



Antibacterial Property of Hanguana Malayana (Bakong) Crude Leaf Ethanolic Extract against Staphylococcus Aureus

Vandon T. Borela¹, Jan Arianne B. Urbano², Andrei Joshua A. Tayag², Myco G. Teresa²

¹Teacher, Parang High School, Philippines

²Student, Parang High School, Philippines

*Corresponding Author: Vandon T. Borela

Email: borelavandon89@gmail.com



Article Info

Article history:

Received 7 November 2020

Received in revised form 16 November 2020

Accepted 19 November 2020

Keywords:

Hanguana Malayana

Staphylococcus Aureus

Inhibition Zone

Abstract

Hanguana Malayana, known as *Bakong* plant is an invasive macrophytic plant found in few regions of the Philippines. Due to its mode of propagation, it occupies large spaces and it invades the space that's allotted for the crops of local farmers. Hence, the researchers investigate this plant for beneficial uses. In this study, the effect of the different concentrations of *H. malayana* crude ethanolic leaf extract on *S. aureus* is evaluated on the basis of the study stating that the plant is a potential resources of bioactive phytoconstituents. The bioactive compounds shows synergistic impact that made it have an compelling antibacterial property against *Staphylococcus aureus*. In this think about, it was as it were demonstrated to have antibacterial property against gram-positive microbes, *Staphylococcus aureus*. This research concluded that the *Hanguana malayana* crude leaf ethanolic extract has an antibacterial property against *Staphylococcus aureus*.

Introduction

Hanguana Malayana, commonly known as “Bakong”, is a wetland macrophyte native in the Philippines and is localized in Laguna de Cagayan, where it is formerly known as a pest that thrives in wetlands and terrestrial habitats until the plant is known to be a good source of fiber (Domingo, 2017).

Hanguana malayana is the prevailing plant within the river. Rasau could be a plant whose territory is on the banks of waterways and lakes in peat overwhelm ranges. His life is clustered in a watery put. Rasau takes off are one of the favorite elective nourishments of proboscis monkeys and orangutans. Daffodils (*Hanguana malayana*) are bushes with a greatest tallness of 2 meters. Daffodils can be found in timberland swamps, waterway banks, lakes, and lakes. The dispersion of daffodils is affected by water profundity. Daffodils are found in waters with a profundity not surpassing 2.5 meters.

A macrophyte is an amphibian plant that fills in or close to water and is either rising, submergent, or gliding. Most macrophytes fill normally in the wild; be that as it may, some are developed and devoured by people as plant food. Around the world, different wetland macrophytes are utilized customarily to treat human infections. Wetland macrophytes are a likely normal asset for the revelation of remedially important regular items. To date, the health-promoting phytochemicals and bioactivities of many macrophytes are underexplored (Ooh et al., 2014; Huang et al., 2007; Santosh & Satya, 2010; Panda & Misra, 2011). Wetlands give a special environment for a few restorative plants. In Muriyad, it is said that Near by individuals utilize a wide assortment of wetland/wetland-associated plants as fixings of conventional home grown therapeutic arrangements.

Customarily, H. WW is utilized remotely to treat fever (Grosvenor et al., 1995). Agreeing to the phytochemical screening, Hanguana Malayana contains the nearness and concentration of four sorts of HBAs, specifically gallic acid (GA), p-HBA, vanillic acid (VA) and protocatechuic acid (PCA) (Ooh et al., 2014). Phenolic constituents of plants are a prominent source of health-promoting phytochemicals. Plant phenolic compounds are structurally diverse and can be divided into different classes, including hydroxybenzoic acids (HBAs), hydroxycinnamic acids, and flavonoids (Perron & Brumaghim, 2009; Teixeira et al., 2013).

Gallic acid may be a well-known characteristic antioxidant that's essentially an auxiliary polyphenolic metabolite. Gallic corrosive could be an exceptionally imperative common antioxidant tea detailing, known as an Ayurvedic herb (Watson et al., 2013). Concurring to ponder, Polyphenols diminished the hazard of the frequency of numerous cancers in people with eat less wealthy in natural products and vegetables. Plant-derived phenolic compounds are used to prevent cancer chemistry. Expensive caustic agents, including anticancer drugs, had organic effects. Gallic acid has also been shown to provide anti-invasive and anti-metastatic exercises against various cancer cells. Potential prevention and recovery for gastric cancer metastasis may be the best.

