ANTIMICROBIAL EFFECTS OF SELECTED HERBAL EXTRACTS ON MULTI-DRUG RESISTANT GRAM-NEGATIVE BACTERIAL STRAINS

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Abstract
The rise in antibiotic resistance has resulted in a decreasing number of fully active antimicrobial agents available to treat infections by multi-drug resistant (MDR) bacteria. This has necessitated a search for new antimicrobial agents. Herbal remedies may offer novel treatment options which elicit little or no transferred resistance if used in optimal concentrations. This study evaluated the antimicrobial properties of ten plants traditionally used as herbal remedies against 27 multi-drug resistant Gram-negative bacterial isolates. The herbal extracts were obtained through extraction with organic (methanol) and inorganic (water) solvents. Susceptibility of the test strains to conventional antibiotics was determined by the disc diffusion technique. Determination of the Minimum Inhibitory Concentrations (MIC) and the sub-lethal concentrations of the most effective extracts against the MDR strains was done by broth inoculation followed by colony count method. The effect of sub-lethal extract concentrations was done by a method modified from McMahon *et al*. (2007). Out of the ten plants, only *Warbugia ugandensis* was active against the MDR strains and its efficacy was significantly different from that of other plant extracts such as *Terminalia brownii, Azaridachta indica, Clausena anisata* and *Strychnos henningsii* ($p<0.001$). The root and bark methanol extracts from *W. ugandensis* were the most effective with an MIC of 42 µg/ml. Susceptibility of test strains to conventional antibiotics was not significantly different before and after habituation to sub-optimal extract concentration ($p>0.005$). Methanol extracts from the root and bark of *W. ugandensis* provide potential sources of effective antimicrobial compounds for further development of alternative safe antimicrobial products in form of chemotherapeutic agents or antiseptics. The optimization and standardization of operation procedures and methods of analysing the efficacy of herbal extracts demands serious consideration.

Key words: Multi-drug resistant (MDR) bacteria, Minimum Inhibitory Concentrations (MICs), Sub-Lethal Concentration (SLC)

1.0 Introduction

Antimicrobial agents have substantially reduced the threat posed by infectious diseases over a period of time since their discovery in the 1940s (Lewis and Ausubel, 2006). However, the escalation of multidrug resistance in bacteria in recent years has seriously jeopardized these gains. This has gained worldwide attention due to the high impact on public health. Increased usage of antimicrobial agents to treat bacterial infections has lead to the emergence of multi drug resistant (MDR) strains (Bonnet, 2004). Such strains are resistant to first line of treatments and also the more expensive second and third-line antibiotics. The high cost of such replacement drugs and the toxicological effects are prohibitive and are out of reach for many Kenyans. Furthermore, newer antimicrobials are losing their effectivity fast due to transfer of resistance markers from resistant to susceptible bacterial strains. This scenario has necessitated a search for new antimicrobial substances from other sources especially plants, which produce diverse chemical compounds with different biological activities (Lewis and Ausubel, 2006).

Plants form an integral part of life in many indigenous African communities as a readily and cheaply available alternative to allopathic medicines. Such plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Somchit *et al.,* 2003), caused by bacteria often known to resist various classes of conventional antibiotics. Due to either limited availability or affordability of pharmaceutical medicines about 80% of the rural population in Sub-Saharan Africa (SSA) depends on traditional herbal remedies for primary health care (PHC) (WHO, 2002). Therefore, provision of safe and effective traditional medicines could become a critical tool to increase access to health care (WHO, 2002). It is expected that plant extracts exhibiting effective inhibition to microbial growth may provide less toxic, cheaper and more affordable alternatives for infection management. Moreover, the more efficient inhibition and lysis of bacteria resulting from the synergistic reactions of the active ingredients in plant extracts decreases the risk of progressively increasing antibiotic resistance (Miyasaki *et al*, 2010).

2.0 Materials and Methods

2.1 Test Strains

The test strains were selected from each of the following species; *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Salmonella typhi*, and their susceptibility patterns to different classes of antibiotics was determined. All the isolates had combined resistance to at least; two quinolones, two or more cephalosporins belonging to different classes, two aminoglycosides, one or two tetracyclines, and to one or more β- lactam/β-lactamase inhibitor.

2.2 Preparation of Plant Materials

The ten plants investigated in the study are those traditionally used for the treatment of bacterial infections by Kenyan communities (Table 1). The leaf, bark and root samples were harvested, packed in clean sterile manila papers, labelled with a voucher specimen and transported to the laboratory for analysis. The materials were oven-dried at 25°C and weighed every day until a constant weight was attained after which they were chopped and ground to fine powder using a motor grinder. The herbal extracts were obtained through extraction with organic (methanol) and inorganic (water) solvents.

