



ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF SENNA DIDYMOBOTRYA LEAVES WATER EXTRACT AS AN ALTERNATIVE REMEDY TO CURB NOSOCOMIAL INFECTIONS

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Abstract: Nature is a paradise of medicinal solutions to all ailments affecting human beings through medicinal plants. Medicinal plants are being used widely to treat against the currently widespread strains of drug resistant microbes. 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 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. *Senna didymobotrya* is widely used traditionally to treat against various illnesses such as abdominal pains, stomach problems and as an anthelmintic. The current study was done to investigate the plants leaves water extract potency to curtail selected nosocomially infectious microorganisms. From the study, the plant was found to inhibit the growth of all the selected nosocomial infectious microorganisms. The antibacterial activity of the plant against the selected microorganisms is notable since the microorganisms have been found to be major causative agents of nosocomial infections. However, additional work needs to be done to isolate the active compounds, determine their structural elucidation and prepare aseptic solutions from the water plant's extract.

Key words: *Senna didymobotrya*, water, nosocomial infections, medicinal plants.

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]. 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 the extent 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 [4,5]. Nosocomial infections in developing countries remain a serious problem on which very little attention is paid to [6].

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 [7]. According to Rojas *et al.*, [8], 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 equally effective or even more active than the currently used commercial antibiotics; however, according to Fabricant [29], some of these traditional ethnobotanicals have not yet been documented.

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 leading to accumulation of this information for thousands of years. Civilization and western education in African countries however has become a threat to this process of knowledge transfer, 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 [10].

According to Gislene [11], 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 [12]. 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 [13]. Plants have been used for their chemotherapeutic effects and as template molecules for synthetic or aliphatic drugs synthesis [14]. The medicinal value 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 [15,16]. It is estimated that about 50,000 to 70,000 plant species

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have medicinal values [17]. 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 [18-20].

Senna didymobotrya is mainly found along lakeshores, streams, rivers, deciduous, bush land and old plantations. The plant is hardly attacked by diseases or pests. *Senna didymobotrya* is locally known as senetwet (Kalenjin, Kenya). It is used in the preparation and preservation of 'mursik' (Kalenjin name for fermented milk) (21,22). Mursik is given to athletes returning home after participating in world athletic games. The current study was done to investigate the potency of *Senna didymobotrya* water extract use in the treatment against selected nosocomially infectious bacteria.

MATERIALS AND METHODS

Sample Collection and Preparation

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The plant samples were identified by a taxonomist in the University of Eastern Africa, Baraton. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance fifty grams of the leaves of *Senna didymobotrya* was put in a conical flask and heated in a water bath at 80°C for 90 minutes. The extract was filtered using Butchner funnel; Whatman no.1 filter paper, a vacuum and pressure 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 50°C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

Bioassay Study:

Preparation of the Bacterial Suspension: The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard, a procedure similar to that used by *Biruhalem et al.*, [23] and *Donay et al.*, [24]. The McFarland standard was prepared by dissolving 0.5 g of BaCl₂ 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. Three-five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and

dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37°C 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 the aid of a UV spectrophotometer to 0.132A° at a wavelength of 600 nm in order to obtain an approximate cell density of 1x10⁸ CFU/ml.

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 100µg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract: Mueller Hinton agar plates were prepared by the manufacturer's instruction 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with penicillin and DMSO control 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 37°C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

Table 1: Antimicrobial activity (Mean Zone of Inhibition ± S.E.) of *Senna didymobotrya* leaves Crude extract

Microorganisms	Plant extract Mean ± S.E	Penicillin	DMSO
<i>E. coli</i>	18.33 ± 0.882	44.00±0.577	0.00±0.000
<i>S. aureus</i>	11.00 ± 0.577	30.33± 0.333	0.00±0.000
<i>E. aerogenes</i>	10.33± 0.577	39.00±0.000	0.00±0.000
<i>B. cereus</i>	14.33 ±0.333	18.33±0.333	0.00±0.000

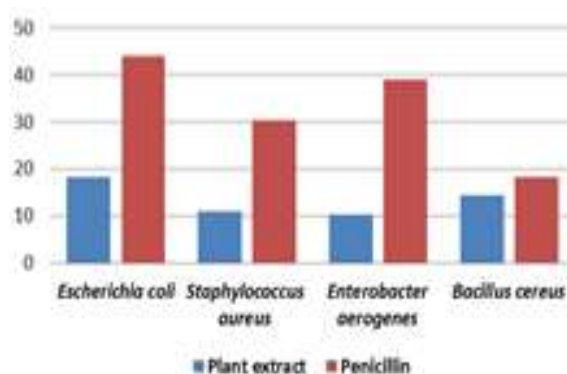


Figure 1: Inhibition of Plant extract and Penicillin (Positive Control) on Bacterial Strains

From study results obtained (Table 1), the plant leaves water extract was found to inhibit all the selected nosocomially infectious bacterial organisms with *Escherichia coli* being the most inhibited microorganism (Fig. 1), followed by *Bacillus cereus*, *Staphylococcus aureus* and *Enterobacter aerogenes*. The negative control (DMSO) did not show any zones of inhibition while the positive control (penicillin) inhibited the growth of all the organisms it was tested against. The comparison of the zones of inhibition among the microorganisms showed that there was significant difference between the zones of inhibition ($p \geq 0.05$).

