Antibacterial Screening of *Kyllinga nemoralis* (Hutch and Dalz) Ethanolic Rhizome Extract

Lotis M. Balala* and Hope Desiree L. Gacita

College of Veterinary Medicine, Visayas State University, Visca, Baybay City, Leyte, Philippines

ABSTRACT

The challenges of global health in controlling new diseases and drug resistance drive pharmaceuticals and researchers alike to discover natural antimicrobial products from plants. Nature is a rich source of medicine waiting to be explored. This study aimed to screen the antibacterial activity of *Kyllinga nemoralis* (Hutch and Dalz) ethanolic rhizome extract against *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Salmonella gallinarum*. Broth microdilution technique was used to determine the minimum inhibitory concentration (MIC) and the inoculation of the clear wells into Mueller Hinton Agar was done to determine the minimum bactericidal concentration (MBC) of the extract. Qualitative phytochemical screening tests were done to detect groups of bioactive compounds available in the extract. The MIC against *S. aureus* and *B. cereus* was 1.88 mg/ml and 15 mg/ml for *E. coli* and *S. gallinarum*. MBC was noted only against *S. aureus* at 3.75 mg/ml which means that at the same dose, *K. nemoralis* rhizome extract was bactericidal to the organism. MBC values were not obtained for the other test bacteria indicating that *K. nemoralis* rhizome extract only exhibited bacteriostatic activity against these organisms. Penicillin and streptomycin displayed superior antibacterial activity than the rhizome extract with MIC ranging from 0.08 mg/ml to 1.25 mg/ml and MBC of zero (0) or no growth demonstrating equal capacity of these antibiotics to inhibit and kill the bacteria. The detection of alkaloids, tannins and flavonoids in the rhizome extract was inferred to be responsible for the antibacterial activity of the plant.

Keywords: *Kyllinga nemoralis, MIC, MBC, phytochemical screening*

1 Introduction

The global health in the current scenario is extremely challenged by the emergence of new diseases and drug-resistant pathogens. Thus, efforts to sustain development of natural products into new drugs must be pursued especially in the developing countries. Since ancient times, traditional medicine has played a central role in forming people's culture of relying to plant-based medicines to treat or prevent illnesses (Fokunang et al. 2011). Undoubtedly, the environment is far the richest source of these natural products with pharmacologic value. While numerous medicinal plants have already been scientifically validated and marketed, a few others with potential effect remained undiscovered and undocumented. One of these is *K. nemoralis* (Hutch & Dalz) of the family Cyperaceae (Sindhu et al. 2014). *Kyllinga nemoralis* (Hutch & Dalz) is a native sedge in the Philippines that is locally known in Tagalog as *anuang* and *barubotones*, and *bontonsilyo* in Cebuano (Stuart 2019). This plant grows in moist, fertile soil and is common in lawns, pastures, garden, in between crops and plantations, roadsides and other wet, sunny, disturbed places (Kumar 2013; Fern 2014). Although ubiquitous with pantropical distribution, it is regarded of no economic importance

(Acevedo-Rodriguez and Strong 2005). Sedges demonstrate notable ecological diversity and thrive well in both wet and dry environments (Mishra et al. 2016). Rhizomes of specific species of *Kyllinga* are shown to exhibit digestive, diuretic, sudorific, antispasmodic, sedative and nerve tonic properties (Basualdo and Zardini 1995). The aromatic leaves and rhizomes are used to provide carminative and digestive effect (Gatti 1985 as cited by Hellión-Ibarrola et al. 2016). Chemically, two flavonoid glycosides (kaempferol 3-O-beta-apiosyl-(1−2)-beta-glucoside and isorhamnetin 3-O-betaapiosyl-(1−2) beta-glucoside) and quercetintriglycoside (quercetin 3-O-beta-apiofuranosyl-(1-->2)- beta-glucopyranoside 7-O-alpharhamnopyranoside) are said to be responsible for the bioactive activity of the rhizome extract (Apers et al. 2002). Anecdotal testimonies in the veterinary practice also account the palliative effect of *K. nemoralis* in small (dogs and cats) and large (ruminants) animals that willingly eat the leaves and rhizomes of this grass-like plant in several occasions including during illness.