2.3 Extraction of Plant Extracts

Warm water extraction was done to simulate the traditional decoction method of preparing herbal preparations. Sixty (60) g of the ground powder was soaked in 300 ml sterile distilled and deionised water at 50ºC for 1 hr, placed in an orbital shaker at 100 rpm for 24 h at 25ºC. The resulting elute was membrane-filtered and lyophilized into granules (LyoBeta range, Telstar, UK). Stock solutions were prepared by dissolving 0.4 g of the granules in 20 ml of deionised sterile distilled water while another set of a similar amount of preparations was dissolved in TE buffer (pH 8), and stored at -20ºC until further use.

Organic extraction was done using methanol. Sixty (60) g of the ground powder was soaked for 72 hr in a beaker containing 300 ml of methanol (Analytical Grade, 99.9%, Sigma-Aldrich, UK) and placed in an orbital shaker (Basic Model, Cole-Parmer, UK) at 100 rpm for 72 h at 25ºC. The resulting extracts were membrane filtered on a whatman paper number 1 then the filtrate concentrated at 50ºC using a vacuum rotary evaporator (Basic Model, Buchi, UK). In order to evaporate the methanol used as the extraction solvent, the concentrates were oven-dried at 25°C until a constant weight was attained. Excess methanol was evaporated by further air-drying the paste at 25°C until a constant weight was attained. Stock solutions of the methanol-free paste were prepared by dissolving 0.4 g in 20 ml of deionised sterile distilled water. Another preparation

was dissolved in TE buffer at pH 8 as an alternative diluent. The preparations were stored at -20ºC until further use.

2.4 Pre-Screening of the Herbal Extracts for Antimicrobial Activity against Test Strain

Preliminary screening of the extracts for antimicrobial effect was done using *E. coli* J53 as the test organism. This strain has a defined genetic background and is susceptible to all conventional antibiotics except Sodium azide. A confluent lawn of the bacteria was prepared from a 0.5 MacFarland equivalent (approximately 10^6 CFU/ml) and placed in an incubator to dry after which 0.5 µl of the test extracts were point inoculated using a micropipette (Eppendorf, Hamburg, Germany). Other sets of preparations were made in a similar way and used for testing the efficacy at each of the following titres: 1 µl, 5 µl, 10 µl, 30 µl, 80 µl or 100 µl. Extract-free sterile distilled water and TE buffer (the diluents used for making the stock solutions of the test extracts), were inoculated in another set of these plates and used as negative controls. The plates were allowed to stand for at least 1 hr at room temperature for the extracts to diffuse at the point of inoculation before incubation at 37°C for 8 h. Observation of a clear zone on bacterial lawn at the point of inoculation of the extract was interpreted as evidence of inhibition of bacterial growth. The diameter of these zones were measured and recorded to the nearest size mm. The plants whose extracts exhibited an inhibition zone > 20 mm were selected for further testing of clinical isolates.

2.5 Determination of MICs and the Sub-Lethal Concentrations of the Herbal Extracts

Duplicate tubes containing 2 ml MH broth were prepared. To one set of these tubes, separate titres of the extract in the range of 5µl-120ul were added to separate tubes before a uniform inoculum of 10^6 CFU/ml of the test isolates was added to each tube. Negative controls were set in a similar way but using distilled water or TE buffer instead of the extracts. The preparations were incubated for 8 h at 37ºC with continous shaking at 100 rpm in an orbital shaker. The tubes were removed from the shakers and arranged sequentially with increasing concentration of the herbal extract titre added. The tubes were then assessed for evidence of bacteria growth by physical examination before streaking 1 µl of the preparations on MacConkey plates. The plates were then incubated for 8 hr and the colonies counts determined. The tube containing the lowest concentration at which no colonies were observed was identified and calculations done to determine the appropriate concentration of the herbal extract added. This concentration was identified as the MIC. The tube containing the preparation at which the last visible growth was observed was identified as the highest amount of extract that does not inhibit bacterial growth. This concentration was therefore identified as the highest sub-lethal concentration of the extract. This sub-lethal concentration was used as the reference concentration in the habituation experiment discussed in section 2.6 below.

2.6 Habituation to Sub-lethal Concentrations (Modified from McMahon et al., 2007)

Two sets of duplicate tubes containing 2 ml MH broth were prepared. The sub-lethal concentration of an extract was added and inoculated with approximately 10^6 CFU/ml bacterial cells of the clinical isolates or controls ATCC *E. coli* strains 25922 and J53. Negative controls were prepared using similar sets of bacterial preparations but instead of the extracts, deionised sterile distilled water or TE buffer (pH 8) was used as blanks. All preparations were incubated for 72 h at 37ºC with shaking at 100 rpm after which colony counts were determined on MacConkey agar.