Another investigation in human glioma cells has demonstrated that gallic acid inhibits cell multiplication and essentially diminishes cell reasonability, attack, and tube arrangement (Watson et al., 2013). p-HBA or p-Hydroxybenzoic is moreover known as 4-Hydroxybenzoic Acid could be a monohydroxybenzoic corrosive, a phenolic subsidiary of benzoic corrosive. It is fundamentally known as the premise for the arrangement of its esters, known as parabens. Parabens are a course of broadly utilized additives in restorative and pharmaceutical items.

Vanilla humus is probably the best organic phenolic fertilizer found in some vanilla and many other plant treatments. It can act as a fragrance agent and create wonderful fragrances. And soft it is an intermediate product that converts organic ferric friction into vanillin in two stages. Vanillic acid, which can encode chlorogen, is an oxidized form of vanillin. It is also an intermediate for the production of vanillin from ferulic abrasives. Vanillic corrosive could be a metabolic byproduct of caffeic corrosive and is regularly found within the pee of people who have expended coffee, chocolate, tea and vanilla-flavored confectionary (Muskiel & Groen, 1979). Vanillic acid specifically and particularly restrains 5'nucleotidase movement (Dhananjaya et al., 2006).

Protocatechuic acid may be a dihydroxybenzoic acid, a sort of phenolic acid. Protocatechuic acid, a polyphenolic compound, encompasses a comparative work to antimicrobial components, i.e., it inhibits the function of bacterial cell layers and represses protein disparity in bacterial cells. It could be a major metabolite of antioxidant polyphenols found in green tea. It has blended impacts on typical and cancer cells in in vitro and in vivo considers (Lin et al., 2007). Protocatechuic acid (PCA) is antioxidant and anti-inflammatory. PCA has been reported to induce apoptosis of human leukemia cells, as well as malignant HSG1 cells taken from human oral cavities (Babich et al., 2002), but PCA was found to have mixed effects on TPA-induced mouse skin tumours. Depending on the amount of PCA and the time before application, PCA could reduce or enhance tumour growth (Nakamura et al., 2000). Similarly, PCA was reported to increase proliferation and inhibit apoptosis of neural stem cells (Guan et al., 2009).

Similarly, PCA was reported to increase proliferation and inhibit apoptosis of neural stem cells. In an in vitro model using HL-60leukemia cells, protocatechuic acid showed an antigenotoxic effect and tumoricidal activity (Anter et al., 2011). Hanguana Malayana contains the concentrations for six hydroxycinnamic acids in the plant extracts, namely, p-CA, ferulic acid

(FA), chlorogenic acid (ChA), caffeic acid (CFA), sinapic acid (SNA) and syringic acid (SA) (Ooh et al., 2014).

Coumaric acid is a hydroxy derivative of cinnamic acid and is found naturally in eggplant carbon dioxide (ortho, meta, meta,). P-Coumaric acid is one of the most common dioxides in nature. P-coumaric acid (4-hydroxycinnamic acid) exists in phenolic or conjugated form, which acts as another phenolic compound. P-coumaric acid makes research into its biological activity. In this review, the occurrence, bioavailability, and bioavailability of p-coumaric acid are discussed and related to monosaccharides, oligosaccharides, monosaccharides, amine alcohols, organic acids, amines and lignin. It compares antioxidant, anticancer, antibacterial, antiviral, anti-inflammatory, platelet, anti-anxiety, antipyretic, analgesic, anti-arthritis and, obesity, hyperlipidemia, and all biological activities.

Cumulative evidence from multiple studies indicates that conjugation of p-coumaric acid greatly strengthens its biological activities; however, the high biological activity but low absorption of its conjugates remains a puzzle (Sarkar, 2018). According to studies, Ferulic Acid is a hydroxycinnamic acid found in cell walls of plants such as rice and oats and the seeds of apples and oranges, where it plays a key role in the plants' protection and self-preservation. Ferulic acid is proven to slow ageing process by reducing the effects of damaging free radicals on the skin, it is said that it also protects skin from sun damage. Another benefit of it is it exhibits synergistic effect alongside other antioxidants like Vitamin C and E.

