

**EVALUATION OF THE *IN VITRO* ANTIBACTERIAL ACTIVITY OF NEEM
(*Azadirachta indica* Juss.) BARK ALCOHOL EXTRACTS AGAINST
PATHOGENIC BACTERIA IN PHILIPPINE NATIVE CHICKEN**

A Thesis

Presented to

the College of Agriculture, Resources, and Environmental Sciences

Central Philippine University

Jaro, Iloilo City



In Partial Fulfilment

of the Requirements for the Degree

BACHELOR OF SCIENCE IN AGRICULTURE

By

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April 2019

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ABSTRACT

The study was conducted from February 8 to 20, 2019 at the College of Medical Technology Laboratory, Central Philippine University, Jaro, Iloilo City to evaluate *the in vitro* antibacterial activity of neem bark alcohol extracts against different pathogenic bacteria. The experimental treatments were laid out in a factorial completely randomized design with three replications. The treatments consisted of neem tree stem bark (NTSB) acetone, ethanol, and methanol extracts, NTSB pure extract, and alcohol extraction solvents, AES, (acetone, ethanol, and methanol). Tetracycline antibiotic was used as a positive control and distilled water as the negative. The potency of the NTSB alcoholic extracts was analyzed through zones of inhibition (ZOI) 24 hours after the application of the controlling treatments. Results of the study revealed that responses of test organisms to NTSB alcoholic extracts varied indicating that *E. coli* and *S. typhimurium* are best controlled by NTSB methanolic extracts, while *S. aureus* by NTSB ethanolic extract. Pure extract of neem had anti *S. typhimurium* but is not significantly different from distilled water and AES in controlling *E. coli* and *S. aureus*. AES and distilled water have the same antibacterial abilities. These ZOI are statistically narrower than that in tetracycline. Based on the interpretative criteria using disc diffusion susceptibility testing, all treatments against all pathogenic bacteria except for tetracycline and NTSB ethanolic extract against *S. aureus* showed resistance.

REFERENCES

- Amada, L. (2018). Evaluation of the potency of neem tree (*Azadirachta indica* Juss.) stem bark using different alcohol extraction solvents against *Staphylococcus aureus* R. Undergraduate *Special Topic Report*. College of Agriculture, Resources, and Environmental Science. Central Philippine University, Jaro, Iloilo City.
- Anderson, J., Blaney, W., Ley, S., Simmonds, M., & Toogood, P. (1990). Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae. *Entomologia Experimentalis et Applicata*, 55 (2), 149-160. doi.org/10.1111/j.1570-7458.1990.tb01358.x
- Azarfar, A., Fallah, R., & Kiani, A. (2013). A review of the role of five kinds of alternatives to infeed. *Academic Journals*, 5 (1), 317-321. doi: 10.5897/JVMAH2013.0237 Retrieved from <http://www.academicjournals.org/journal/JVMAH/article-full-text-pdf/DD44F0740992>
- Bandyopadhyay, U., Banerjee, R., Biswas, K., & Chattopadhyay, I. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 82 (11), 1336-1345. Retrieved from <http://repository.ias.ac.in/5193/1/305.pdf>
- Banerjee, C. K., Gandhi, M., Lal, R., Sankaranarayanan, A., & Sharma, P. L. (1998). Acute toxicity study of the oil from *Azadirachta indica* seed (neem oil). *Science Direct*, 23 (1), 39-51. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0378874188901134>
- Bonsu, F., Kagya-Agyemang, J., Kwenin, W., & Zanu, H. (2012). Medicinal response of broiler chickens to diets containing neem (*Azadirachta indica*) leaf meal, haematology and meat sensory analysis. *World Applied Science Journal*, 19 (6), 800-805. doi: 10.5829/idosi.wasj.2012.19.06.827
- Chamber, J. & Gong, J. (2011). The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Science Direct*, 44 (10), 3149-3159. doi.org/10.1016/j.foodres.2011.08.017
- Chattopadhyay, R. & Banyopadhyay, M. (2005). Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. *African Journal of Biomedical Research*, 8 (2), 101-104. Retrieved from <http://www.bioline.org.br/pdf?md05018>
- Chattopadhyay, R., Chattopadhyay, R., & Maitra, S. (1993). Possible mechanism of anti-inflammatory activity of *Azadirachta indica* leaf extract. *Indian Journal of Pharmacology*, 25 (2), 99-100. Retrieved from <http://www.ijp-online.com/article.asp?issn=02537613;year=1993;volume=25;issue=2;spage=99;epage=100;aulast=Chattopadhyay;type=0#>
- Clinical Laboratory Standards Institute (2014.) Zone of Inhibition. Retrieved from <http://www.who.int/heli/risks/toxics/chemicalsdirectory/en/>