Antibacterial studies of *K. nemoralis* against human pathogens had already been reported (Sindhu et al. 2014) but data is limited on the antibacterial activity of the plant rhizome against pathogens affecting animals. This study, therefore, investigated the antibacterial activity of *K. nemoralis* rhizome extract against gram-positive (*B. cereus* and *S. aureus*) and gram-negative (*E. coli* and *S. gallinarum*) bacteria of veterinary importance. The results provide information on the plant's potential as an antibacterial agent for veterinary use.

2 Materials and Methods

Collection and Preparation of K. nemoralis

Whole plant of *K. nemoralis* (Figure 1A) was collected from the vicinity of Visayas State University campus, Visca, Baybay City, Leyte and identified based on the plant description and illustration by Stuart (2019). The plant was washed thoroughly with running water to remove soil, debris and other contaminants. The rhizomes were selected, weighed, cut into small pieces and air dried until around 75% of its moisture was removed. Moisture loss on drying (LOD) was computed using this formula (Gatenby 2013; IRRI n.d.):

$$LOD = \frac{Beginning\ Weight - Ending\ Weight}{Beginning\ Weight} \times 100$$

Preparation of Plant Crude Extract

Air-dried rhizomes (Figure 1B) were soaked in ethanol (1:2 w/v) for 48 hours and strained using Whatman® filter paper No. 1. The plant residue was discarded and the crude ethanol rhizome extract was concentrated in a rotary evaporator at 60°C until the volume was around 10 ml. The extract was further air-dried to completely remove the solvent.

Test Organisms

Bacterial stock culture of *S. aureus*, *B. cereus*, *E. coli*, and *S. gallinarum* were obtained from the Microbiology Laboratory of the College of Veterinary Medicine, Visayas State University, Visca, Baybay City, Leyte. Some of these bacteria were purchased from BIOTECH, UPLB, College, Laguna and some were isolated from clinical samples and identified by biochemical tests. The bacteria were re-inoculated into fresh selective culture media to ensure the viability of the organisms. Newly cultured organisms were gram-stained and the colony characteristics were assessed to ensure the culture's purity. The inoculum density for the antibacterial assay was standardized by inoculating a loopful colony of the fresh culture into 10 ml of Mueller-Hinton Broth (MHB) incubated at 37°C until turbidity was comparable to 0.5

Figure 1. *K. nemoralis* (A) and the air-dried rhizomes (B)

McFarland standard (CLSI 2012) or equivalent to a bacterial suspension containing between 1×10^8 and 2×10^8 CFU/ml (Hudzicki 2009).

Tube Preparation of Rhizome Extract

The initial concentration of the rhizome extract was prepared by dissolving 90 mg of the concentrated air-dried extract in 3 ml of distilled water. The concentration was based on preliminary testing of antibacterial activity of the extract. From this stock solution, serial dilution was carried out to produce the different concentrations of the crude extract. Initially, 1 ml of MHB was dispensed in 10 test tubes. After which, 1 ml of the crude extract (30 mg/ml) was added to the first test tube and then mixed. Using another pipette, 1 ml from the first test tube was transferred to the second tube, then mixed, and the process was repeated until all 10 tubes were filled. The final concentrations of the extract after 10 dilutions ranged from 30 mg/ml to 0.06 mg/ml (Figure 2). The antibacterial testing of each concentration was performed in microtiter plates replicated four times. Sterility and growth controls were also included in the experimental setup.
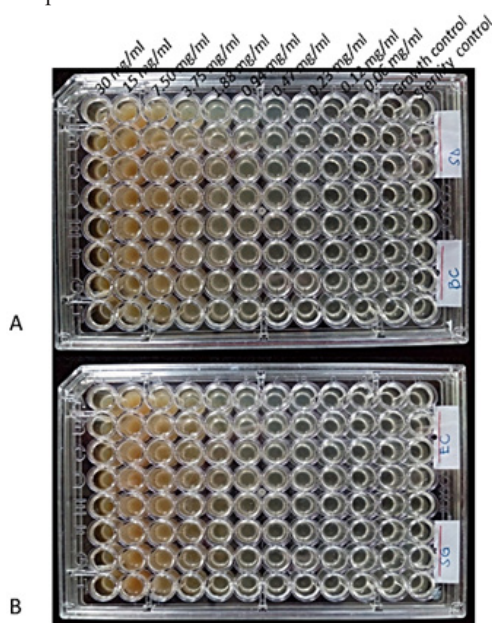

