Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants

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ABSTRACT
Measles is a killer disease of children in Uganda. The treatment of the disease is mainly directed at the secondary microbial infections. A large proportion of the population in Uganda still relies on the use of herbal remedies, which have been claimed to produce beneficial responses. In this study, the efficacy of *Warburgia ugandensis* and *Zanthoxylum chalybeum* against common bacteria and fungi was investigated. Bactericidal and antifungal assays were done using extracts derived from *Z. chalybeum* and *W. ugandensis* (agar well diffusion, disc diffusion and colony count assays). All extracts (ethanolic, petroleum ether and aqueous) of *Z. chalybeum* did not show antimicrobial activity. Phytochemical investigations of *Zanthoxylum chalybeum* (seed) yielded a pure crystalline alkaloid (27-135D) which was characterized as skimianine based on ^{1}H-NMR spectroscopy and comparison with spectra of authentic samples. Skimmianine did not have antimicrobial activity in this test system. *W. ugandensis* water extracts elicited antibacterial activity against both *Escherischia coli* and *Staphylococcus aureus* in the agar well assay but not in the disc diffusion assay. *Warburgia ugandensis* water extracts and fraction 27-163D also showed antifungal activity against *Candida albicans*. Chromatography of extracts of *Warburgia ugandensis* stem bark afforded compound 49-169K, which was characterized as the sesquiterpine muzigadial (by ^{1}H-NMR spectroscopy), which did not show antibacterial activity but had antifungal activity against *C. albicans*. Therefore, the claimed efficacy of *W. ugandensis* could be attributable to antibacterial and antifungal activity of its components. Since *Z. chalybeum* extracts had neither antifungal nor antibacterial activities, its mode of action is unclear from these results.
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INTRODUCTION

Despite the wide availability of clinically useful antibiotics and semisynthetic analoques, a continuing search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. Moreover, the combination of genetic versatility of microbes and widespread overuse of antibiotics has led to increasing clinical resistance of previously sensitive microorganisms and the emergence of previously uncommon infections. Studies aimed at discovery and characterization of the substances that exhibit activity against infectious micro-organisms, yet showing no cross resistance with existing antibiotics, are highly called for.

Human and veterinary health services in Uganda are still very poor and are compounded by many people living in rural areas several kilometers from health centers. This has resulted in a large proportion of the population relying on traditional methods of treatment, using herbal extracts, which have been claimed to produce beneficial responses. This is in consonance with past experience of many useful drugs being derived from plants (such as atropine, ergometrine and the antibiotics).

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Measles is one of the major killer diseases of children in Uganda[1]. There is currently no treatment for this primarily viral disease. Intervention treatment is usually directed at controlling secondary bacterial infections using antibacterial compounds. In Uganda, plants may represent a potential source of antibacterial drugs since extracts from some plants have been used in treatment of measles. The claimed efficacy of *W. ugandensis* and *Z. chalybeum* extracts in the treatment of measles was studied and here we report the evaluation of the antimicrobial properties of these plant extracts.

MATERIALS AND METHODS

i) Phytochemical studies

Plant collection and pre-extraction preparation

Zanthoxylum chalybeum specimens were collected from Ongino, Kumi district, Eastern Uganda. Botanical identification was done by a botanist at the natural chemotherapeutic research laboratories (Wandegeya), Ministry of Health, Kampala. Voucher specimens have been kept at the Department of Pharmacy, Medical School (Makerere University), for reference purposes. *W. ugandensis* stem bark was collected from Mabira Forest, 20 Km on Kampala-Jinja road and also identified and voucher specimens kept as above.

Z. chalybeum stem bark was chopped into small pieces and dried under the sun for three weeks. The seeds

were already dry when collected, but they were also further sun dried for one week.

W. ugandensis stem bark was likewise chopped and sundried for three weeks. The dried specimens were then crushed by pounding in a wooden mortar into fine powder, ready for extraction.