- Crandall, P., Howard, Z., O'Bryan, C., & Ricke, S. (2012). *Salmonella enteritidis* in shell eggs: current issues and prospects for control. *Science Direct*, 45 (2), 755-764. doi.org/10.1016/j.foodres.2011.04.030
- Desin, T., Koster, W., & Potter, A. (2013). *Salmonella* vaccines in poultry: past, present and future. *Expert Review of Vaccines*, 12 (1), 87-96. doi: 10.1586/erv.12.138.
- Elangovan, A. V., Sastry, V. B., Singh, S. D., & Verma, S. S. (2000). Effect of feeding neem (*Azadirachta indica*) kernel meal on growth, nutrient utilization and physiology of japanese quails (*Coturnix coturnix japonica*). *Asian-Australasian Journal of Animal Science*, 13 (9), 1272-1277. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/20003000380>
- Lab Protocols (2017). *Molecular and cell biology protocols*. Retrieved from <http://mcblabprotocols.com/stock-solution-preparation/preparation-stock-solution-tetracycline/>
- Langata, L. M., Maingi, J., Musenyo, H. A., Kiiru, J., & Nyamache, A. K. (2019). Antimicrobial resistance genes in *Salmonella* and *Escherichia coli* isolates from chicken droppings in Nairobi, Kenya. *BMC Research Notes*, 12 (22), __. doi.org/10.1186/s13104-019-4068-8
- Mamman, P. H., Mshelia, W. P., Susbatrus, S. C., & Sambo, K. w. (2013). Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *Salmonella spp.* and *Staphylococcus aureus*. *International Journal of Medicine and Medical Sciences*, 5 (1), 14-18. doi: 10.5897/IJMMS12.017
- Maragathavalli, S., Brindha, S., Kaviyarasi, N., Annadurai, B., & Gangwar, S. (2012). Antimicrobial activity in leaf extract of neem (*Azadirachta indica*). *International Journal of Science and Nature.*, 3 (1), 110-113. Retrieved from https://www.researchgate.net/publication/278667499_Antimicrobial_activity_of_Azadirachta_indica_neem_leaf_bark_and_seed_extract
- Montet, D., Hazm, J. E., Ouadia, A., Chichi, A., Diop, M. B., Mbaye, M. S., et al. (2019). Contribution of the methodology of collective expertise to the mitigation of food safety hazards in low- or medium-income countries. *Science Direct*, 99 (1), 84-85. doi.org/10.1016/j.foodcont.2018.12.009
- Murry, A., Hinton, A., & Morrison, H. (2004). Inhibition of Growth of *Escherichia coli*, *Salmonella typhimurium*, and *Clostridia perfringens* on Chicken Feed Media by *Lactobacillus salivarius* and *Lactobacillus plantarum*. *Science Alert*, 3 (9), 603-607. doi: 10.3923/ijps.2004.603.607
- Murthy, K., Rompicherla, R., Roplekar, P., Shah, D., Sharma, N., & Weer, C. (2014). To study the antibiotic susceptibility of the isolated strains of *Staphylococcus aureus* and comparative analysis with natural herbs. *International Science Journal*, 1 (3), 2348-6058. Retrieved from https://www.researchgate.net/publication/310457447_TO_STUDY_THE_ANTIBIOTIC_SUSCEPTIBILITY_OF_THE_ISOLATED_STRAINS_OF_STAPHYLOCOCCUS_AUREUS_AND_COMPARATIVE_ANALYSIS_WITH_NATURAL_HERBS