Figure 2. Experimental setup of the broth microdilution technique of *K. nemoralis* rhizome extract against gram-positive (A) and gram-negative (B) bacteria. SA – *S. aureus*, BC – *B. cereus*, EC – *E. coli*, SG – *S. gallinarum*

Antibacterial Assay

The antibacterial activity of the rhizome extract was evaluated through the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Briefly, 0.1 ml each of the extract concentrations was poured into corresponding wells in the 96-well microtiter plate using a micropipette. Subsequently, 0.1 ml of the prepared bacterial inoculum was added to each well and incubated at 37°C for 24 hours. After incubation, the wells were examined for turbidity or sedimentation as a sign of bacterial growth. The MIC of the extract is the lowest concentration that inhibited the growth of the organism as determined by the absence of turbidity or clear well (CLSI 2012). The wells with clear content were streaked into Mueller-Hinton Agar (MHA) to determine the MBC and incubated at 37°C for 24 hours. The MBC is the lowest concentration of the extract that prevents the growth of the organism after its inoculation into an antibiotic-free media (Andrews 2001). Penicillin and streptomycin were used as reference control for gram-positive and gram-negative bacteria, respectively.

Qualitative Phytochemical Screening of K. Rhizome Extract

Phytochemical screening of the crude *K. nemoralis* rhizome extract was carried out to determine the presence of active compounds such as alkaloids (Wagner's test), flavonoids (lead acetate test), saponins (froth test), and tannins (ferric chloride test). The reaction of each test was recorded as (+) for presence or (-) for absence of the compound.

Wagner's test: Initially, the extract was dissolved in 1% hydrochloric acid solution and filtered in Whatman Filter paper No. 1. The Wagner's reagent was made by dissolving 2 g of potassium iodide and 1.27 g of iodine in 5 ml distilled water and the solution diluted in 100 ml of distilled water. The filtrate was then treated with few drops of the Wagner's solution and the presence of alkaloid was indicated by the formation of brown or reddish precipitate (Abdullahi et al. 2013).

Lead acetate test: Few drops of lead acetate solution was added into the extract. The formation of yellow color precipitate indicated the presence of flavonoids (Tiwari et al. 2011).

Froth test: Dissolved extract (0.5 g in 10 ml distilled water) was heated in a water bath for 5

min. The presence of saponins was indicated by the formation of froth (Banso and Adeyemo 2006).

Ferric chloride test: Extract (0.5 g) was dissolved in distilled water (10 ml) and filtered. A few drops of 5% ferric chloride were added to the filtrate. Tannins were indicated by the production of black or blue green color (Banso and Adeyemo 2006).

Statistical Analysis

Gathered data were recorded in Microsoft Excel. The final MIC and MBC were determined as the statistical mode or the concentration of the rhizome extract that frequently inhibited the growth or killed each bacterium after four replications.

3 Results and Discussion

Table 1 indicates the physical characteristics of *K. nemoralis* crude rhizome extract after air-drying. The final product appeared dark brown in color with slightly thick consistency. It took about 55 min to achieve the target yield which was around 10 to 11 ml.