Extraction, purification and spectroscopy

The powdered materials were macerated in petroleum ether, alcohol and water. One hundred grams of powdered plant materials (*Z. chalybeum* and *W. ugandensis*) was soaked in petroleum ether, ethanol and water respectively in Ehlenmeyer flasks, corked and allowed to stay overnight. The marcerate thus obtained was filtered with Whatman No. 1 filter paper and the filtrate (ethanol and petroleum ether) concentrated *in vacuo* in a round bottomed flask using a rotary evaporator (Heidolph model) under low temperature.The water extract filtrate was poured onto watch glasses and petridishes and dried in air in a hood. The dried extract was subsequently scrapped off onto prescription bottles. Melting points were determined on Koffler hot stage apparatus and on a Thomas Hoover capillary melting point apparatus. Infra-Red spectra were run using KBr discs on Perkin Elmer Infrared spectrophotometer, model 727B. ^{1}H NMR spectra were determined for solutions in deuteriochloroform and D_2O with a Jeol 90MHz, Tetramethylsilane as internal standard. Analytical thin layer Chromatograms were run on 0.2mm thick layer of silica gel, (Merck). The products isolated were detected by UV fluorescence. Preparative layer chromatography was run on 1.0mm thick layer of silica gel. Column chromatography was performed on silica gel 60 (70-230 mesh) and Sephadex LH-20.

Extraction and isolation of compounds from *Z. chalybeum.*

Crushed seeds of the plant (100g) were soaked in ethanol, placed in a shaker for 24 hr, filtered, concentrated under reduced pressure, to obtain 6.9g of the crude extract. Compound 27-135D crystallised out of the crude extract, was filtered off and recrystallised from methanol. Stem bark(100gms) was soaked in ethanol, placed in shaker overnight, filtered and concentrated under reduced pressure to obtain 7.6g. of the crude extract.

Extraction and isolation of compounds from *W. ugandensis*

The stem bark (100g) of *W. ugandensis* was soaked in EtOH, placed on a shaker for 24 hours, filtered and then concentrated to obtain the crude extract (13.2g). The crude extract (6.6g) was dissolved in 80% aqueous MeOH (100ml) and extracted with petrol (100ml x 3). The petrol extract was dried over anhydrous Na_2SO_4., filtered, concentrated under reduced pressure to obtain 0.48g of extract (27-163A). To the aqueous layer, distilled water (33.3ml) was added and then extracted with chloroform (2 x 100ml).The chloroform extract was dried over anhydrous Na_2SO_4., filtered and concentrated to obtain 1.42g of extract (27-163B). The remaining aqueous extract was dried in a hood to obtain 2.40g of 27-163D.

To obtain muzigadial, ground stem bark (200g) was macerated first with petrol and then with Ethyl acetate (EtOAC). The petrol extract was concentrated to yield 10g of crude extract which was fractionated over silica gel column eluting with petrol and then petrol containing increasing amounts of EtOAC.In order of elution, the following fractions were obtained: 49-169b (1.17g), 49-169c (500 mg), 49-169d (500mg), 49-169e (1.15g), 49-169f (1.15g), 49-169g (1.16g), 49-169h (150mg), 49-169i (651mg), 49-169j (1.56mg), 49-169k (850mg), 49-181a (665mg). Fraction 49-169k was pure muzigadial.

ii) Antibacterial and antifungal studies

Test organisms and media

The standard reference bacteria used were obtained from the University of Nairobi, Medical School, Department of Microbiology (courtesy, Prof. Ndinya Achola). Wild type strains were not used because they often have unpredictable sensitivity to antimicrobial compounds. Some may even have variable degrees of drug resistance. The following standard bacteria were obtained: *Staphylococcus aureus* ATCC No. 13709. *Escherischia coli* ATCC No. 9637. The fungus used was *Candida albicans* ATCC No. 10231 (obtained from the department of biology, Addis Ababa University. The media used were gelatin, peptone bacteriological, Muller Hinton and Nutrient agars.

Extracts tested and the standard bactericidal agent

Crude extracts (ethanolic, water and petroleum ether) and fractions of stem bark and seed of *Z. chalybeum* were tested. Most reports suggested that extracts of the seed and stem were most often used. There was no report of use of leaves at all. Extracts of only stem bark of *W. ugandensis* were tested. No other part of the plant was reported to be medicinally useful. Broad spectrum tetracyline antibiotic capsules (Kampala pharmaceuticals, Ltd.) was used to produce a standard bacterial killing percentage for comparison with that of the medicinal plant extracts.

