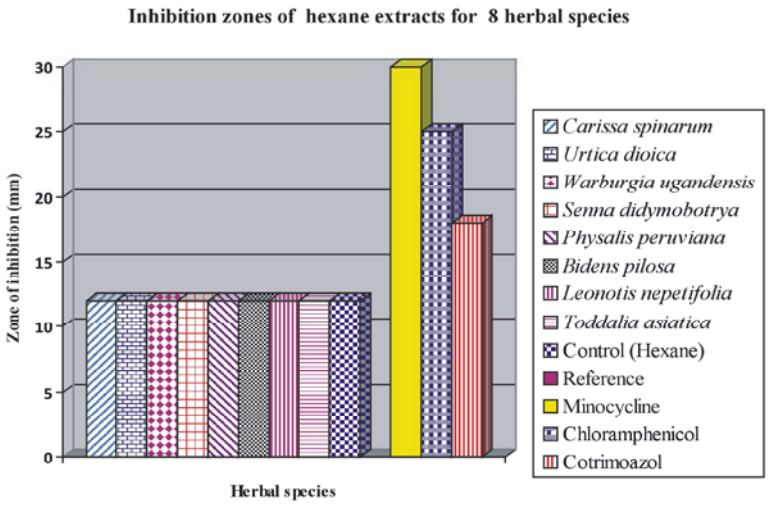

Fig. 2: Zones of inhibition of hexane extracts against *Escherichia coli*

Table 1: The antimicrobial activity of hexane extracts of the eight herbs

Well diffusion (Hexane extract)	Volume of Extract (ml)	Diameter of zone of inhibition (mm) *Staphylococus aureus*	*Escherichia coli*
Carissa spinarum	0.2	24±1.0	12±0.0
Urtica dioica	0.2	16±1.0	12±0.0
Warburgia ugandensis	0.2	23±1.0	12±0.0
Senna didymobotrya	0.2	16±1.0	12±0.0
Physalis peruviana	0.2	20±0.0	12±0.0
Bidens pilosa	0.2	24±1.0	12±0.0
Leonotis nepetifolia	0.2	24±1.0	12±0.0
Toddalia asiatica	0.2	25±1.0	12±0.0
Control (Hexane)	0.2	12±0.0	12±0.0
Reference			
Erythromycin(Ery)	15 µg	31±1.0	12±0.0
Minocycline	30 µg	32±1.0	30±1.0
Chloramphenicol (Chl)	30µg	33±1.0	25±1.0
Cotrimoazol	25µg	12±0.0	18±1.0

Table 2: Antimicrobial activity of dichloromethane extracts of eight herbs

Well diffusion (Dichloromethane extract)	Volume of Extract (ml)	Diameter of zone of inhibition (mm) *Staphylococcus aureus*	*Escherichia coli*
Carissa spinarum	0.2	20±0.0	12±0.0
Urtica dioica	0.2	20±0.0	12±0.0
Warburgia ugandensis	0.2	20±0.0	12±0.0
Senna didymobotrya	0.2	20±0.0	12±0.0
Physalis peruviana	0.2	23±1.0	12±0.0
Bidens pilosa	0.2	20±0.0	12±0.0
Leonotis nepetifolia	0.2	25±1.0	19±1.0
Toddalia asiatica	0.2	24±1.0	12±0.0
Control (CH_2Cl_2)	0.2	12±0.0	12±0.0
Reference			
Erythromycin(Ery)	15 µg	31±1.0	12±0.0
Chloramphenicol	30 µg	33±1.0	25±1.0
Minocycline	30 µg	32±1.0	30±1.0
Cotrimoazol	25µg		18±1.0

measured 12 mm, which is zero zone of inhibition. The reference antibiotic compounds tested against gram-negative bacteria *Escherichia coli* namely, minocyline, chloramphenicol and cotrimoazol measured (mm) 30, 25 and 18 zone of inhibition respectively (Table 1, Figure 2).