MIC and MBC of K. nemoralis Rhizome Extract

Table 2 reflects the MIC and MBC activities of *K. nemoralis* rhizome extract against the test organisms. All pathogens were inhibited by the rhizome extract with MIC ranging from 1.88 mg/ml

to 15 mg/ml. The MIC for gram-positive bacteria was 1.88 mg/ml lower than the MIC for gram negative bacteria at 15 mg/ml. This made these organisms generally more sensitive to the crude rhizome extract at lower concentration than their gram-negative counterpart.

The MBC of the rhizome extract for *S. aureus* was two-fold higher (1.88 mg/ml:3.75 mg/ml) than its MIC; however, bacterial growth was not exhibited by the other three organisms when inoculated into the MHA indicating a zero (0) MBC (Figure 3). The inhibitory activity of the *K. nemoralis* rhizome extract affirmed its antibacterial potential in controlling the growth of selected bacteria.

The MIC of penicillin against gram-positive bacteria ranged from 0.63 mg/ml to 1.25 mg/ml. Streptomycin inhibited the gram-negative bacteria at MIC range of 0.08 mg/ml to 0.63 mg/ml. MBC assay of the bacteria inhibited by the antibiotics showed no growth in MHA indicating equal magnitude of these reference antibiotics in killing the test organisms.

Phytochemical Screening

The qualitative phytochemical screening of the extract to various tests is presented in Table 3. Out of the different compounds evaluated, the rhizome extract demonstrated the presence of three major active compounds including flavonoids, tannins and alkaloids.

Table 1. Physical characteristics of *K. nemoralis* rhizome extract

Characteristics	Rhizomes
Before infusion	
Initial weight	1,000 g
Weight after air-drying	250 g
Amount of moisture loss on drying	75%
During infusion	
Weight	250 g
Volume of solvent	500 mL
No. of hours of infusion	48 h
After infusion	
Volume of filtrate	275 mL
Color of filtrate	Light, clear brown
Consistency	Slightly sticky
After concentration	
Volume of filtrate	11 mL
No. of minutes used in concentration	55 min
Color of extract	Dark brown
Consistency	Slightly sticky

Table 2. MIC and MBC values of *K. nemoralis* rhizome extract against test bacteria

Bacteria	Gram characteristics	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	Positive	1.88	3.75
Bacillus cereus	Positive	1.88	0
Escherichia coli	Negative	15	0
Salmonella gallinarum	Negative	15	0

Table 3. Phytochemical contents of *K. nemoralis* rhizome extract

Test	Phytochemical compound	Result	Reaction
Wagner's test	Alkaloid	+	Reddish brown precipitate
Lead acetate test	Flavonoid	+	Yellow precipitate
Froth test	Saponin	-	No bubble/froth formation
Ferric Chloride Test	Tannin	+	Red precipitate

(+) presence of bioactive compounds;(-) absence of bioactive compounds
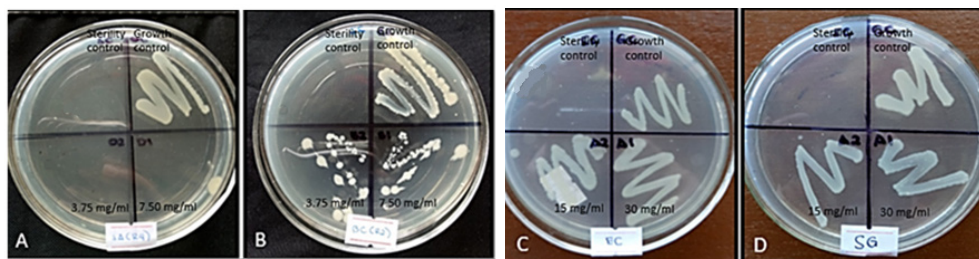

Figure 3. Minimum bactericidal concentration of *K. nemoralis* rhizome extract against A) SA - *S. aureus,* B) BC - *B. cereus*, C) EC - *E. coli*, and D) SG - *S. gallinarum*.