The term "chlorogenic acids" refers to a related polyphenol family of esters, including hydroxycinnamic acids (caffeic acid, ferulic acid and p-coumaric acid) with quinic acid. Despite the "chloro" of the name, chlorogenic acids contain no chlorine. Instead, the name comes from the Greek *χλωρός* (light green) pertaining to the green color produced when chlorogenic acids are oxidized (Clifford et al., 2003). Chlorogenic Acid in Green Coffee have health benefits. People take green coffee by mouth for obesity, diabetes, high blood pressure, Alzheimer's disease, and bacterial infections (Greenberg et al., 2006; Watanabe et al., 2006; Almeida et al., 2006; Bassoli et al., 2008; Oboh et al., 2013). Even though it's already proven that the plant have health-promoting phytochemicals, the researchers still aim to identify the effect of *H.malayana* ethanolic crude leaf extract against *S.aureus*. The phytochemicals present in *H.malayana* might exhibit synergistic or antagonistic effect.

Methods

Plant Collection and Identification

Hanguana malayana was obtained from Sta. Teresita, Cagayan. The plant was brought to the University of the Philippines for its identification. Leaf tests of *Hanguana malayana* was discuss dried at room temperature for almost a month. The dried leaf tests where powdered using mechanical blender. The *H. malayana* powder test was macerated in 95 per cent ethyl liquor in jostle for three days and then sifted using channel paper. The filtrate dissipated and accumulated using a hot plate at 40 degrees Celsius and in a vacuum.

Preparation of Treatment

Two experimental treatments was made: 100% and 50%, after the filtrate was concentrated it was weighed using an analytical balance and it was dissolved in distilled water until it is saturated. Further, the percent extraction yield is obtained using the formula as per Terblanche and company (2017).

Percent extraction yield = [amount (g) of dried crude extract
(obtained)/(amount (g) of finelygrounded plant material used] x 100

Preparation of Culture Plates

Mueller Hinton Agar was cooked on the label of the manufacturer and then sterilized with a pressure cooker for 15 PSI for 15 minutes and then transferred to 12 petri dishes. Pour the amount that is enough to cover the bottom of the petri dish, leave it to harden for a while. Bacteria have been given to the researchers in a vial. The researchers prepared the nutrient broth according to the manufacturer's label and then sterilized it with a 15 PSI pressure cooker for 15 minutes. The bacteria were transferred to the broth using a sterile wooden applicator stick, aseptically. It was placed in a sterile storage box for 24 hours to allow the bacteria to grow. The bacteria have been tran.

Preparation of Filter Discs

The filter disks were prepared using a paper punch. It was sterilized with a 15 PSI pressure cooker for 15 minutes. The researchers used 12 petri dishes. 6 for 100 percent of the extract and 6 for 50 percent of the treatment. There are 12 filter disks (whatman filter paper no.1) all in all, 6 for 100 percent and 6 for 50 percent. Using a syringe, 0.02ml of each treatment will be injected to the filter discs and will be dried for a couple of minutes. Treated filter discs were applied aseptically to working culture plates using a tweezer. It was sealed using a masking tape and labeled to avoid error. It was placed in a clean storage box or cabinet at room temperature, and the inhibition zone around the filter discs was observed for about two to three days.

Quantification

The inhibition zone was measured using a ruler. The radius or the diameter of the inhibition zone was recorded. The antibacterial property of *Hanguana Malayana* was based on the size of the inhibition zone.