Bactericidal and antifungal tests

Agar well diffusion assay

Wells were made on Muller Hinton agar plates using asterile

borer. Two to five hour broth cultures of *S. aureus* and *E. coli* were centrifuged at 1956.5g for 15 mins (3x) and reconstituted in 1% gelatin in normal saline. The turbidity of the reconstituted organisms was adjusted to that of turbidity standard[2] (prepared by adding 0.5ml of 1% $Bacl_2$ to 99.5ml of 0.36N H_2S0_4). Both the standard and bacterial suspensions were agitated a vortex mixer immediately prior to use. A sterile cotton swab (on a wooden applicator stick) was dipped into the standardized bacterial suspension. Broth was expressed from the swabs by pressing and rotating the swabs firmly against the inside of the tube above the fluid level. The swab was then evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums; a final sweep of the agar rim was made with the cotton swabs[2]. The plates were allowed to dry for 3-5 minutes after which 20µl of the test samples and the controls were dispensed into each well. The concentration of the test samples were 50 mg/ml of 1% GNS. The plates were incubated at 37°C for 24hrs during which activity was evidenced by the presence of a zone of inhibition surrounding the well. Zone sizes were measured in millimeters compared to standard tetracycline.

Filter paper disc assay

The procedure for inoculation of the plates was essentially as given above (agar well diffusion assay). However, instead of drilling wells on the agar, filter paper discs (Whatman No. I) impregnated with 100µg of extracts were applied on the surface of the agar and the plate incubated overnight. Plates on which *Candida albicans* had been applied were also tested [3].

Tube incubation and colony plate assays

Staphylococcus aureus and *E. coli* were cultured on Mueller Hinton Agar. Four colonies of the organisms were taken and grown in 10ml of peptone water for 4 hrs in a shaker to obtain logarithmic phase organisms. The organisms were centrifuged for 15 minutes at 1956.5g and washed twice in 1% GNS. The organisms were finally centrifuged to obtain a pellet. The pellet was reconstituted with 1% GNS and the turbidity adjusted in order to obtain a turbidity visually comparable to that of the standard (prepared by adding 0.5ml of 1% $Bacl_2$ to 99.5ml of 0.36N H_2S0_4). This was crosschecked spectrophotometrically (470nM filter). Serial dilutions were made from this point to 10^{-4}. 10µl of the organisms were plated in duplicate unto Mueller Hinton plates and incubated overnight. A colony count was done on a colony counter after 18hrs. This gave a value of 150 colony forming units (CFU's). 100µl of these organisms at this dilution (equivalent to $1.5x10^3$ CFU's) were incubated with 200µl of plant extract. Preliminary studies indicated that 20µl of this mixture when plated at zero time gave a growth of about 100 CFU's. The mixture was incubated at 37°C on a shaker and 20 ul samples removed and plated at varying periods of time of 0 mins, 30 mins, 60 mins and 90mins onto Mueller Hinton plates in duplicate. The plates were then incubated at 37°c and the colonies counted after 18hrs. Tetracycline capsules (BP) obtained from Kampala pharmaceuticals Ltd were used as positive controls. Following standard methods of colony counting of the American Public Health Association[4]. mainly colonies between 30-300 were considered. A final concentration of bacteria killed or which grew were calculated using the formula:

$$\text{Percentage of organisms killed} = \left(1 - \frac{\text{CFU per ml of test}}{\text{CFU per ml in GNS at Omins}}\right) \times 100.$$

The analysis of all the differences between means was performed using a paired t-test[5]. The confidence interval was set at 95% ($p<0.05$). In all cases means are shown with ± standard error.

RESULTS

Phytochemical analyses

Compounds 27-135D and 27-149K were characterized as the alkaloid skimmianine and the sesquiterpine muzigadial respectively [7].

Bactericidal and antifungal studies

Warburgia ugandensis showed antibacterial activity against *S. aureus* and *E. coli* in the agar well diffusion assay. This effect was not demonstrable in the paper disc assay (Table 1).

Table 1. Antifungal and antibacterial activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*: paper disc and agar well diffusion assays.

Exract or sample code	Extract concentration mg/ml	Test organisms					
		S. aureus		*E. coli*		*C.. albicans*	
		P.D.	A.W.	P.D.	A.W.	P.D.	A.W.
1.Zanthoxylum							
i) Water extract (stem)	100	-	-	-	-	-	N.D.
ii) Ethanol extract (stem)	100	-	-	-	-	-	N.D.
iii) Pet. ether extract (stem)	100	-	-	-	-	-	N.D.
iv) Water extract (seed)	100	-	-	-	-	-	N.D.
v) Ethanol extract (seed)	100	-	-	-	-	-	N.D.
vi) Pet.ether extract (seed)	100	-	-	-	-	-	N.D.
vii) 27-135D (stem)	50	-	-	-	-	-	N.D.
2) *Warburgia* (Stem)							
i) Water extract	100	-	+	-	+	+	N.D.
ii) Pet ether extract	100	-	-	-	-	-	N.D.
iii) Ethanol extract	100	-	-	-	-	-	N.D.
iv) 27-163D	100	-	+	-	+	+	N.D.
v) 49-169K	50	-	-	-	-	-	N.D.