3.0 Results

3.1 Susceptibility Profiles of Test Strains to Conventional Antibiotics

All the 27 test isolates had combined resistance to at least; two quinolones, two or more cephalosporins, two aminoglycosides, tetracyclines, trimethoprim, and to at least one or more β- lactam/β- lactamase inhibitor. All isolates were resistant to ampicillin, 26 resistant to augmentin and only 8 isolates were resistant to cefoxitin (Table 2).

3.2 Pre-Screening of the Herbal Extracts for Antimicrobial Activity

The extracts from the 10 plants exhibited antibacterial effect and this depended on the plant species, part of the plant used, method of extraction and the volume of extract used (Table 3). Methanol extracts of *W. ugandensis* had a high antimicrobial effect even at titres as low as 0.5 µl from the 1 ug/ml of the plant extract preparation. There was a strong correlation between the amount of titre used and the zone of inhibition. Plants whose extracts of 100 µl produced an inhibition zone of greater than 24 mm were selected for further

testing using the clinical strains and their MICs determined. The plants whose extracts met this criterion were: *W. ugandensis; T. brownii; A. indica; C. anisata* and *S. henningsii* (Table 3).

3.3 Characterization of Plants with Inhibitory Effect

The five plants whose extracts produced an inhibition zone ≥24 mm at 100 µl were further tested using clinical Gram-negative isolates. Although extracts from these plants had been tested to give inhibitory effect on *E. coli* J53, only methanol extracts from the root and bark of *W. ugandensis* were inhibitory to the MDR strains. Mean colony counts were obtained by averaging the colony counts for the duplicate sets at 100 µl of the extracts and their standard error (SE) obtained (Figure 1)**.** Inhibition of the other clinical isolates was similar to that of *E. coli* isolate, E_1.

3.4 Inhibitory Characteristics of *W. ugandensis*

Figure 2 indicates a comparison of inhibitory characteristics of different parts of *W.ugandensis.* Methanolic extracts from the bark of *W. ugandensis* exhibited better inhibitory properties than those obtained using water extraction ($p<0.001$). Similarly, the root extracts obtained using methanol from this plant were more effective than those obtained using water extraction method ($p < 0.01$), However, there were no significant differences in the methanolic and water extracts from the leaf ($p=0.298$). The inhibition characteristics of root and bark extracts obtained using methanol were not significantly different (t-test, $p = 0.908$)

Figure 3 shows the inhibition trend, MIC and sublethal concentrations of extracts from *W. ugandensis*. There was a gradual decrease in the number of colonies as the amount of titre (root and bark methanol extracts) increased. The colony counts from all other non-effective plants were not significantly different from those of the negative controls ($P > 0.05$) indicating that there was no inhibition. Colony counts decreased as methanol extract titres for the root and bark increased**.** Mean colony represents average colony counts for the duplicate sets at 100 µl of *W. ugandensis* extracts against a clinical *K. pneumoniae* isolate, K_1, and their standard errors (SE) (Figure 2)**.** Inhibition was similar for the other clinical isolates. There were no colonies at titres > 42 µl for the bark and root extracts. Therefore, the MIC for *W. ugandensis* methanolic root and bark extracts was 42.5 µg/ml. Consequently, the highest sublethal extract concentration for both the root and bark was thus 32.5 µg/ml **(**Figure 2**).**

Table 4 indicates mean inhibition zones of 15 conventional antibiotics against a *S. typhi* isolate, S3, before and after exposure to sub-optimal concentration (32.0 µg/ml) of *W. ugandensis* methanolic root extract. All the other clinical isolates had a similar trend. Bacterial strains exposed to a sub-optimum concentration of *W.ugandensis* methanolic extracts from the root and bark exhibited no significant differences in their antibiotic susceptibility profiles before and after exposure (t-test, $p > 0.005$).

4.0 Discussion

This study reports efficiency of extracts from *W. ugandensis* among the 27 Gram-negative bacterial isolates analysed. Varied antibacterial activity between the root/bark and leaf extracts observed are attributed to the different secondary metabolites found in these plant parts. The fact that the study also reports resistance by these isolates to different groups of antibiotics show that our extracts are a potential to offering effective, less expensive treatment options to diseases caused by these Gram-negative MDR strains. Characterization of specific anti-bacterial substances from the root and bark extracts will be an important step for potential development of novel anti-microbial agents against related strains.

On the basis of zones of inhibition, our results also revealed that extracts from five plants inhibited the growth of *E. coli* strain J53 indicating that such plants have a potential as anti-microbial agents and may require further testing for drug development. Other studies on medicinal plants (Thakurta *et al*., 2009; Osuga *et al*., 2006) have also realized a potential for these plants in the control of bacterial strains that exhibit resistance to conventional antibiotics.

