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Antibacterial Potency of Ethnobotanical Plants as Alternative Remedies to Curtail Nosocomial Infections: A Case Study of Five Native Plants in Kenya

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors fully participated in designing the study, performance of the experimental work, analysis of the obtained data and preparation of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The current study was done to investigate the antibacterial potentiality of five ethnobotanical plants used in Kenya as remedies in the treatment against selected nosocomial infectious bacteria.
Materials: The plant samples were collected in the months of June and July. The samples were identified in the Department of Biological Sciences. Voucher specimens were prepared and stored in the department of biological sciences herbarium.
Methodology: The plant leaves samples were dried, powdered and extracted using methanol and water in the ratio 9:1. The samples were vacuum filtered using Whatman no.1 filter paper. The solvents were removed using a rotar vapor with a water bath at 40ºC. The bioassays were done

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using well diffusion method followed by incubation at 37ºC for 24hrs and then the zones of inhibition diameters measured with the aid of a ruler in millimeters (mm).
Results: From the results *Tetradenia riparia* had the best zones of inhibition as compared to all of the other four plants used in the study. *Tetradenia riparia* highly inhibited *Staphylococcus epidermidis* 27.67±0.333 mm, followed by *Bacillus cereus* 18.00±0.577 mm and *Escherichia coli* with an inhibition zone of 13.33±0.333 mm. Penicillin which was used as the positive control inhibited the growth of all the microorganisms while dimethylsulfoxide (DMSO) did not inhibit the growth any of the microorganisms.
Conclusion: The results obtained in this research may be an indication that the five plants can be good sources for antiseptic solutions and new drugs in the fight against hospital acquired infections. However, further research needs to be done to isolate the pure compounds from the plants, study their structural elucidation and their mode of action. Formulations of solutions with aseptic activity need to be done especially on *Tetradenia riparia* which demonstrated the highest activity against the microorganisms.

Keywords: Plants; nosocomial infections; bacterial; ethnobotany; alternative medicine.

1. INTRODUCTION

Nosocomial infections are defined as the infections acquired by a patient or a health professional in the hospital or any other health care setting. Nosocomial infections have been known to cause illness, prolonged hospital stay, excess costs, disability and even death [1,2,3].

Plants have been used for a long period of time to maintain human health especially in developing countries. The knowledge on the use of medicinal plants has been passed from generation to generation by the old grandmothers and grandfathers leading to accumulation of this information for thousands of years. The quick civilizations and western education in African countries however has become a threat to this process of knowledge transfer therefore, creating the need for documentation of information on ethnobotany. The emergence of drug resistant microorganisms has also increased, therefore, creating the need for continued search for new antibiotics [4].

According to Gislene [5], WHO recommends medicinal plants as the best source to obtain a variety of drugs. Despite the great achievements made in the search for new antibiotics, disease infections still remain to be a major threat to human health [6]. There is renewed interest in the use of plants as therapeutic agents due to the belief that green medicine is save, cheap and dependable as compared to allopathic drugs [7]. Plants have been used for their chemotherapeutic effects and as template molecules for synthetic or aliphatic drugs synthesis [8]. The medicinalvalue of plants is associated with the presence of important pharmacological compounds commonly known as phytochemicals which have been found to have little purpose in the biological activities and also nutritional value of plants but research has proved them to have great medicinal importance. The production of these compounds by plants is as a result of protection response of the plant against pathogens [9,10]. It is estimated that about 50,000 to 70,000 plant species have medicinal values [11]. Globally millions of people from developing countries use medicinal plants as a source of basic medical health care. It is also estimated that about 80% of people living in developing countries and 40% of those living in developed countries use plants as a source of medicine [12,13,14].