Discusssion

The essence of screening antimicrobial properties of medicinal plants is relevant nowadays to secure a good alternative for the treatment of bacterial infections (Delahaye et al. 2009). Various literatures demonstrated the capacity of phytoconstituents in inhibiting bacterial growth and reducing the number of many serious pathogens that have plagued mankind (Kang et al. 2014). The antibacterial activity of *K. nemoralis* in this study could be supported by the presence of certain phytochemical agents in the plant's rhizome extract including alkaloids, flavonoids and tannins. Saponins, however, were not detected. Active phytochemicals are secondary metabolites of the plants that possess a broad range of bioactivities including antibacterial activity (Bernhoft 2008).

All test bacteria were inhibited by the rhizome extract at concentration range of 1.88 mg/ml to 15 mg/ml. This affirmed the antibacterial potential of the extract against these pathogens. Antibacterial activity of *K. nemoralis* extract against *S. aureus* and other gram-positive bacteria (*Staphylococcus saprophyticus, Streptococcus mutans, Streptococcus pneumonia,* and *Enterococcus faecalis*) was reported by Sindhu et al. (2014). Chloroformic phase extract of *K. odorata* also revealed inhibitory activity against *S. aureus* (0.125 mg/ml) and *P. aeruginosa* (0.500 mg/ml) (Bezerra et al. 2019).

Although the extract exhibited inhibitory activity to test bacteria, MBC assay showed that only *S. aureus* was completely killed at 3.75 mg/ml (Figure 3A). This activity of the rhizome extract is significant as *S. aureus* is one of the most important human pathogens associated with hospital and community-acquired infections (Fonkeng et al. 2015). The rise of methicillin-resistant *S. aureus* epidemics in humans (Lee et al. 2011) and animals (Smith et al. 2011) also requires the development of novel, alternative drugs. Comparison of results, however, appeared that commercial antibiotics displayed better antibacterial activity than the *K. nemoralis* rhizome extract. This response is expected as several aspects of the drug including concentration, nature of pathogens, etc. are optimized to confer effective potency and bioactivity prior to their production and marketing.

The inherent resistance of *B. cereus* to the

extract could be the result of endospore production. Endospore-forming organisms such as *B. cereus* (Quinn et al. 2003) respond to harsh conditions by developing protective endospores to render bacteria dormant for extended periods and reactivate to become fully active bacteria when conditions are favorable (Cornell University 2017). The general resistance of gram-negative bacteria, on the other hand, has been demonstrated in several studies and is attributed to the outer membrane and extra lipopolysaccharide of the cell wall (Delahaye et al. 2009). The outer membrane provides an efficient permeability barrier (Nikaido 1998) without compromising continuous exchange of material required for sustaining life (Delcour 2008). Gram-positive organisms lack an outer membrane but are surrounded by layers of peptidoglycan (Silhavy et al. 2010).

Most antibacterial agents are generally described by Pankey and Sabath (2004) as potentially bacteriostatic (inhibitory) or bactericidal (effectively kill). Traditionally, the antibacterial plant is considered bactericidal against the test organism if the MIC:MBC ratio is less than or equal to four or the MBC is similar in magnitude to the MIC; bacteriostatic, if the MIC:MBC ratio is greater than four and less than 32 and lastly, tolerant, if the MBC: MIC ratio is greater than or equal to 32 (Cutler et al. 1994; Derendorf and Hocchaus 1995). Levison (2004) interpreted that the MBC for bactericidal drugs is not more than fourfold higher than the MIC while the MBC of bacteriostatic drugs are many times higher than their established MIC. This means that bacteriostatic activity ratio of MBC to MIC is >4 while bactericidal activity is <4. Using this principle, it can be inferred that K. nemoralis rhizome extract at 3.75 mg/ml was bactericidal (MBC 2-fold higher than MIC) to S. aureus. However, the growth of *B. cereus, E. coli* and *S. gallinarum* in MHA was indicative that the extract could be bacteriostatic in these organisms and further implied that at 15 mg/ml, the extract could only inhibit their growth but cannot kill them effectively. The *in vitro* microbiological determination whether an antibacterial agent is bactericidal or bacteriostatic is, however, influenced by growth conditions, bacterial density, test duration, and extent of reduction in bacterial numbers (Pankey and Sabath 2004).