Zones of Inhibition of Dichloromethane Extracts Against *Staphylococcus aureus*: In the dichloromethane crude extract, the results showed *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* against gram-positive *Staphylococcus aureus* measured maximum zone of inhibition (mm) in the order, 20.0, 20.0, 20.0, 20.0, 23.0, 20.0, 25.0 and 24.0. The dichloromethane crude extracts of *Leonotis nepetifolia* displayed the highest activity. The antibacterial activity of dichloromethane crude extracts of the eight herbs is in the range of 20mm to 25mm zone of inhibition. The reference antibiotic compounds, minocyline, chloramphenicol and cotrimoazol against gram-positive *Staphylococcus aureus* displayed positive results of zone of inhibition (mm), 16, 12 and 25 respectively. The control pure dichloromethane solvent against gram-positive *Staphylococcus aureus* measured 12mm which is zero zone of inhibition. The results of dichloromethane extracts of the eight herbs tested against gram-positive *Staphylococcus aureus* indicate that the herbal extracts have high potential antibacterial activity than the reference antibiotics (Table 2, Figure 3).

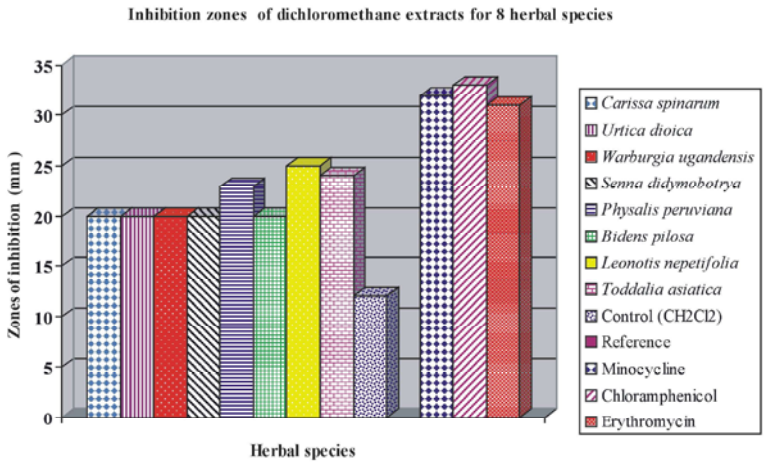

Fig. 3: Zones of inhibition of dichloromethane extracts against *Staphylococcus aureus*

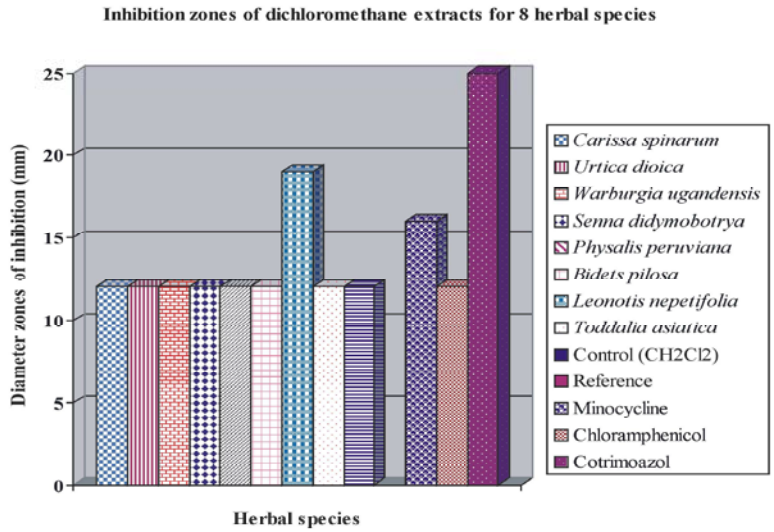

Fig. 4: Zones of inhibition of dichloromethane extracts against *Escherichia coli*

Zones of Inhibition of Dichloromethane Extracts Against *Escherichia coli:* Results obtained for dichloromethane extracts of the eight herbs against gram-negative bacteria *Escherichia coli* have two herbs with significant antibacterial activity namely, *Bidens pilosa* and *Leonotis nepetifolia* whose zone of inhibition measured (mm) 16 and 19 respectively. In this case the dichloromethane extracts of *Bidens pilosa* showed less activity while *Leonotis nepetifolia* displayed higher activity. The dichloromethane extracts of other herbs and pure dichloromethane solvent displayed no zone of inhibition against gram-negative bacteria *Escherichia coli*. The reference antibiotic compounds, minocycline, chloramphenicol and cotrimoazol against gram-negative bacteria *Escherichia coli* measured (mm) 30, 25 and 18 zone of inhibition (Table 2, Figure 4).