P.D. = Paper disc assay.; Pet = Petroleum
A.W. = Agar well assay;. 27-135D = Skimmianine;
- = Not active; 27– 163D = W. ugandensis fraction.
+ = Active; 49 –169K = Muzigadial
N.D. = Not done.

Both the crude and the purified fractions of *Z. chalybeum* had no antibacterial activity against *E. coli* and *S. aureus* (Table 1). This was true in all assays - i.e. paper disc, agar well diffusion as well as the colony count assay. The alkaloid skimmianine isolated and characterised from the seed did not have any antibacterial activity either. It also had no antifungal activity against *C. albicans* (Table 1). All attempts to do the colony count assay resulted in very heavy growth of bacteria.

DISCUSSION

Warburgia ugandensis extracts were bactericidal. It was not possible to demonstrate this effect in the paper disc assay, probably because the paper disc retains the active component and does not allow it to diffuse into the Muller Hinton Agar. In the agar well assay, the water extract showed a slightly higher activity as compared to the ethanolic extract. This was to be expected since the aqueous phase of the Muller Hinton agar would be expected to more easily dissolve water-soluble extracts and hence enable easier diffusion. This emphasizes the fact that agar well diffusion assay is ideal for water extracts. The antibacterial activity of *W. ugandensis* is only detectable at very high concentration of the extract (i.e. upto 50 mg/ml of crude extract). This highlights the problem of deciding on the starting point of assaying crude extracts. Most assays of extracts start at 50-100ug/ml, at which level it is not possible to detect activity in *W. ugandensis* extracts. This may explain why there is as yet no report of antibacterial activity of *W. ugandensis*. It is probably not proper to start assaying crude extracts at concentration levels comparable to tetracycline since tetracycline is a known pure compound. It is quite likely that the active ingredient is present in *W. ugandensis* in very low quantities, requiring the use of large amounts of crude extracts.

Warburgia ugandensis is known to have potent antifeedant and antifungal activity [8]. Warburganal and Muzigadial have been characterized as the active compounds. This study has also demonstrated antifungal activity in *W. ugandensis* (Table 1). To the best of our knowledge this is the first report of antibacterial activity in extracts derived from *W. ugandensis*. The extracts inhibited *S. aureus* more than *E. coli*.

In the colony count assay, both the alcohol and the water extracts exhibited bactericidal activity against both *S. aureus* and *E. coli*. There was, however, a marked difference in the activity of the two. This was rather paradoxical since both ethanol and water are highly polar solvents, which would be expected to extract similar components from the plants. It was also noted that the agar well diffusion assay revealed a low activity of ethanolic extracts. In the tube incubation test, however, the bactericidal activity of the crude extracts against both *S. aureus* and *E. coli* was quite high. *Staphylococcus aureus* was more sensitive than *E. coli* to *W. ugandensis* extracts (agar well assay). Since *S. aureus* is more often associated with secondary bacterial infections in measles than *E. coli* [10], it may explain the value of the plant in measles therapy.

Zanthoxylum chalybeum had no antibacterial activity against *E. coli* and *S. aureus*. This was true of the crude as well as the purified fractions. The extracts also had no antifungal activity against *Candida albicans*. In a study carried out by Odebiyi and Sofowora [11], antibacterial activity was demonstrated in extracts of *Zanthoxylum zanthoxyloides*, a closely related plant to the one in this study. There are a number of possible explanations for the differences in biological activities. Firstly, it is known that similar plants growing in different geographical locations may be phytochemically very different [12]. Secondly, the active constituents of various *Zanthoxylum* species vary greatly [16,4,3,9] and the taxonomy in certain cases is somewhat obscure (both scientifically and by local momenclature). This makes it usually necessary to specify not only the botanical sources used but also give some indication of the chemical composition. This is because, apart from the interspecific variation in chemistry, the extent of the variations of active constituents within a species (particularly from different geographical locations) is still not clear [13].