The secondary metabolites such as flavonoids, tannins and alkaloids found in *K. nemoralis* rhizome extract could have a role in the plant's defense mechanism against herbivores, pests, and pathogens (Bennet and Wallsgrove 1994). These chemicals are not required for the immediate survival of the plant but are synthesized to increase the fitness of the plant to survive by allowing it to interact with its environment, including pathogens and herbivorous and symbiotic insects (Kennedy and Wightman 2011). Rajagopal et al. (2016) demonstrated the presence of phenolic compounds, glycosides and flavonoids in *K. nemoralis* ethanolic extract, but the absence of saponins, oils and fats, carbohydrates, proteins and amino acids, and phytosterols. Other *Kyllinga* species also revealed the presence of steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, and amino acids in *Kyllinga triceps* (Aneela et al. 2014) and alkaloids, flavonoids, saponins, terpenoids, quinones, cardiac glycosides, phenolic compounds, phytosterols, and coumarins in *K. erecta* extract (Augustus et al. 2014). The antibacterial property of flavonoids is believed to be due to their capacity to inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Cushnie and Lamb 2005). Deprivation of extracellular microbial enzymes and substrates required for microbial growth, and direct action on microbial metabolism through inhibition of oxidative phosphorylation are few of the different mechanisms in which tannins derive their antimicrobial activity (Scalbert 1991). Most alkaloids act through efflux pump inhibition that consequently alter DNA and RNA synthesis (Silva et al. 2010).

The threat of the emergence of new diseases and antimicrobial resistance in conventional antibiotics underscores the importance to develop potential plant-derived products to provide long term solutions. The antibacterial activity of *K. nemoralis* rhizome extract against the test organisms provides basic information that could be useful in the development of *K. nemoralis*-derived antimicrobials.

4 Conclusion and Recommendations

The neglected sedge, *K. nemoralis*, exhibited potential antibacterial activity against selected pathogens of animals. The susceptibility of the bacteria could be attributed to the limited phytoconstituents of the extract detected in the study. Future exploration could be directed to

further investigation of its antimicrobial properties against a range of pathogens and the extraction and testing of phytochemical contents from other plant parts using different solvents.

5 Acknowledgement

Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

6 Literature Cited

Abdullahi, M. N., Ilyas, N. & Ibrahim, H. (2013). Evaluation of phytochemical screening and analgesic activity of aqueous extract of the leaves of *Microtrichia perotitii* dc (Asteraceae) in mice using hotplate method. *Medicinal Plant Research*, **3** 37-43.

Acevedo-Rodriguez, P. & Strong, M. (2005). Monocotyledons and gymnosperms of Puerto Rico and the Virgin Islands. *Contributions from the United States National Herbarium*, 52: 1-415. Retrieved from:http://botany.si.edu/antilles/prflora/monocots/vol52web.pdf

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Therapy,* **4**(1) 5-16.

Aneela, S., Dey, A. and De, S. (2014). Gas chromatography -mass spectrometry analysis of *Kyllinga triceps. International Journal of Pharmaceutical Sciences and Research,* **5**(7) 2999-3003.

Apers, S., Huang, Y., Van Miert, S., Dommisse, R., Berghe, D.V., Pieters, L., and Vlietinck, A. (2002). Characterization of new oligoglycosidic compounds in two Chinese medicinal herbs. *Phytochemical Analysis,* **13** (4) 202–206.