Zones of Inhibition of Ethyl Acetate Extracts Against *Staphylococcus aureus:* The results of pure ethyl acetate solvent measured 12mm, which is no zone of inhibition. The reference antibiotic compounds, minocyline, chloramphenicol, erythromycin and cotrimoazol against gram-positive *Staphylococcus aureus* measured (mm) 32, 33 31 and 18 zone of inhibition. Results obtained when ethyl acetate extracts tested against gram-positive *Staphylococcus aureus* indicated that the herbs *Warburgia ugandensis* and *Bidens pilosa* have the highest activity with zone of inhibition 25mm, followed by *Carissa spinarum, Senna didymobotry* and *Physalis peruviana* with less activity of zone of inhibition 24mm. Results obtained when crude ethyl acetate extracts of *Urtica dioica, Leonotis nepetifolia* and *Toddalia asiatica* tested against gram-positive *Staphylococcus aureus* measured (mm) 23, 20 and22 zone of inhibition respectively (Table 3, Figure 5).

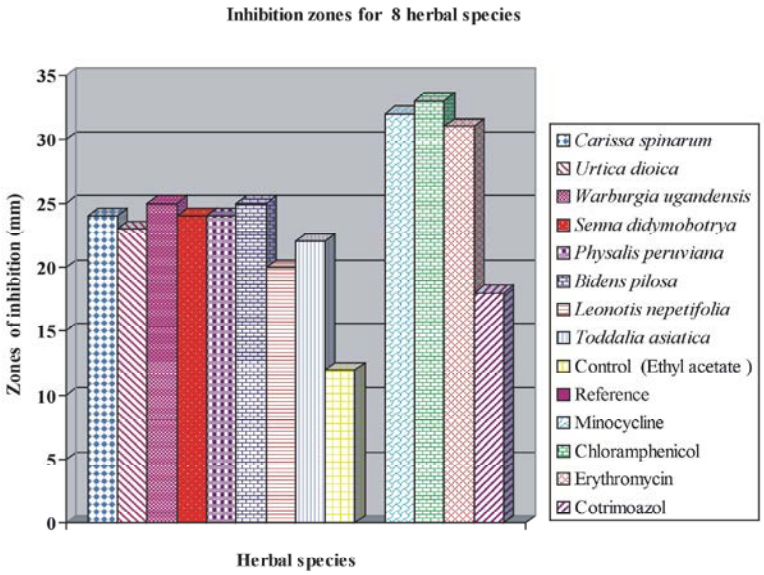

Fig. 5: Zones of inhibition of ethyl acetate extracts against *Staphylococcus aureus*

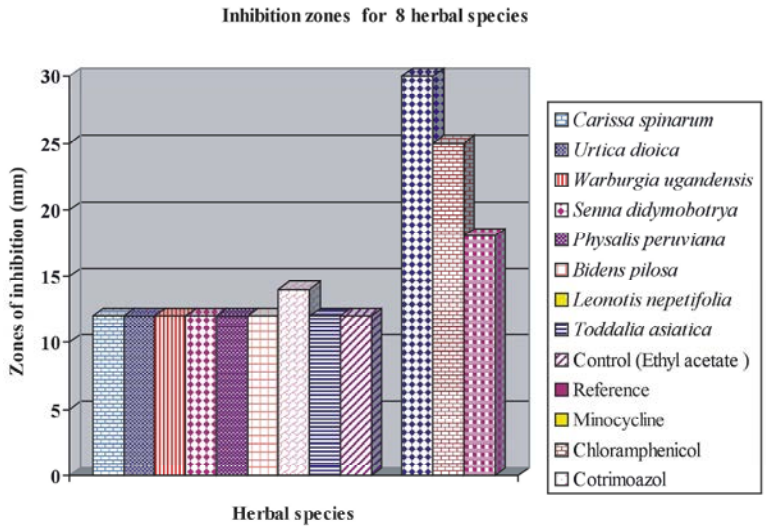

Fig. 6: Zones of inhibition of ethyl acetate extracts against Escherichia coli

Zones of Inhibition of Ethyl Acetate Extracts Against *Escherichia coli:* Results of crude ethyl acetate extracts of eight herbs against gram-negative bacteria *Escherichia coli* indicated that *Leonotis nepetifolia* measured 14 mm zone of inhibition. The crude ethyl acetate extracts of other herbs and pure ethyl acetate solvent measured 12 mm, meaning no zone of inhibition. The reference antibiotic compounds, minocyline, chloramphenicol and cotrimoazol against gram-negative bacteria *Escherichia coli* measured (mm) 30, 25 and 18 zone of inhibition, indicating that crude ethyl acetate extract of *Leonotis nepetifolia* has less activity than the reference (Table 3, Figure 6).