The finding in our study that the MDR strains were only inhibited by methanol preparations from root and bark of *W. ugandensis* indicate that the extracts provide potential sources of effective anti-microbial compounds for further development of alternative safe anti-microbial products in form of chemotherapeutic agents or antiseptics. *W. ugandensis* has been used traditionally to treat bacterial infections and previous studies have reported its anti-bacterial activity. Crude extracts and purified compounds from *W. ugandensis* have been

reported to be effective against *Mycobacterium tuberculosis*, *E. coli* and *Vibrio cholerae* (Mbwambo *et al.,* 2009). Wube *et al.* (2005), reported antimycobacterial activity of dichloromethane stem bark extracts against *M. aurum*, *M. fortuitum*, *M. phlei* and *M. Smegmatis.* The active constituents showed MIC values ranging from 4 to128 µg/ml compared to the antibiotic drugs ethambutol (MIC range from 0.5 to 8 µg/ml) and isoniazid (MIC range from 1 to 4 µg/ml). This closely corroborate with the results of our study in which methanolic root and bark extracts showed strong anti-bacterial activity with an MIC value of 42.5 µg/ml. Such an MIC indicates antibacterial potential comparable to that of conventional antibiotics like gentamicin (Tadeg *et al*., 2005).

Phytochemical investigations of this plant have identified drimane-type sesquiterpenoids as the main active components. Published work (e.g. Brooks and Draffan, 1969; Wube *et al*., 2005), has reported the presence of sesquiterpenoids in heartwood (e.g. ugandensolide, ugandesidial, warburgin and warburgiadione) and stem bark (e.g. muzigadiolide, deacetylugandensolide, cinnamolide, mukaadial, ugandensidial, muzigadial and waburganal). Flavonol glycosides and monoterpenes have been detected from the leaves of this plant (Manguro *et al.* 2003). Based on this information, this study did not carry out any phytochemical analysis but rather screened the crude extracts for their antimicrobial potential.

It is known that, in general, plant extracts are more effective against Gram-positive than Gram-negative bacteria (Suffredini *et al.,* 2006). However, our study showed that the Gram-negative organisms used were sensitive to the plant extracts even at low MICs. Therefore, these findings further support the idea that *W. ugandensis* may be an important source of compounds with broad-spectrum anti-microbial properties.

Results obtained with extracts from other plants including *T. brownii, A. indica, C. anisata, S. henningsii, Z. chalybeum, A. remota, T. indica, R. communis* and *A. Secundiflora* were significant in their own accord. From these results we note that although they are traditionally used in management of bacterial diseases, not all prescribed anti-bacterial medicinal plants may be effective against multi-drug resistant Gram-negative pathogens. The results may also suggest that the resistance observed for other plant extracts may partially be due to efflux pumps in the bacteria. Many efflux systems are multidrug transporters capable of expelling a wide spectrum of structurally unrelated drugs and anti-microbials, thus contributing significantly to bacterial multi-drug resistance (Veen and Konings, 2007).

The contrasting results of this study with those published by other investigators on anti-microbial activities of other plant extracts apart from *W. ugandensis* could be due to methodological discrepancy. For instance, Thakurt *et al*., (2007) reported significant antibacterial activity of *Azadirachta indica* leaf extracts against multi-drug-resistant *Vibrio cholerae*, while Tajamul *et al*. (2010) reported good anti-bacterial activity of *Ricinus communis* against pathogenic bacterial strains like *K. pneumoniae* and *E. coli.* This could be attributed to differences in bioassay techniques used and diluents used for extraction and elution. Thakurt *et al*. (2007) and Tajamul *et al*. (2010) used the disc diffusion technique to assay the anti-microbial activity of the plant extracts. Such standard anti-microbial susceptibility testing methods like the agar diffusion and Kirby-Bauer may result to misinterpretation of results especially for extracts with low anti-microbial activity or in cases where the active ingredient(s) may irreversibly bind to the paper discs (Das *et al.,* 2010). To overcome these drawbacks, the anti-microbial activity of the crude plant extracts was detected by performing viable counting following direct inoculation. This method allows for maximum action by the extract on the bacterial cells hence generating more reliable data. Besides the methodological approaches used by various investigators, the contrasting results could also be attributed to the locality of plant species, storage conditions, test strains used, and lack of appropriate positive and negative controls in some of these studies.