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 [25&26]. *Escherichia coli* has shifted from the easily treatable strains to highly complicated and strongly resistant strains [27]. This trend has created the need for new therapeutic compounds to curb the menace. The inhibition of *Bacillus cereus* by the plant extracts is noteworthy since the bacterium has been known to cause respiratory tract infections and blood stream infections among immune-compromised patients [28]. According to the study conducted by Lequin et al., [29], *Bacillus cereus* is a potential causative agent of severe late onset hemorrhagic meningoenkephalitis in preterm infants.

Enterobacter aerogenes is major causative agent of a wide variety of nosocomial infections viz, pneumonia, urinary tract infections, meningitis, wound infections and intravascular and prosthetic devices infections [30-32]. The inhibition of the plant against the bacterial therefore demonstrates that the plant's water extract could be used in the treatment against these infections. *Staphylococcus aureus* is a major nosocomial pathogen in the world. The cases of nosocomial infections by the bacterium in US health facilities increased from 2.4% in 1975 to 29% in 1991 a trend which was also coupled with increased drug resistance by the microorganism [33]. Recent data indicate a 260% increase in nosocomial infections caused by *S. aureus* from 1990 through 1997 in health facilities which participated in the International Network for the Study and Prevention of Emerging Antimicrobial Resistance (INSPEAR) [34,35].

The results obtained in this study are in conformity with those obtained by Ngule et al., [36], in which the plant leaves were found to inhibit the growth *Bacillus cereus* 19.00 ± 0.258 , *Escherichia coli*

12.17 ± 0.477 and *Enterobacter aerogenes* 10.33 ± 0.615 . The data also correlates with that recorded by Nyaberi et al., [36], in which the stem charcoal of the plant inhibited the growth of *E. coli* (15.3 ± 0.6). The antibacterial activity of the plant could be associated to the wide variety of phytochemicals found in the plant's water extract Anthony [37]. According to this study the aqueous extract was found to contain tannins, saponins, terpenoids, flavonoids and alkaloids. The present study is however different and in contrast with previous studies which discredited the use of water as good solvent in the extraction of active compounds from plants [38-40]. In this study therefore we affirm the use of traditional method of extraction by using decoction method to extract plant active compounds.

Plant extracts have been found to act against microorganisms by interfering with peptidoglycan bacterial cell wall synthesis [41]. They may also inhibit protein synthesis, interfere with nucleic acid synthesis, breaking the peptide bonds, preventing the utilization of available nutrients, lysis of microbial cells and acting as chelating agents inhibiting metabolic pathway [42].

CONCLUSION

The antibacterial activity of the plant against the selected microorganisms is remarkable since the microorganisms 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. Formulations of aseptic solutions also need to be done.