Zones of Inhibition of Ethanol Extracts Against *Staphylococcus aureus*: It is observed that pure ethanol solvent showed no zone of inhibition when tested against gram- positive *Staphylococcus aureus*. Results of crude ethanol extracts against gram-positive *Staphylococcus aureus* indicate that *Warburgia ugandensis, Physalis peruviana, Bidens pilosa* and *Toddalia asiatica* showed the highest antibacterial activity with inhibition zone measured 27mm. It is also observed that the crude ethanol extracts of *Urtica dioica* and *Leonotis nepetifolia* against gram-positive *Staphylococcus aureus* measured 26 mm zone of inhibition. The crude ethanol extracts

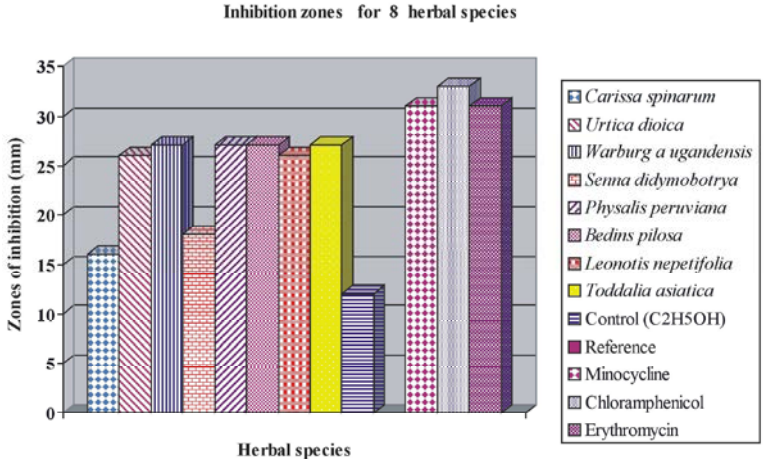

Fig. 7: Zones of inhibition of ethanol extracts against *Staphylococcus aureus*

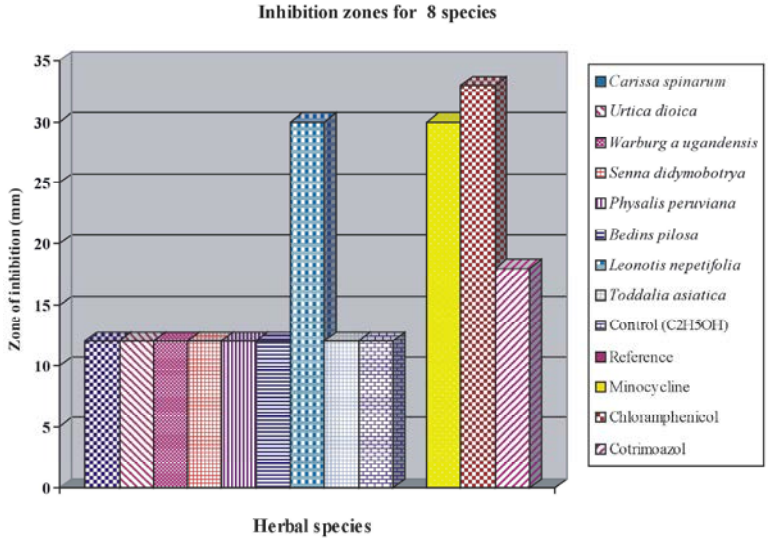

Fig. 8: Zones of inhibition of ethanol extracts against *Escherichia coli*

Table 3: Antimicrobial activity of ethyl acetate extracts of eight herbs

Well diffusion (Ethyl acetate extract)	Volume of extract (ml)	Diameter of zone of inhibition (mm) *Staphylococcus aureus*	Diameter of zone of inhibition (mm) *Escherichia coli*
Carissa spinarum	0.2	24±1.0	12±0.0
Urtica dioica	0.2	23±1.0	12±0.0
Warburgia ugandensis	0.2	25±1.0	12±0.0
Sunni didymobotrya	0.2	24±1.0	12±0.0
Physalis peruviana	0.2	24±1.0	14±1.0
Bidens pilosa	0.2	25±1.0	12±0.0
Leonotis nepetifolia	0.2	20±1.0	12±0.0
Toddalia asiatica	0.2	22±1.0	12±0.0
Control(Ethyl acetate)	0.2	12±0.0	12±0.0
Reference			
Erythromycin(Ery)	15 µg	31±1.0	12±0.0
Chloramphenicol	30 µg	33±1.0	25±1.0
Minocycline	30 µg	32±1.0	30±1.0
Cotrimoazol	25µg	18±1.0	18±1.0

Table 4: The antimicrobial activity of ethanol extracts of the eight herbs.

Well diffusion (Ethanol)	Volume of Extract (ml)	Diameter of zone of inhibition (mm) *Staphylococcus aureus*	Diameter of zone of inhibition (mm) *Escherichia coli*
Carissa spinarum	0.2	16±1.0	12±0.0
Urtica dioica	0.2	26±1.0	12±0.0
Warburgia ugandensis	0.2	27±1.0	12±0.0
Senna didymobotrya	0.2	18±1.0	13±1.0
Physalis Peruvian	0.2	27±1.0	13±1.0
Bidens pilosa	0.2	27±1.0	13±1.0
Leonotis nepetifolia	0.2	26±1.0	17±1.0
Toddalia asiatica	0.2	26±1.0	12±0.0
Control (C_2H_5OH)	0.2	12±0.0	12±0.0
Reference			
Erythromycin(Ery)	15 µg	31±1.0	12±0.0
Chloramphenicol	30 µg	33±1.0	33±1.0
Minocycline	30 µg	32±1.0	30±1.0
Cotrimoazol	25µg	18±1.0	18±1.0

of *Carissa spinarum* and *Senna didymobotrya* against gram-positive *Staphylococcus aureus* measured (mm) 16 and 17 zone of inhibition respectively (Table 4, Figure 7).