The plant *Warburgia ugandensis* is commonly known as Uganda green heart. The plant belongs to the family Canellaceae. The plant is natively found in Africa. *Warburgia ugandensis* is ever green and resistant to insect attack [15]. The plant is used by the Tugen community in Kenya for the treatment against visceral leishmaniasis [16]. The back of the plant is chewed for the treatment of stomach problems, tooth ache, muscle pains and constipation. The decoction of the plant roots is used in the control of diarrhea [17]. Previous studies done on *Warburgia ugandensis* have shown the plant to have great antimicrobial activity [18]. *Acacia mearnsii* belongs to the family fabaceae. The plant is common in lowlands, heavy woodland, open forest and cleared lands. The plant is used ethnobotanically for the treatment of microbial infections [19]. *Bridelia micrantha* bark decoction is taken by the Zulu community as an emetic and in east Africa for the treatment of stomach ache,

tape worm infections, diarrhea and as tonics for children [20].

The plant *Tetradenia riparia* is commonly known as ginger bush. The plant belongs to the *Lamiaceae* family. It is aromatic tall shrub growing up to 3-5 m in height. The plant is slightly succulent with irregular branch pattern. The plant stems are brown and smooth, except for the younger portions which have glandular hairs and a ruby tinge. The surfaces of the leaves are also covered with glandular hairs making them slightly sticky to the touch. The leaves of the plant are brightly green and slightly heart shaped with an irregularly margin and bluntly toothed. It is used ethnobotanically in the treatment against malaria and dengue fever, cough, respiratory problems, stomach ache, diarrhea, dropsy, angina pectoris, fever, yaws, headache, toothache and as an antiseptic [21,22]. *Justicia flava* (Acanthaceae) is used traditionally in the treatment of cough, fever, paralysis, epilepsy, spasm, convulsion and diarrhea [23].

Nosocomial infections remain to be a problem in the health of human beings with approximately 5 to 15% of all patients in regular wards and more than 50% of the patients in the intensive care units in the developed countries affected. The lack of scientific data on theextend of nosocomial infections in developing countries complicates the whole matter hence creating a problem in handling these hospital acquired infections. The lack of data in these developing countries is attributed to deficiencies in both social and economic status [24,25]. Nosocomial infections in developing countries remain a serious problem on which very little attention is paid to [26].

The increased cases of drug resistant microorganisms necessitates the need for the invention of new antibiotic compound sources to fight against these microorganisms which mostly cause nosocomial infections hence increase mortality in hospitalized patients [27]. According to Rojas et al. [28], the use of alternative antibiotics other than the currently used ones remains to be a better option in the fight against emergence of drug resistance microorganism. Plants species have been believed to be more active than the currently used commercial antibiotic; however, according to Fabricant [29], some of these traditional ethnobotanicals have not yet been documented.

The current study demonstrates the potentiality of five ethnobotanical plants used in Kenya to treat against selected nosocomial infectious bacteria. The plants used in this study include *Tetradenia riparia, Warburgia ugandensis, Brideliami crantha, Acacia mearnsii* and *Justicia flava* were selected based on their ethnobotanical use in Kenya.

2. MATERIALS AND METHODOLOGY

2.1 Sample Collection and Preparation

The plant samples leaves were randomly harvested by Ngule Chrispus in the month of June and July 2014 from the natural forest around University of Eastern Africa, Baraton. The samples were identified by taxonomist Mr Joel Ochieng from the Department of Biology, University of Eastern Africa, Baraton. Voucher specimens were prepared and stored in the biology department herbarium. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks and then ground into fine powder. The powdered samples were stored in transparent polythene bags.

2.2 Extraction Procedure

From the powdered samples 100 g were placed in 500 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours with frequent shaking for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40ºC. The residue was obtained and stored at 4ºC for the study [10].

3. BIOASSAY STUDY

3.1 Bacteria Source and Media Preparation

The bacteria (*B. cereus, E. coli* and *S. epidermidis*) used in the study were commercial pure cultures from Carolina biological supply company (USA). The colonies for use in the study were obtained from the pure cultures and then transferred into blood agar plates. The blood agar media was prepared according to the manufacturer's instructions (Himedia, India). The plates were sterilized by the use of an autoclave

at 121ºC. Approximately 20 ml of the prepared media was poured into the sterilized plates and then the surface of the media was flamed using a Bunsen burner flame to remove air bubbles. The Mueller Hinton broth was prepared according to the manufacturer's instructions (Himedia, India). About 5ml of the broth was transferred in to sterile test tubes. The transfer of the media to the plates and test tubes was done under sterile biohazard hood. The plates were then incubated at 37ºC for 24 hours to check on their sterility [28].