- Nandita, D. & Ifeoma, E. (2001). Antimicrobial effects of components of the bark extract of neem (*Azadirachta indica* A. Juss). *A Journal of Scientific and Technological Research*, 8, (23-28). Retrieved from <http://eprints.covenantuniversity.edu.ng/id/eprint/5103>
- Pandey, G., Singh, M., & Verma, K. (2014). Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* (neem) leaves. *Academic Sciences*, 6 (2). Retrieved from <https://innovareacademics.in/journal/ijpps/Vol6Issue2/8912.pdf>
- PSA. (2018). Chicken Situation Report, October-December 2018. Philippine Statistics Authority. Retrieved from <https://psa.gov.ph/livestock-poultry-ipsr/chicken/inventory>.
- Ragland, W. L., Janjecic, Z., Franciosini, M. P., & Koz, I. (2015). Antibiotic Growth Promoters in Poultry, and their Potential Alternatives. pp. 1-7. Retrieved from <https://www.openaccessgovernment.org/wp-content/uploads/2014/06/Zagreb-Biotek-ebook-web.pdf>
- Rathod, G., Kotecha, B., Sharma, R., Amin, H., & Prajapati, P. (2012). In vitro antibacterial study of two commonly used medicinal plants in ayurveda: neem (*Azadirachta indica*) and tulsi (*Ocimum sanctum*). *International Journal of Pharmaceutical & Biological Archives*, 3 (3), 582-586. Retrieved from <http://www.ijpba.info/ijpba/index.php/ijpba/article/download/677/459>
- Riego, J. & Maramba-Untalan, C. (2001). In vitro analysis of the antibacterial properties of *Basella rubralinn* (alugbati) and *Plumiera acuminata* (kalachuchi) against most common bacterial wound isolates among pediatric patients. *PIDSP Journal*, 5 (2), 15-19. Retrieved from http://www.pidsphil.org/pdf/Journal_05100703/jo20_ja03.pdf
- Sarmiento, W., Maramba, C., & Gonzales, L. (2011). An in-vitro study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *Research Gate*, 12 (1), 40-45. Retrieved from https://www.researchgate.net/publication/285867827_An_invitro_study_on_the_antibacterial_effect_of_neem_Azadirachta_indica_leaf_extract_on_methicillin-sensitive_and_Methicillin-resistant_Staphylococcus_aureus
- Scharff, R. (2012). Economic Burden from Health Losses Due to Foodborne Illness in the United States. *WDAS country Access Consortium*, 75 (1), 123-131. doi:10.4315/0362-028X.JFP-11-058
- Sundararaj, N., Kalagatur, N. K., Mudili, V., Krishna, K., & Antonysamy, M. (2019). Isolation and identification of enterotoxigenic *Staphylococcus aureus* isolates from Indian food samples: evaluation of in-house developed aptamer linked sandwich ELISA (ALISA) method. *Springer Link*, 1 (1), 1-11. doi.org/10.1007/s13197-019-03568-1

Upadhyaya, I., Upadhyaya, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S., Yin, H.-B., et al. (2014). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken egg-borne transmission of *Salmonella enterica* serovar *enteritidis*. *Applied and Environmental Microbiology*, 81 (1), 2985-2994. doi: 10.1128/AEM.03809-14

WHO. (2017). Agrochemicals, Health and Environment. Retrieved from <http://www.who.int/heli/risks/toxics/chemicalsdirectory/en/>

Wilonsky, L. & Sote, E. (1984). Isolation of natural plaque-inhibiting substances from Nigerian chewing sticks. *Caries Research*, 18 (1), 216-225. Retrieved from doi.org/10.1159/000260768