Augustus, S.O.K., Janet, J.O., Ebenezer, T.B., and Ogboma, U.J. (2015). Antioxidant activities, total flavonoid and total phenolic contents of whole plant of *Kyllinga erecta. Journal of Food and Nutrition Research,* **3**(8) 489-494.

Banso, A. & Adeyemo S. (2006). Phytochemical screening and antimalarial assessment of *Abutilon mauritianum, Bacopa monnifera and Datura stramonium. Biokemistri*, **18** 39-44.

Basualdo, I. & Zardini, E.M. (1995). Medicinal plants of Paraguay: underground organs II. *Economic Botany,* **49** (4) 387–394.

Bennett, R. N. & Wallsgrove, R. M. (1994). Secondary metabolites in plant defense mechanisms. *New Phytologist*, **127**(4) 617-633.

Bernhoft, A. (2008). A brief review on bioactive compounds in plants- Bioactive compounds in plants-benefits and risks for man and animals. *Proceedings of the Norwegian Academy of Science and Letters, Oslo,* **50** 11-17.

Bezerra, J.J.L., do Nascimento, T.G., Kamiya, R.U., Prata, A.P.D., de Medeiros, P.M., da Silva, S.A.S., and de Mendonca, C.N. (2019). Phytochemical screening, chromatographic profile and evaluation of antimicrobial and antioxidant activities of three species of the Cyperaceae Juss. Family. *Journal of Medicinal Plant Research,* **13** (14) 312-320.

Clinical and Laboratory Standards Institute (CLSI) (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard—Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute.

Cornell University. (2017). Bacterial endospores. Retrieved from https://micro.cornell.edu/research/epulopiscium/bacterial-endospores

Cushnie, T. & Lamb, A. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, **26** 343-356.

Cutler, N. R., Sramek, J. J. & Narang, P.K. (1994). *Pharmacodynamics and drug development: Perspectives in clinical pharmacology.* USA: John Wiley and Sons.

Dafale, N.A., Semwal, U.P., Rajput, R.K., & Singh, G.N. (2016). Selection of appropriate analytical tools to determine the potency and bioactivity of antibiotics and antibiotic resistance. *Journal of Pharmaceutical Analysis,* **6**(4) 207-213.

Delahaye, C., Rainford, L., Nicholson, A., Mitchell, S., Lindo, J., & Ahmad, M. (2009). Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis. Journal of Medical and Biological Sciences,* **3**(1) 1-7.

Delcour, A. (2009). Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta,* **1794** (5) 808-816.

Derendorf, H. & Hocchaus, G. (1995). *Handbook of Pharmacokinetic/Pharmacodynamic Correlation.* USA: CRC Press.

Fern, K. (2014). Tropical plants database: *Kyllinga brevifolia.* Retrived from tropical.theferns.info/view tropical.php?id=Kyllinga+brevifolia&redir=Cyperus+brevifolius

Fokunang, C. N., Ndikum, V., Tabi, O. Y., Jiofack, R. B., Ngameni, B., Guedje, N. M., Tembe-Fokunang, E.A., Tomkins, P., Barkwan, S., Kechia, F., Asongalem, E., Ngoupayou, J., Torimiro, N. J., Gonsu, K. H., Sielinou, V., Ngadjui, B. T., Angwafor, F. III, Nkongmeneck, A., Abena, O. M., Ngogang, J., & Kamsu-Kom. (2011). Traditional medicine: past, present and future research and development prospects and integration in the National Health System of Cameroon. *African Journal of Traditional, Complementary, and Alternative Medicines*, **8**(3) 284-295.

Fonkeng, S., Mouokeu, L., Tume, R.S., Guy, C., Njateng, S.S., Kamcthueng, M.O., Ndonkou, N.J., & Kuiate, J.R. (2015). Anti-*Staphylococcus aureus* activity of methanol extracts of 12 plants used in Cameroonian folk medicine. *BMC Research Notes* **8**, 710.