Thirdly, the difference in extraction procedures could also explain the differences in the findings. For instance, previous studies [14] indicated that chelerythrine, berberine and canthin-6-one are antibacterial components of *Zanthoxylum spp.* There has been also a report of antibacterial activity of two groups of compounds (phenolic acids and alkaloids), which occur in the root of *Z. zanthoxyloides* [11]. In other studies [11], in which antibacterial activity was reported, the *Zanthoxylum* species was Soxhlet-extracted and the extract concentrated to a low volume before defatting with light petroleum. The precipitate observed was passed through a column of alumina. In all cases of this study [11], the alcoholic extracts produced the highest zones of inhibition. The antibacterial compound was characterized as canthin-6-one, which has also been isolated from *Zanthoxylum elephantiasis*. The observed differences in activity of extracts may, therefore, be attributable to the differences in extraction procedures.

Finally, the absence of antibacterial activity may suggest that the extracts of *Z. chalybeum* may act in an indirect way; the active ingredient may exist as a precursor, which requires activation in the body by some as yet unknown mechanism. It is known that skimmianine (isolated in this study from the seed of *Z. chalybeum*) has ephedrine-like action [15]. and antihistaminic effect. It is possible that the extract may achieve effect via an immunopharmacological mechanism. This, however, requires further investigation.

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The antibacterial effect on *S. aureus* was higher than that on *E. coli* in the agar well assay. Both the alcohol and the water extracts showed antibacterial activity against both *E. coli* and *S. aureus* in the colony count assay (Tables 2 and 3).

Table 2: Cidal studies for *Staph aureus* and *E. coli* in tetracycline and 1% Gelatin as positive and negative controls respectively.

Tet/gelatin Concentration	Reaction mixture	% CFUs ± S.E.					
		0 min	15 min	30 min	60 min	90 min	120 min
1% GNS	10µl S.A 200µl GNS	100.0±2.0	ND	96.1±1.0	168.5±20.0	TNTC	TNTC
1% GNS	50µl S.A 200µl GNS	100.0±3.0	ND	71.0±10.0	116.9±14.0	TNTC	TNTC
1% GNS	100µl (S.A) 200µl GNS	100.0±3.0	55.2±10.0	9.2±5.0	46.0±15.0	167.5±3.0	TNTC
1% GNS	10µl (E.C) 200µl GNS	100.0±5.0	ND	115.1±12.0	146.2±4.0	200.8±6.0	TNTC
1% GNS	50µl (E.C) 200µl GNS	100.0±52.0	ND	81.5±8.0	65.4±4.0	87.7±5.0	TNTC
1% GNS	100µl (E.C) 200µl GNS	100.0±20.0	69.2±12.0	56.6±8.0	28.3±6.0	128.3±14.0	169.2±52.0
0.025mg/ml tetracycline	10µl (S.A) 200µl Tet	100.0±20.0	ND	101.1±8.0	51.1±12.0	6.7±3.0	26.7±2.0
0.025mg/ml tetracycline	50µl (S.A) 200µl Tet	100.0±8.0	ND	53.6±12.0	102.9±16.0	78.3±11.0	98.6±12.0
0.025mg/ml tetracycline	100µl (S.A) 200µl Tet	100.0±1.0	65.7±8.0	40.4±7.0	22.2±4.0	51.5±8.0	70.70±6.0
0.025mg/ml tetracycline	10µl (E.C) 200µl Tet	100.0±20.0	ND	87.5.0±21.0	41.7±2.0	20.0±8.0	5.4±5.0
0.025mg/ml tetracycline	50µl (E.C) 200µl Tet	100.0±21.0	ND	85.9±16.0	81.3±15.0	26.6±10.0	18.6±5.0
0.025mg/ml tetracycline	10µl (E.C) 200µl	100.0±50.0	69.4±23.3	59.3±40.0	53.2±30.0	11.1±10.0	2.8±2.0

ND = Not done; TNTC = too numerous to count; GNS = % gelatin in normal saline; S.E = Standard error of the mean; CFUs = Colony forming units, S.A = *S. aureus*; E.C. = *E. coli.*

Table 3: Cidal studies for *Staph. aureus* and *E. coli* in *Warburgia ugandensis* extracts.