However, this study does not rule out the potential of such plants as agents for the treatment of infections caused by other agents. Besides, the plants may be effective while in combination with other medicinal plants or in combination with conventional antibiotics but further research to support this opinion is needed. Furthermore, some plants found not to have inhibitory effects *in vitro* may undergo enzymatical processing to make them effective *in vivo.* Others may become more potent in the presence of other components involved in immune response (Gonzalez-Lamothe *et al*., 2009).

In a previous study by McMahon *et al*. (2007), the continued use of tea tree oil as a herbal remedy in sub-optimal concentrations was shown to elicit the emergence of resistance to conventional antibiotics. The current study shows otherwise in which exposure of the MDR strains to sub-optimal extract concentration of

W. ugandensis extracts had no effect on their susceptibility to conventional antibiotics and conjugative efficiency. Very little has been done to screen other herbal extracts for similar effects and it is not known if such herbal-elicited antibiotic resistance is transferable via conjugation or whether the presence of herbal agents stimulate or inhibit conjugal transfer of antibiotic resistance. Therefore, this study indicates the safety of using the plant extracts as herbal remedies in treatment of bacterial infections as they do not increase the selective pressure that potentiate bacterial resistance. In addition, this study provides useful data that will shed light on the use of the selected herbal extracts as alternatives to conventional anti-microbials.

5.0 Conclusion

The 27 under investigation strains were multi-drug resistant. Methanol extracts from root and barks of *W. ugandensis* exhibited better anti-microbial properties than other plant extracts tested. The study established that the MIC of methanol extracts from the root and bark of *W. ugandensis* against the MDR gram-negative bacterial strains was 42.5 µg/ml. Exposure of test strains to sub-lethal extract concentrations of the effective extracts of *W .ugandensis* does not induce resistance to conventional antibiotics.

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References

Bekele, T. (2008). Antidiabetic activity and phytochemical screening of crude extracts of Ajuga remota. Masters Thesis. Addis Ababa University, Ethiopia.

Brooks, C., J. W. and Draffan, G. H. (1969). Sesquiterpenoids of Warburgia species-II ugandensolide and ugandansidial (cinnamodial). *Tetrahedron.* **25,** pp 2887-2898.

Daniyan, S. Y. and Muhammad, H. B. (2008). Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. *African Journal of Biotechnology,* **7** (14), pp 2451-2453.

Das, K., Tiwari, S. K. R. and Shrivastav, K. D. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*. **4**(2), pp 104-111.

Gonzalez-Lamothe, R., Mitchell G., Gattuso M., Diarra S. M., Malouin, F. and Bouarab, K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. *International Journal of Molecular Sciences*. **10**, pp 3400-3419.

Haraguchi, H. (1998). Mode of action of antimicrobial substances in plants. *Journal of Recent ResearchDevelopments in Agricultural and Food Chemistry.* **2** (1), pp 258-268.

Jaki, B. U. Franzblau, S. G., Chadwick, L. R., Lankin, D. C., Zhang ,F., Wang Y. and Pauli G. F. (2008). Purity-activity relationships of natural products: The case of anti-TB active ursolic acid. *Journal of NaturalProducts.* **71**, pp 1742-1748.

Krishnaiah, P. Devkota., Etienne, T. and Norbert, S. (2007). Anthraquinones from the stem bark of S*tereospermum zenkeri* with antimicrobial activity. *Journal of Phytochemistry*. **68**(11), pp 1595-1599.

Lewis, K. and Ausubel, F. M. (2006). Prospects of plant derived antibacterials. *Jouranal of Natural Biotechnology.* **24**, pp 1504-1507.

Manguro, L. O. A., Ugi, I., Lemmen, P. and Hermann, R. (2005). Flavonol glycosides of *Warburgia ugandensis* leaves. *Journal of Phytochemistry*. **64**, pp 891-896.

Mascolo, N., Izzo A. A., Borrelli F., Capasso, R., Di Carlo, G., Sautebin, L. and Capasso, F. (2004). Healing powers of aloes. **In**: Reynolds, T. (Editor). Aloes: The genus Aloe. CRC Press, Boca Raton, Florida, United States. pp. 209–238.

Mbwambo Z. H., Erasto P., InnocenT E. and Masimba P. J. (2009). Antimicrobial and cytotoxic activities of fresh leaf extracts of *Warburgia Ugandensis. Tanzania Journal of Health Research.* **11**(2).

McMahon, S. A., Blair, S. I., Moore, E. J. and McDowell, A. D. (2007). Habituation to sub-lethal concentrations of tea tree oil (Melaleuca alternifolia) is associated with reduced susceptibility to antibiotics in human pathogens. *Journal of Antimicrobial Chemotherapy.* **59**, pp 125–127.

Michel, M. and Gutmann, L. (1997). Methicillin-resistant *Staphylcoccus aureus* and vancomycin-resistant *Enterococci:* Therapeutic realities and possibilities. *Lancet.* **349**, pp 1901–1906.