Gatenby, A. (2013). What can I use to get the moisture content in my product? Retrieved from https://www.cscscientific.com/csc-scientific-blog/bid/95592/what-can-i-use-to-get-the-moisture-content-in-my-product

Hellión-Ibarrola, M.C., Montalbetti, Y., Heinichen, O.Y., Kennedy, M.L., Campuzano, M.A., Alvarenga, N., & Ibarrola, D.A. (2016). Antidepressant-like effect of *Kyllinga brevifolia* rhizomes in male mice and chemical characterization of the components of the active ethyl acetate fraction. *Journal of Ethnopharmacology,* **194** 1005-1011.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. Retrieved from https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

International Rice Research Institute (IRRI) (n.d.). Moisture content calculations. Retrieved from http://www.knowledgebank.irri.org/step-by-step-production/postharvest/harvesting/measuring-moisture-content/moisture-content-calculations

Kang, C.G., Hah, D. S., Kim, C. H., Kim, Y. H., Kim, E. & Kim, J.S. (2011). Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. *Official Journal of Korean Society of Toxicology,* **27**(1) 31-36.

Kennedy, D.O. & Wightman, E. L. (2011). Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition*, **2** 32-50.

Kumar, B. (2013). Kyllinga nemoralis. the IUCN red list of threatened species 2013. Retrieved from: http://www.iucnredlist.org/details/177219/0

Lee, A.S., Huttner, B. & Harbarth, S. (2011). Control of Methicillin-resistant *Staphylococcus aureus. Infectious Disease Clinics of North America,* 25(1) 155-179.

Levison, M. E. (2004). Pharmacodynamics of antimicrobial drugs. *Infectious Disease Clinics of North America,* **18** (3) 451-465.

Mishra, S., Tripathi, A., Kumar, & Chauhan, D.K. (2016). Role of sedges (Cyperaceae) in wetlands, environmental cleaning and as food material: Possibilities and future perspectives. In M.M. Azooz & P. Ahmad (Eds.), *Plant-Environment Interaction: Responses and Approaches to Mitigate Stress,* (1st Ed.). John Wiley & Sons, Ltd. pp. 327-338.

Nikaido, H. (1998). Antibiotic resistance caused by gram-negative multidrug efflux pumps. *Clinical Infectious Diseases,* **27** 32-41.

Pankey, G.A. & Sabath, L.D. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. *Clinical Infectious Diseases,* **38**(6) 864-870.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., & Fitzpatrick, E.S. (2011). *Veterinary microbiology and microbial disease,* 2nd ed. USA: Wiley-Blackwell.

Rajagopal, P. L., Kumar, S., Sreejith, P.N. & Premaletha, K. R. (2016). Phytochemical and antioxidant screening of the aerial parts of *Kyllinga nemoralis. International Journal of Science and Research Methodology,* **4**(2) 66-76.

Scalbert, A. (1991). Antimicrobial activity of tannins. *Phytochemistry*, **30**(12) 3875-3883.

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspective in Biology,* **2** 5.

Silva, R.B., Almeida, C.R., Chavasco, J.M. & Chavasco, J.K. (2010). Antimycobacterial activity evaluation and MIC determination of lyophilizated hydroalcoholic extracts of *Bixa orellana* L., Bixaceae. Revista Brasileira de Farmacognosia, **20**(2): 171-174.

Sindhu, T., Rajamanikandan, S. & Srinivas, P. (2014). In vitro antioxidant and antibacterial activities of methanol extract of *Kyllinga nemoralis. Indian Journal of Pharmaceutical Sciences,* **76**(2) 170-174.

Smith, T.C. & Pearson, N. (2011). The emergence of *Staphylococcus aureus* ST398. *Vector Borne*

Zoonotic Diseases, **11** 327–339.

Stuart, G. U. (2019). Anuang. Retrieved from http://stuartxchange.ph/Anuang.html

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A Review. *Internationale Pharmaceutica Sciencia*, **1**(1) 98-106.