Extract Concentration	Reaction mixture	% CFUs ± S.E.					
		0 min	15 min	30 min	60 min	90 min	120 min
Alcohol extract 50mg/ml	200µl W 100µl SA	100.0±12.0	86.4±16.0	66.1±15.0	15.5±10.0	8.7±5.0	0.0
Alcohol extract 50mg/ml	200µl W 100µl EC	100.0±24.0	0.0 ±1.0	0.0	0.0	0.0	0.0
Water extract 10mg/ml	200µl W 100µl SA	100.0±12.0	53.3±8.0	53.3±13.0	22.5±6.0	16.7±5.0	18.3±4.0
Water extract 20mg/ml	200µl W 100µl EC	100.0±23.0	66.2±16.0	50.4±18.0	35.0±10.0	23.6±14.0	17.1±6.0
Water extract 10mg/ml	200µl W 100µl SA	100.0±12.0	75.0±10.0	43.8±8.0	0.0	0.0	0.0
Water extract 20mg/ml	200µl W	100.0±14.0 100µl EC	41.7±8.0	24.2±6.0	0.0	0.0	0.0

W = *W. ugandensis*; S.E = Standard error of the mean; CFUs = Colony forming units, S.A = *S. aureus*; E.C. = *E. coli.*

Incubation of *E. coli* in 1% GNS resulted in a rise of % CFU's from 100.0 ± 20.0% to 169.2 ± 52.0 at 120 minutes of incubation. Tetracycline (0.25mg/ml) on the other hand caused a fall from 100.0 ± 50.0% CFU's to 2.8 ± 2.0% CFU's at 120 minutes (Table 4). *W. ugandensis* extracts exhibited differences in activity against *E. coli*. The water extract (10mg/ml) caused a fall in % CFU's from 100.0 ± 12.0% to 43.8 ± 8.0% at 30 minutes of incubation, while the water extract (20mg/ml) caused a fall from 100.0 ± 14.0% CFU's to 24.2 ± 6.0% CFU's at 30 minutes of incubation (Table 4).

Table 4: Percentage bacterial kill or growth following incubation with *W. ugandensis* extracts (1% gelatin in normal saline and tetracycline used as negative and positive controls respectively).

Tet/gelatin/extract Concentration	Reaction mixture	% kill or growth ± S.E.			
		30 min	60 min	90 min	120 min
Alcohol extract 50mg/ml	200µl W 100µl SA	34.0±4.0 (K)	84.5±0.0 (K)	91.3±0.0 (K)	100.0±0 (K)
Alcohol extract 50mg/ml	200µl W 100µl EC	100.0±0.0 (K)	100.0±0.0 (K)	100.0±0.0 (K)	100.0±0.0 (K)
Water extract 10mg/ml	200µl W 100µl SA	47.0±8.0 (K)	77.5±5.0 (K)	83.3±6.0 (K)	82.0±12.0 (K)
Water extract 20mg/ml	200µl W 100µl SA	50.0±7.0 (K)	65.0±5.0 (K)	74.4±2.0 (K)	83.0±12.0 (K)
Water extract 10mg/ml	200µl W 100µl EC	57.0±7.0 (K)	100.0±6.0 (K)	100.0±0.0 (K)	100.0±0.0 (K)
Water extract 20mg/ml	200µl W 100µl EC	76.0±11.0 (K)	100.0±12.0 (K)	100.0±0.0 (K)	100.0±0.0 (K)
0.025% tetracycline	200µl W 100µl SA	60.0±12.0 (K)	77.8±1.0 (K)	48.5±12.0 (K)	30.0±14.0 (K)
0. 25 % tetracycline	200µl W 100µl EC	41.0±6.0 (K)	46.8±5.0 (K)	79.2±6.1 (K)	100.0±6.0 (K)
1% GNS	200µl W 100µl SA	91.0±8.0 (K)	54.7±6.0 (K)	67.5±1.0 (G)	TNTC (G)
1% GNS	200µl W 1 00µl EC	43.0±10.0 (K)	71.7±11.0 (K)	28.0±4.0 (G)	69.6±3.0 (G)

TNTC = too numerous to count; GNS = % gelatin in normal saline; S.E = Standard error of the mean; S.A = *S. aureus*; E.C. = *E. coli.*; W. = *Warburgia ugandensis;* K = Kill; G = Growth

The alcohol extracts inhibited *E. coli* more; so that by 15 minutes of incubation, there was a fall in % CFU's from 100.0 ± 24.0% (at zero time) to 0.0% CFU's (Table 3). *W. ugandensis* showed antibacterial activity against *S. aureus* and *E. coli* in the agar well diffusion assay. This effect was not demonstrable in the paper disc assay (Table 1). The antibacterial effect on *S. aureus* was more pronounced than on *E. coli* in the agar well assay. Both the alcohol and the water extracts showed antibacterial activity against both *E. coli* and *S. aureus* in the colony count assay. The alcohol and water extracts also had antifungal activity against *C. albicans* (paper disc assay, Table 1).