Miyasaki1, Y., Nichols1 W. S., Morgan1, M. A., Kwan1, J. A., Van Benschoten, M. M., Kittell, P. E. and Hardy1, W. D. (2009). Screening of Herbal Extracts against Multi-Drug Resistant *Acinetobacter baumannii. Jouranal of Phytotherapy research*. **DOI**: 10.1002/ptr.3113

Olila, D., Olwa, O. and Opuda-Asibo, J. (2001). Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. *Journal of African Health Science*. **1**(2), pp 66–72.

Raji Y, Oloyo, A. K. and Morakinyo, A. O. (2006). Effect of methanol extract of *Ricinus communis* on reproduction of male rats. *Asian Journal of Andrology.* **8** (1), pp 115 – 121.

Somchit, M. N., Reezal, I., Nur I. E. and Mutalib, A. R. (2003). In vitro Antimicrobial Activity of Ethanol and Water Extracts of *Cassia alata. Journal of Ethnopharmacology.* **84**, pp 1-4.

Suffredini, I. B., Paciencia, L. B., Nepomuceno, D. C., Younes, R. N. and Varella, A. D. (2006). Antibacterial and cytotoxic activity of Brazilian plant extracts – Clusiaceae. Mem Inst Oswaldo Cruz. **101**, pp 287–290.

Tadeg H., Mohammed E., Asres K. and Gebre-Mariam T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology.* **100,** pp 168-175.

Thakurta, P., Bhowmik, P., Mukherjee, S., Hajra, T., Patra, A., Bag, P. (2009). Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India *Journal of Ethnopharmacology*. **111**(3), pp 607-612.

Veen, H. W. and Konings, W. N. (2007). Drug efflux proteins in multidrug resistant bacteria. *Journal of Biological Chemistry.* **378**, pp 769–777.

Wube, A. A., Bucar, F., Gibbons S. and Asres, K. (2005). Sesquiterpenes from *Warburgia ugandensis* and their antimycobacterial activity. *Journal of Phytochemistry*. **66**(19), pp 2309.

Table 1: Plant species screened for their antimicrobial activity

Plant name	Plant family	Phytochemicals	Medicinal uses
Clausena anisata	Rutaceae	Flavonoids, tannins, saponins f	Stomach aches, whooping cough, malaria [f]
Aloe secundiflora	Aloceae	Phenolic compounds: chromone, anthraquinone or anthrone derivatives [k]	Disinfectant, treatment of Pneumonia, malaria, chest pains [k]
Strychnos henningsii	Loganiaceae	Alkaloids, saponins [j]	Mouth antiseptic, wounds, gastrointestinal disorders, malaria [j]
Ajuga remota	Lamiaceae	Flavonoids, tannins and sterols [i]	Bacterial infections [i]
Ricinus communis	Euphorbiacea	Alkaloids, saponins, tannins, phenolic [h]	Stomach aches, diarrhoea**,** boils, burns, worms, venereal diseases [h]
Zanthoxylum chalybeum	Rutaceae	Alkaloids and saponins [g]	Bacterial infections, malaria, colds, cough, toothache [g]
Tamarindus indica	Caesalpinaceae	Alkaloids, flavonoids, saponins, tannins [f]	Treatment of scabies, boils, diarrhoea, dysentery [f]
Warburgia ugandensis	Canellaceae	Sequiterpene dialdehydes: warburganal, muzigadial, polygodial [e]	Constipation, treatment of bacterial infections, stomach aches, coughs, muscle pains, weak joints, body pains [e]
Terminalia brownii	Combretaceae	Tannins, saponins, flavonoids, alkaloids [c]	Diarrhoea, stomach ache, fevers, hepatitis, colds [d]
Azadirachta indica	Maliaceae	Tannins, saponins, flavonoids, terpenoids, alkaloids [a]	Abdominal colic, treatment of wounds, boils, scabies, malaria, rheumatism [b]

[a]Krishnaiah *et al.* 2008; [b]Thakurta *et al.* 2009; [c]Osuga *et al*. 2006 ; [d]Zakaria *et al*., 2007; [e]Haraguchi, 1998; [f]Daniyan and Muhammad, 2008; [g]Olila *et al.* 2001; [h]Raji *et al*., 2006; [i]Bekele, 2008; [j]Michel *et al*. 1999; [k]Mascolo *et al.,* 2004.