3.2 Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard [30,31]. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Approximately 3-5 identical colonies of each bacterium were taken from a blood agar plate (Himedia) culture using a sterile swab into Mueller Hinton broth (Himedia). The broth culture was incubated at 37ºC for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with UV spectrophotometer to 0.132Aº at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^8$ CFU/ml.

3.3 Preparation of the Extract Concentrations and Antibiotic

Extracts stock solutions were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of penicillin in 1ml of sterile distilled water. DMSO (100%) served as a negative control.

3.4 Determination of the Bioactivity of the Extract

Mueller Hinton agar plates were prepared as per the manufacturer's instructions (Himedia, India). The media was measured into a conical flask and double distilled water added. The flask contents were slightly warmed to allow complete dissolving of the media in to the water. The media and the plates were sterilized in an autoclave at 121ºC for 15 minutes. The media was then poured into the plates. The plates were flamed on the surface using a non-luminous flame to remove air bubbles. The cork borer was sterilized using a non-luminous flame. The plates and all the equipment's to be used for the experiment were transferred in to a biohazard hood. The germicidal lamp was put on for 30 minutes to sterilize the surface of the plates and other equipments. The bacterial suspension was smeared on the media and five wells with a diameter of 6mm each were drilled on each agar plate using a cork borer. Three of the wells were filled with 0.1 ml of the 500 mg/ml of the extracts. The other wells were filled with 0.1 ml 0f 500 mg/ml of penicillin and 0.1 ml of 100% DMSO positive and negative controls respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37ºC for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler [28].

4. RESULTS

From the results (Table 1) all the plants inhibited all the selected microrganisms used except *Justicia flava* which did not inhibit the growth of *Staphylococcus epidermidis*. *Tetradenia riparia* (Fig. 1) showed the best zones of inhibition as compared to all of the other four plants used in the study. The plant *Tetradenia riparia* inhibited *Staphylococcus epidermidis* the most, with an inhibition zone of 27.67±0.333 mm, followed by *Bacillus cereus*18.00±0.577 mm and *Escherichia coli* with an inhibition zone of 13.33±0.333 mm. The plant *Warburgia ugandensis* showed the same tread as *Tetradenia riparia* in terms of the zones of inhibition with *Staphylococcus epidermidis* having the highest zone of inhibition (19.33±0.333 mm) followed by *Bacillus cereus* 17.00±0.882 mm and *Escherichia coli* within inhibition zone of 11.67±0.333 mm. Similar trend (Table 1) was also observed in the zones of inhibition caused by the *Brideliami crantha*. The plant *Acacia mearnsii* inhibited the growth of *Bacillus cereus* the most with an inhibition zone 20.33±0.333 mm, followed by *Staphylococcus epidermidis* 17.00±0.577 mm and *Escherichia coli* 10.33±0.33 mm. *Jasticia flava* inhibited the growth of *Bacillus cereus* most and followed by *Escherichia coli* 9.00±0.00 mm but did not inhibit the growth of *Staphylococcus epidermidis*. Among all the plants used in this study *Justicia flava* showed the weakest antibacterial activity.

Table 1. Antibacterial activity of *Tetradenia riparia*, *Wubergia ugandensis*, *Brideliami crantha*, *Acacia mearnsii* and *Justicia flava* against selected nosocomial pathogenic microorganisms

Bacteria	TR zones of inhibition (mm)	WU zones of inhibition (mm)	BM zones of inhibition (mm)	AM zones of inhibition (mm)	JV zones of inhibition (mm)	Penicillin zones of inhibition (mm)	DMSO zones of inhibition (mm)
Escherichia coli	13.33±0.333	11.67±0.333	12.67±0.577	10.33±0.333	9.00±0.000	31.33±0.333	0.00±0.000
Staphylococcus epidermidis	27.67±0.333	19.33±0.333	13.00±0.333	17.00±0.577	0.00± 0.000	26.67±0.333	0.00±0.000
Bacillus cereus	18.00±0.577	17.00±0.882	12.67±0.577	20.33±0.333	10.33+0.577	23.67±0.882	0.00±0.000