Zones of Inhibition of Ethanol Extracts Against *Escherichia coli:* The results of crude ethanol extracts of the eight herbs against gram-negative bacteria *Escherichia coli* indicate that *Leonotis nepetifolia* measured 30 mm zone of inhibition. Results of the extracts for other herbs measured 12 mm, indicating no zone of inhibition. The reference antibiotic compounds, minocyline, chloramphenicol and cotrimoazol against gram-negative bacteria *Escherichia coli* measured (mm) 30, 33 and 18 zone of inhibition. The pure ethanol solvent against gram-negative bacteria *Escherichia coli* measured 12mm, meaning no zone of inhibition (Table 4, Figure 8).

The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be attributed to the differences in morphological constitutions between these microorganisms, gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components [16]. This makes the cell wall impermeable to antimicrobial chemical substances. The gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [1]. Therefore, the cell walls of gram negative organisms are more complex in lay out than the gram positive ones acting as a diffusional barrier and making them less susceptible to the antimicrobial agents than are gram positive bacteria [17]. In spite of this permeability differences, however, some of the extracts had still exerted some degree of inhibition against gram-negative organisms as well.

CONCLUSIONS

The extracts of hexane, dichloromethane, ethyl acetate and ethanol for the herbs *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica,* have antimicrobial properties because they controlled gram-positive bacteria *Staphylococcus aureus*. However, only the crude extract of dichloromethane and ethanol of *Leonotis nepetifolia* controlled gram-negative bacteria *Escherichia coli* indicating the herbs differ in their antimicrobial activity. The antimicrobial activity profile of all the eight plants against the two strains of bacteria, *Staphylococcus aureus* and *Escherichia coli*, showed that *S. aureus* is the most susceptible while *E. coli* is the most insensitive to the herbal extracts. The antibacterial activity of the herbal extracts was more pronounced on the gram-positive bacteria *S. aureus* than the gram-negative bacteria *E. coli*. This knowledge is likely to improve the use of traditional herbs if integrated with that of the traditional healers. Future work should target the isolation and purification of bioactive constituents of the hexane, dichloromethane, ethyl acetate and ethanol extracts of the eight herbs to identify the active compounds associated with the antibacterial activities.

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