Table 2: Susceptibility of the 27 clinical Gram-negative strains to 15 conventional antibiotics

Antibiotics	Test Strains																									
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	K_1	K_2	K_3	K_4	K_5	K_6	K_7	S_1	S_2	S_3	S_4	S_5	S_6	S_7	P_1	P_2	P_3	P_4	P_5	P_6
NOR	S	R	R	S	S	S	R	S	R	R	R	R	S	R	S	R	S	S	R	S	S	S	S	S	S	S	S
SMX	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	S	R
NA	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	S	S	S	R	S	S	R	R	S	S	R	R
TM	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C	R	R	R	R	R	S	R	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
N	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	S	S	S	S
S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S
MN	R	R	R	R	S	R	I	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R
CXM	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	S	S	R	R
CFM	R	R	R	R	R	R	R	S	R	S	R	R	S	R	S	R	S	S	S	S	S	S	R	S	S	R	R
CRO	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	S	S	S	R	S	R	R	S	S	S	R
ATM	R	R	R	R	R	R	R	S	R	R	R	R	S	R	S	R	S	S	S	S	R	S	S	S	S	R	R
FOX	R	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	R	S	S	S	R	R	S	S	R	R
AUG	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

R: Resistant (intermediate phenotypes were recorded as resistant); S: Sensitive; **Antibiotics**: NOR: Norfloxacin; SMX: Sulfamethoxazole; NA: Nalidixic acid; TM: Trimethoprim; C: Chloramphenical; N: Neomycin; S: Streptomycin; MN: Minocycline; CXM: Cefuroxime; CRO: Ceftriaxone; ATM: Aztreonam; FOX: Cefoxitin; AUG: Augmentin; AMP: Ampicilin. Gram-negative clinical isolates of the species: *E. coli:* E_1-E_7; *K. pneumoniae:* K_1-K_7; *P. aeruginosa:* P_1-P_6; *S. typhi:* S_1-S_7

Table 3: Diameter of inhibition zones at 100 µl of the 10 plant extracts against E. coli J53

Plant	Plant Part	Diameter of Inhibition Zones (mm)	
		Methanol Extracts	Water Extracts
W. ugandensis	Root	30*	22
	Bark	28*	20
	Leaf	16	14
T. brownii	Root	16	17
	Bark	26*	22
	Leaf	27*	23
A. indica	Root	17	16
	Bark	25*	22
	Leaf	24*	21
C. anisata	Root	23	21
	Bark	25*	20
	Leaf	15	14
S. henningsii	Root	24*	20
	Bark	21	20
	Leaf	17	12
Z. chalybeum	Root	14	10
	Bark	17	10
	Leaf	15	11
A. remota	Root	15	10
	Bark	17	11
	Leaf	16	10
T. indica	Root	15	11
	Bark	16	11
	Leaf	17	10
R. communis	Root	11	10
	Bark	10	10
	Leaf	11	11
A. secundiflora	Leaf	10	10

Table 4: Mean inhibition zones of 15 conventional antibiotics against a S. typhi isolate, S3, before and after exposure to sub-optimal concentration of W. ugandensis methanolic root extract

	Antibiotics														
	NOR	SMX	NA	TM	C	N	S	MN	CXM	CFM	CRO	ATM	FOX	AUG	AMP
Mean zones of isolate S_3 before exposure	23	11	13	10	12	15	9	15	12	18	14	14	15	9	8
Mean zones of isolate S_3 after exposure	22	12	14	9	13	16	10	16	13	17	15	16	16	8	6
t-test (p value)	0.03	0.14	0.27	0.13	0.96	0.21	0.45	0.35	0.56	0.92	0.91	0.61	0.9	0.17	0.16

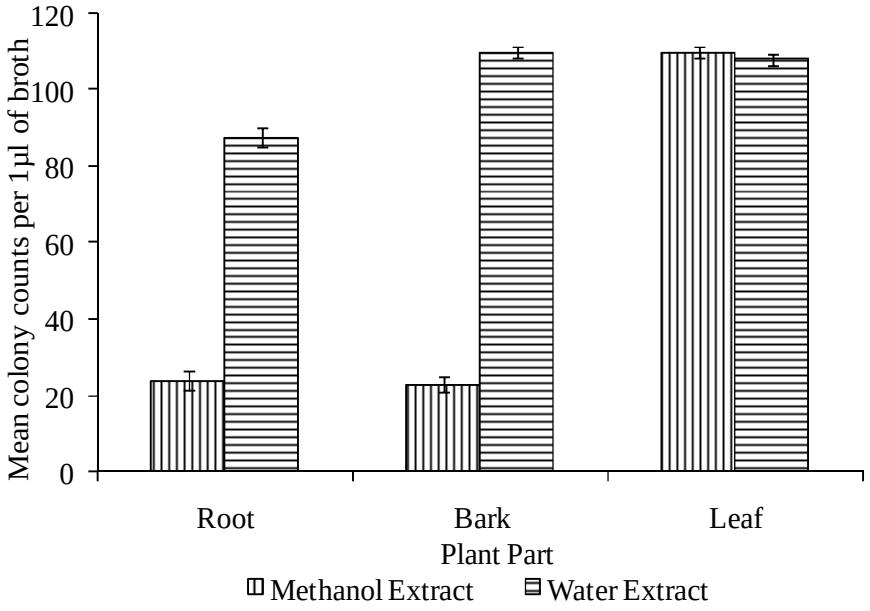

Figure 1: Differences in the effectiveness of water and methanol extracts of W. ugandensis against a clinical S. typhi isolate, S_1