Key: TR= Tetradenia riparia, WU= Wubergia ugandensis, BM= Brideliami crantha, AM= Acacia mearnsii and JV= Justicia flava

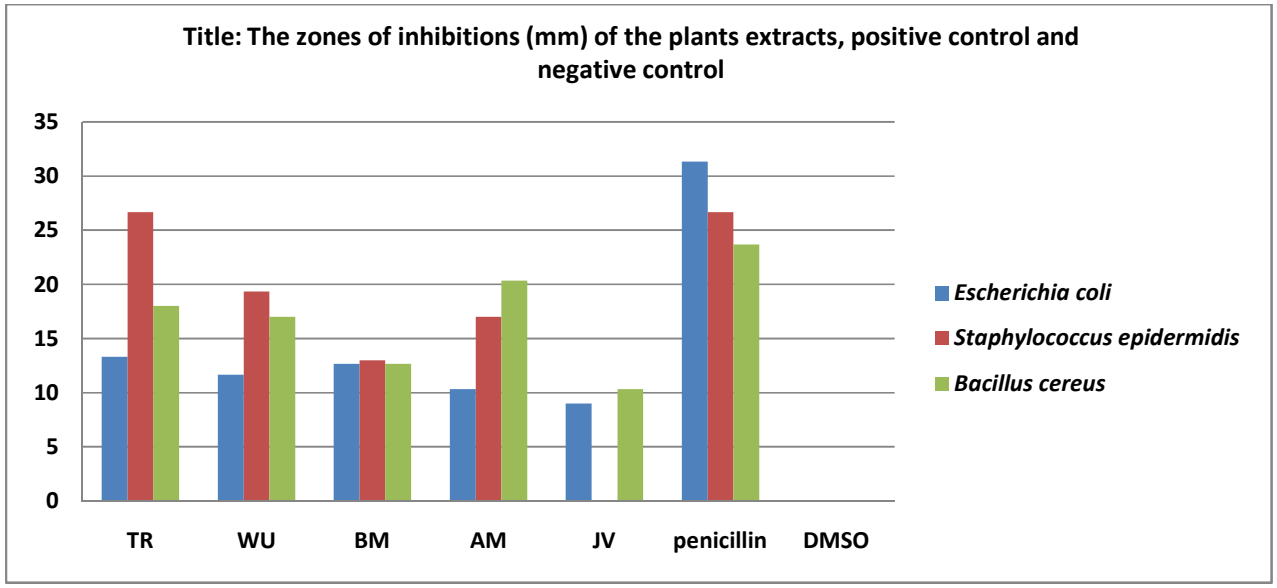

Fig. 1. Comparison on the zones of inhibition caused by the plants

5. DISCUSSION

The inhibition of the plant extracts against the used microorganisms is noteworthy since they have been found to be among the commonly isolated bacterial organisms in nosocomial infections, which include surgical site infections (SSIs), urinary tract infections (UTIs), pneumonias and blood stream infections. *Escherichia coli* is the most abundantly isolated in SSIs and UTIs [32,33]. *Escherichia coli* has shifted from the easily treatable strains to highly complicated and strongly resistant strains [34]. This trend has created the need for new therapeutic compounds to curb the menace. The inhibition of *Bacillus cereus* by the plants extracts is noteworthy since the bacterium has been known to cause respiratory tract infections and blood stream infections among immune-compromised patients [35]. According to the study conducted by Lequin et al. [36], *Bacillus cereus* is a potential causative agent of severe late onset hemorrhagic meningoencephalitis in preterm infants.

Staphylococcus epidermidis is one of the most common multi-resistant nosocomial pathogen. It is the most common nosocomial coagulase – negative staphylococci isolated from blood stream infections [37]. The bacterium is commonly known to cause infections of cerebrospinal shunts, prosthetic joints, peritoneal catheters, prosthetic cardiac valves and vascular grafts [38,39]. *Staphylococcus epidermidis* is the most causative agent of bacteremia in most neutropenic cancer patients [40].