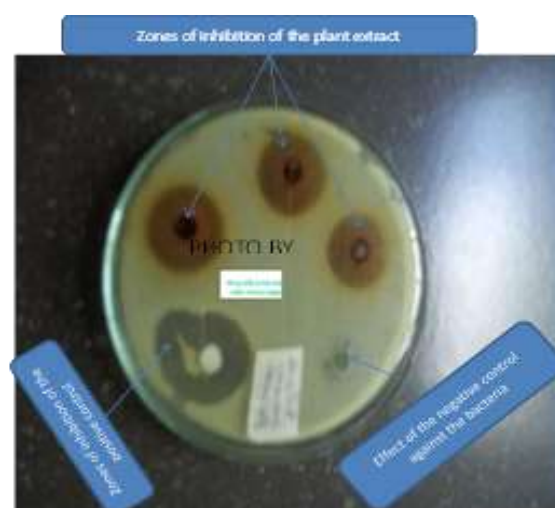


Figure: Sample Photographs of the zones of inhibition

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REFERENCES

1. Nejad SB, Allegranzi B, Syed SB, Ellis B, 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/S0140-6736 (08)61715-8 PMID: 19013310.
4. WHO. *Prevention of hospital acquired infections. A Practical Guide* (2nd edition). 2002.12.
5. 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.
6. Bottone EJ, *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology reviews*, 2010; 23(2): 382-398.
7. Lequin MH, Vermeulen JR, Elburg VMR, et al. *Bacillus cereus* meningoencephalitis in preterm infants. Neuroimaging characteristics. *American Society of Neuroradiology*, 2005; 26: 2137-2143. -250.
8. 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.
9. Raad I, Bodey G, Infectious complications of indwelling vascular catheters. *Clin Infect Dis*. 15:197-208 1992 1520756 West 1986.
10. Cohen ML, Epidemiology of drug resistance: Implications for a post-antimicrobial era. *Science*, 1992; 257: 1050-1055.
11. 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.
12. Akyuz M, Onganer AN, Erecevit P, Kirbag S, Entimicrobial activity of some edible mushrooms in the eastern and southern Anatolia region of Turkey. *Gazi University Journal of Science*, 2010; 23(2): 125-130.
13. 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.
14. 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.
15. 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.
16. Ngule CM, Swamy A, Obey JK, Phytochemical and bioactivity evaluation of *Sennadidymobotryafresen* Irwin used by the Nandi community in Kenya. *International Journal of Bioassays*, 2013, 2(7): 1037-1043.
17. 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.
18. Singh V, Ethnobotanical observations on Dards tribe of Gurez valley in Kashmir Himalaya. *Ethnobiology in Human Welfare: abstracts of the fourth international congress of ethnobotany*, Lucknow, Uttar Pradesh, India, 1994, 17-21: 316.
19. Adongo SO, Murungi J, Wanjau R and Ndengwa F, Analysis of selected essential elements of medicinal plants used in Chuka community, Tharaka Nithi county, Kenya. *The Scientific Journal of Science and Technology*. Special issue: 2010, 87-94.
20. 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 PMID: 19952319.
21. Tabuti JRS, *Senna didymobotrya* (Fresen). H.S Irwin & barneby. In: Schmeltzer, G.H & Gurib. Fakim, A (editors) *Prota 11(i): medicinal plants 1. {CD-ROM}*. PROTA, wageningen, Netherlands, 2007.
22. Ngule CM, Anthony ST, Jackie O, Phytochemical and bioactivity evaluation of *Senna didymobotrya* Fresen Irwin used by the Nandi community in Kenya. *International Journal of Bioassays*, 2013, 2(07):1037-1043.
23. 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.
24. Biruhalem T., Giday M., Anmut 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.
25. Ndamane Y, Kambizi L, Songca SP and 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.
26. Njau EFA, Alcorn JM, Buza J, Chirino-Trejo M Ndakidemi P, Antibacterial activity of *Tetradenia riparia* (HOCHST.) lamiaceae, a medicinal plant from Tanzania. *European Journal of Medicinal Plants*, 2014;4(12): 1462-1478.
27. 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.
28. 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.
29. Olajuyigbe OO, Afolayan AJ, *In vitro* antibacterial and time-kill assessment of crude methanolic stem bark extract of *Acacia mearnsi* wild against Bacteria in Shigellosis. *Molecules* 2012, 17, 2103-2118.

30. Blot SI, Vandewoude KH, Colardyn FA, Evaluation of outcome in critically ill patients with nosocomial *Enterobacter* bacteremia: results of a matched cohort study. *Chest.*, 2003, 123:1208-13.
31. Donnenberg MS, *Enterobacteriaceae*. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas, and Bennett's. (2005). Principles and practice of infectious diseases. 6th ed.* Philadelphia: Churchill Livingstone, 2579.
32. Sanders WE Jr., Sanders CC. *Enterobacter* spp: pathogens poised to flourish at the turn of the century. *Clin Microbiol Rev.* 1997, 10:220-41.
33. Graffunder ME, Venezia RA, Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infections including previous use of antimicrobials. *Journal of Antimicrobial Chemotherapy*, 2002, 49: 999-1005.
34. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Hender-son T, et al., Methicillin –resistant *Stapylococcus auereus* in U.S hospitals, 1975-1991. *Infection Control and Hospital Epidemiology*, 1992, 13: 582-586.
35. Archibald L, Phillips L, et al., Antimicrobial resistance in isolates from in-patients and outpatients in the United States: Increasing importance of the intensive care unit. *Clinical Infectious Diseases*, 1997, 24: 211-215.
36. Nyaberi MO, Onyango CA, et al., Bioactive fractions in the stem charcoal of *Senna didymobotrya* Fresen Irwin and Barney used by pastoral community of west Pokot to preserve milk. *Natural Resource Management*, 2013, PP 980-985.
37. Anthoney TS, Ngule CM, Obey SJ, (2014). Qualitative Assessment of Phytochemicals of Infused *Senna didymobotrya* leaves. *International Journal of Pharmacology (IJP)*, 2014, 4 (1), pp: 40-45. e-ISSN: 2249-7684; Print ISSN- 2249-7692.
38. Iqbal JP, Phytochemical screening of certain Medicinal plants for their application in alternate medicines. *Thesis submitted to Dr. B. R. Ambedkar University, Agra.*, 2009, p 59-67.
39. Iqbal PJ, Phytochemical screening of certain plant species of Agra city. *Journal of Drug Delivery & Therapeutics*, 2012, 2(4), 135-138.
40. Romero CD, Chopin SF, Buck G, Martinez E, Garcia M, Bixby L, Antibacterial properties of common herbal remedies of the Southwest. *J. Ethnopharmacol.*, 2011, 99: 253-257.
41. Jeyaseelan EC, Pathmanathan MK, Jeyadevan JP, Inhibitory effect of different solvent extracts of *Vitex negundo* and *Allium sativum* L., on phytopathogenic bacteria. *Arch. Appl. Sci. Res.*, 2010, 2(6): 325-331.
42. Gobalakrishnan R, Kulandaivelu M, Bhuvaneshwari K, Kandavel D, Kannan L, Screening of Wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrate* L. *Journal of Pharmaceutical Analysis*, 2013, 7(1): 1-6.

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