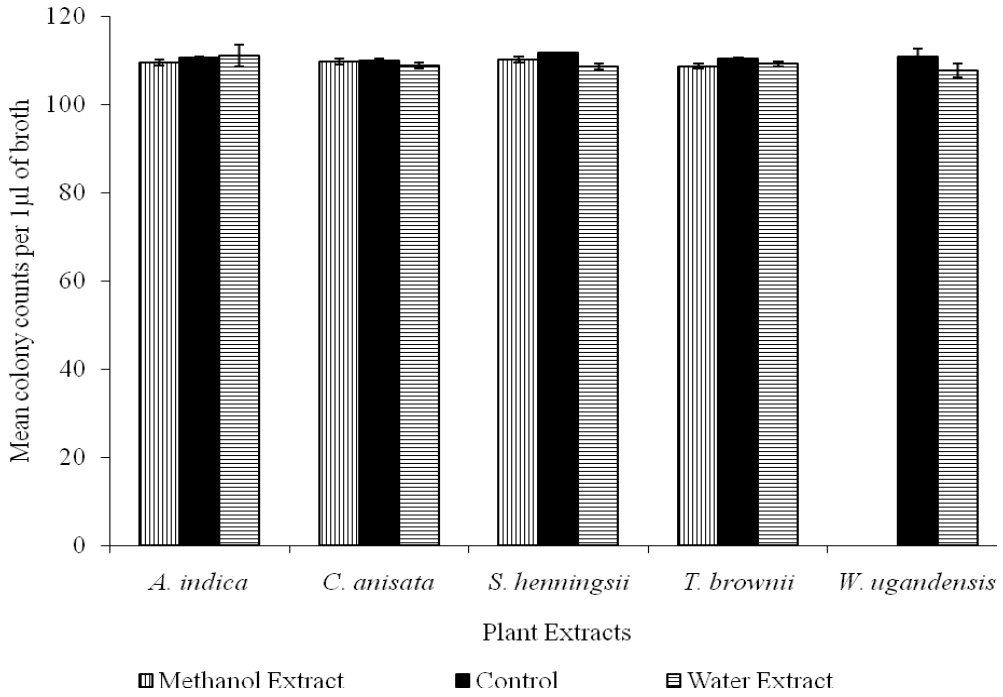

Figure 2: Inhibitory characteristics of the total extracts from five plants against a clinical E. coli isolate, E_1

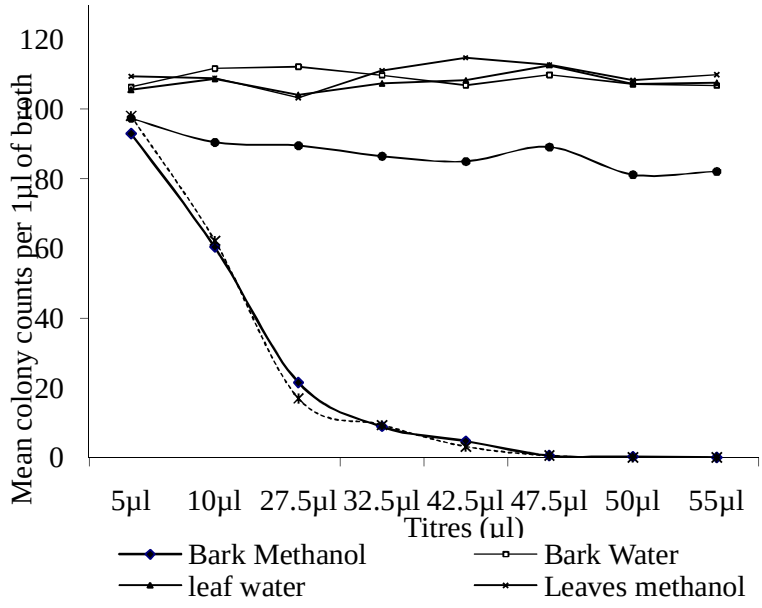

Figure 3: Growth characteristics of a clinical K. pneumoniae isolate, subjected to different titres of W. ugandensis extracts