This study is inconformity to the previous researches in which the plant *Tetradenia riparia* hydromethanolic fractions inhibited the growth of all the microorganisms tested [41,42,43], however the current study is different since we present the use of a solvent system in the extraction of the active compounds from the plant. The study is also in conformity with that done by Njau et al. [33], in which the methanol extract inhibited the growth of *Escherichia coli* 10.66±0.33 mm and *S. aureus* 21.66±0.33 mm. According to the same study the plant leaves water extract inhibited the growth of *Escherichia coli* and *S. aureus,* however the zones of inhibition obtained in the current study are slightly higher as compared to those obtain by Njau [33]. This might be attributed to the difference in the solvent used since in the current study a solvent system of methanol and water was used to extract the compounds from the plant leaves. Antibacterial activity of *Warburgia ugandensis* observed in the current study is consistent with what was observed by Mbwambo et al. [44], in which the plant's leaves ethanol extract was found to inhibit the growth of *Escherichia coli*, *Staphylococcus* sp and *Bacillus cereus*. The current study is however different in that the methanolic water extract used had higher zones of inhibition than those observed in the study. The antibacterial activity of *Brideliami crantha* observed in the current study is also consistent with what was observed by Piéboji et al. [45] in which the bark of the plant was found to have antibacterial activity against the tested microorganisms, however the current research is different since it reports the antibacterial activity of the plant leaves. The observation made on the antibactetial activity of *Acacia mearnsii* is in line with what was observed by Olajuyigbe and Afolayan [46], in which the plant inhibited the growth of *Bacillus pumilus, Escherichia coli* and *Staphylococcus aureus*. The current study is also in agreement with the previous studies which *Justicia flava* plant was found to have antibacterial activity [47], however, different since contrary to the study the current study does not report inhibition of *Escherichia coli*. This difference could be attributed to the type of solvent used and locality of the plant samples.

6. CONCLUSION

The antibacterial activity of the plants against the selected microorganisms isremarkable since the microorganism have been found to be major causative agents of nosocomial infections. However,additional work needs to be done to isolate the active compounds and to determine their structural elucidation. The action mode the active compounds also needs to be determined. Further research also needs to be done to fractionate the methanol water extracts from the plants in an attempt to purify the extracts further. Formulations of aseptic solutions need to be done especially on *Tetradenia riparia* which demonstrated the highest activity against the microorganisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nejad SB, Allegranzi B, Syed SB, Ellis B and Pittet D. Health care associated infection in Africa: A systematic review. Bull World Health Organ. 2011;89:757-765.
2. Bates DW, Larizgoitia I, Prasopa-Plaizier N, Jha AK. Global priorities for patient safety research. BMJ. 2009,338:b1775. Doi: 10.1136/bmj. B1775.
3. Allegranzi B, Pittet D. Preventing infections acquired during health care delivery. Lancet. 2008;372:1719-1720 DOI: 10. 1016/50140-6736(08)61715-8 PMID:19013310.
4. Cohen ML. Epidemiology of drug resistance: Implications for a post-antimicrobial era. Science. 1992;257: 1050-1055.
5. Gislene GF, Locatelli J, Freitas CP, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology. 2000;31:247-256.
6. Akyuz M, Onganer AN, Erecevit P and Kirbag S. Entimicrobial activity of some edible maushrooms in the eastern and souther Anatolia region of Turkey. Gazi University Journal of Science. 2010;23(2): 125-130.
7. Nair GB, Ramamurthy T, Bhattacharya SK, Dutta B, Takeda Y, Sack DA. Global dissemination of Vibrio parahaemolyticus serotype O3: K6 and its serovariants. Clin Microbiol Rev. 2007;20:39–48.
8. Ameyaw Y, Duker – Eshun G. The alkaloid contents of the ethno-plant organs of species in the eastern region of Ghana. Int. J. Chem. Sci. 2009;7(1):48-58.
9. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research. 2011;5(31):6697-6703.
10. Ngule CM, Swamy A and Obey JK. Phytochemical and bioactivity evaluation of Senna didymobotrya fresen Irwin used by the Nandi community in Kenya. International Journal of Bioassays. 2013; 2(7):1037-1043.
11. Kargroglu M, Cenkci S, Erliyaoglu N, Konuk M, Kok MS, Bagci Y. An ethnobotanical survey of inner west Anatolia, Turkey. Hum. Ecol. 2008;36:763-777.
12. Singh V. Ethnobotanical observations on Dards tribe of Gurez valley in Kashmir Himalaya. Ethnobiology in Human Welfere: abstracts of the fourth international congress of ethnobotany, Lucknow, Utter Pradesh, India. 1994; 316.
13. Adongo SO, Murungi J, Wanjau R and Ndengwa F. Analysis of selected essential elements of medicinal plants used in Chuka community, Tharaka Nithicounty, Kenya. The Scientific Journal of Science and Technology. Special issue: 87-94.
14. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD. International study of the prevalence and outcomes of infection in intensive care units. JAMA, 2009;302:2323-9. Doi:10.1001/jama. 2009. 1754. PMD:19952319.
15. Ngule MC, Ndiku MH, Ramesh F. Chemical constituents screening and *In vitro* antibacterial assessment of *Prunus africana* bark hydromethanolic extract. Journal of Natural Sciences. 2014;4(16): 85-90.
16. Pittet D, Allegranzi B, Sax H, Bertinato L, Concia E, Cookson B. Considerations for a WHO European strategy on health care associated infection, surveillance and control. Lancet Infect. Dis. 2005;5:242.
17. Rosenthal VD. Health-care – associtaed infections in developing countries. Lancet. 2011;377:186- 188.
18. Moreno HPR, Da Costa-Issa FI, Rajca-Ferreira AK, Periera MA, Kanek TM. Native Brazilian plants against nosocomial infections: A critical review on their potential and antimicrobial methodology. Curr. Top. Med. Chem. 2013;13(24):3040-78.
19. Rojas JJ, Ochao VJ, Ocampo SA, Munoz FJ. Screening for antimicrobial activity of

ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complementary and Alternative Medicine. 2006;6(2):1-6.

20. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Enviromental Health Perspectives Supplements. 2001;109:69-75.
21. Biruhalem T, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pacific Journal of Tropical Biomedicine. 2011; 370-375.
22. Donay JL, Fernandes P, Lagrange PH, Herrmann JL. Evaluation of the inoculation procedure using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System. Journal of Clinical Microbiology. 2007;45(12):4088-4089.
23. Sikka R, Mann JK, Vashist MG, et al. Prevalence and antibiotic sensitivity pattern of bacteria isolated from nosocomial infections in a surgical ward. Indian Journal of Clinical Practice. 2012; 22(10):519-525.
24. WHO. Prevention of hospital acquired infections. A Practical Guide (2nd edition). 2002;12.
25. Hsueh P, Chen M, Sun C, Chen W, et al. Antimicrobial drug resistance in pathogens causing nosocomial infections at University Hospital in Taiwan 1981-1999. Emerging Infectious Diseases. 2002;8(1):63-68.
26. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. Clinical Microbiology Reviews. 2010;23(2):382-398.
27. Lequin MH, Vermeulen JR, Elburg VMR, et al. *Bacillus cereus* meningoencephalitis in preterm infants. Neuroimaging characteristics. American Society of Nueroradiology. 2005;26:2137-2143.-250.
28. Archer GL. *Staphylococcus epidermidis and other coagulase-negative*. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. *S. epider-* 4th ed. New York: Churchill Livingstone. 1995;1777–84.
29. Raad I, Bodey G. Infectious complications of indwelling vascular catheters. Clin Infect Dis. 15:197-208 1992 1520756 West; 1986.
30. West P, Krismer M, Fischhut B. Recurrence of infection after revision of infected hip arthroplasties. J Bone Joint Surg Br. 7706354. 1995;77:307.
31. Koll BS, Brown AE. The changing epidemiology of infections at cancer hospitals. Clin Infect Dis. 1993;17(suppl 2):322–327.
32. Ndamane Y, Kambizi L, Songca SP, Oluwafemi OS. Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system. J. Med. Plants Res. 2013;7(37):2755-2760.
33. Njau EFA, Alcorn JM, Buza J, Chirino-Trejo M and Ndakidemi P. Antimicrobial activity of *Tetradenia riparia* (HOCHST.) lamiaceae, a medicinal plantfrom Tanzania. European Journal of Medicinal Plants. 2014;4(12):1462-1478.
34. Mbwambo ZH, Erasto P, Innocent E, Masimba PJ. Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and *Warburgia ugandensis*, Ugandan medicinal plants. Tanzania Journal of Health Research. 2009;11(2): 75-78.
35. Piéboji JG, Eze N, Djintchui NA, Ngameni B, Tsabang N, Pegnyemb DE, Biyiti L, Ngassam P, Shiro SK, Galleni M. The *In vitro* antimicrobial activity of some traditionally used medicinal plants against beta lactam resistant bacteria. J Infect Dev Ctries. 2009;3(9):671-680.
36. Olajuyigbe OO, Afolayan AJ. *In vitro* antibacterial and time-kill assessment of crude methanolic stem bark extract of *Acacia mearnsii*de wild against Bacteria in Shigellosis. Molecules. 2012;17:2103-2118.
37. Agyare C, Bempah SB, Boakye DY, Ayande PG, Adarkwa-Yiadom M, and Mensah KB. Evaluation of Antimicrobial and Wound Healing Potential of *Justicia flava* and *Lannea welwitschii.* Evidence-Based Complementary and Alternative Medicine. Article ID 632927. 2013;10.
38. Ngure KP, Ng'ang'a Z, Ingonga J, Geoffrey Rukunga G, Willy Kiprotich Tonui WK. *In vivo* efficacy of oral and intraperitoneal administration of extracts of *Warburgia ugandensis* (Canellaceae) in experimental treatment of old world *Cutaneous leishmaniasis* caused by Leishmania Major. African Journal of Traditional, Complementary, and Alternative Medicines. 2009;6(2):207–212.

39. Olila D, Odyek O, Asibo OJ. Antibacterial and antifungal activities of extrcats of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. Afr. Health Sci. 2001;1(2):66-72.
40. Wamalwa NL, Oballa P, Gicheru J. Genetic variation of *Waburgia ugandensis* in Kenya and implications for its cultivation. Kenya Forestry Research Institution (KEFRI), Nairobi; 2006.
41. Mwitari PG, Ayeka PA, Ondicho J, Matu NE, Bii CC. Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: *Withania somnifera, Warburgia ugandensis, Prunus africana* and *Plectranthus barbatus.* PLOS One. 2013;8(6):e6519. DOI:10.1371/Journal.
42. Codd LE. *The genus Tetradenia*. Flora of Southern Africa. 1985;28(4):113-116.
43. Ngule MC, Ndiku MH, Ramesh F. Chemical constituents screening and *in vitro* antibacterial assessment of *Prunus africana* bark hydromethanolic extract. Journal of Natural Sciences. 2014;4(16): 85-90.
44. Watt JM, Breyer-Brandwijk MG. The medicinal and poisonous plants of southern and eastern Africa. 2nd ed., E. and S. Livingstone Ltd., London; 1962.
45. Olajuyigbe OO, Afolayan AJ. Pharmacological assessment of the medicinal potential of *Acacia mearnsii*: antimicrobial and toxicity activities. Int. J. Mol. Sci. 2012;13:4255-4267.
46. Steenkamp V, Mokoele TL, Rensburg CEJ. Toxicity testing of two medicinal plants, *Brideliami crantha* and *Antidesma venosum*. The Open Toxicology Journal. 2009;3:35-38.
47. Agyare C, Bempah SB, Boakye YD, et al. Evaluation of antimicrobial and wound healing potential of *Justicia flava* and *Lannea welwitschii*. Evidence-based Complementary and Alternative. Article ID 632977. 2013;10.

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