Results and Discussion



Figure 1. Plate 1 of 100% Plant extract concentration



Figure 2. Plate 1 of 50% Plant extract concentration



Figure 3. Plate 2 of 100% Plant extract concentration



Figure 4. Plate 2 of 50% Plant extract concentration



Figure 5. Plate 3 of 100% Plant extract concentration



Figure 6. Plate 3 of 50% Plant extract concentration

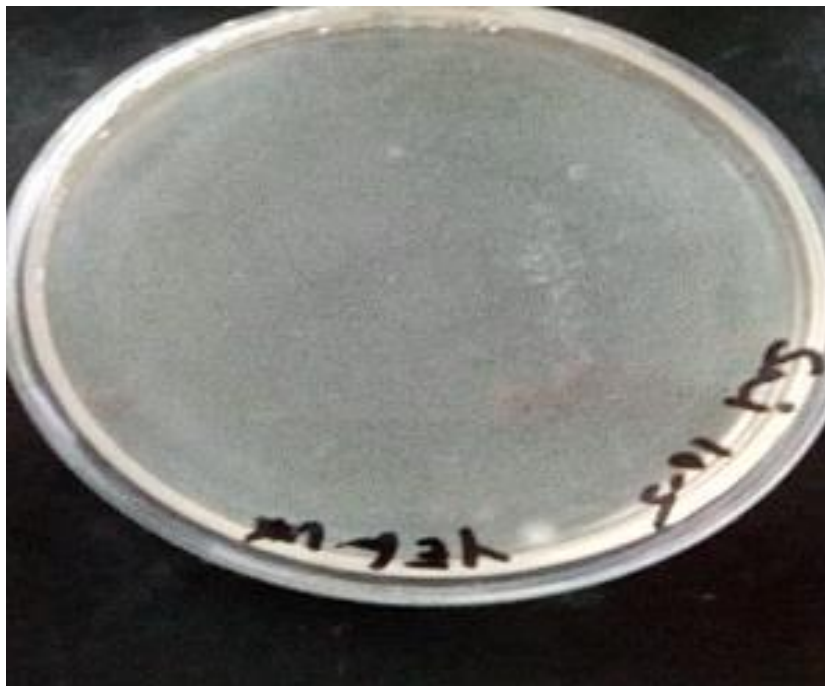


Figure 7. Plate 4 of 100% Plant extract concentration



Figure 8. Plate 4 of 50% Plant extract concentration

Based from the data gathered by the researcher, Table 1 shows the effect of the extract of the plant. Using the 50 % of plant extract of *H. malayana* the *S. aureus* doesn't inhibit because it is observable on the colony of the *S. aureus* present on the four replicate. While on 100% plant extract of *H. malayana*, from plate 1 to 3 there less number of colony while at plate 4 there are less number of colony. *Staphylococcus aureus* (*S. aureus*) is a bacterium that plays a role in the development of infectious diseases such as mastitis, eczema, respiratory infections and toxic shock syndrome. Treatments that can be given to an infected patient *Staphylococcus aureus* is an antibiotic. But improper use of antibiotics can lead to drug resistance. It is not yet known to be effective against gram-negative bacteria like *Escherichia Coli*. In the future studies, the efficacy of antibacterial property of *Hanguana malayana* on any type of bacteria should be evaluated to further know the ability of the plant. Antibacterial activity was measured in vitro with the agar diffusion method using paper discs, and the antibacterial activity was tested. large contrast test (ANOVA) to determine if there was an effective treatment. This differed with an inhibition area of antibacterial activity. A test for antibacterial activity showed that n-hexane extract and chloroform can inhibit growth. *Staphylococcus aureus* at a concentration and inhibition. The best active extract was determined by the chloroform extract with the lowest growth inhibitory concentration (MIC) for stew leaves. *Staphylococcus aureus* at a concentration with a suppression area bacteria. Even though it's already proven that the plant have health-promoting phytochemicals, the researchers still aim have been identified the effect of *H. malayana* ethanolic crude leaf extract against *S. aureus*. The phytochemicals present in *H. malayana* might exhibit synergistic or antagonistic effect. According to Ooh et al. (2014)

Hanguana Malayana contains the concentrations for six hydroxycinnamic acids in the plant extracts, namely, p-CA, ferulic acid (FA), chlorogenic acid (ChA), caffeic acid (CFA), sinapic acid (SNA) and syringic acid (SA). HCAs have phenylpropanoid C6-C3 structure as the most

chemical framework and are recognized by the nearness of hydroxyl group(s) on the fragrant ring(s) and a carboxyl bunch within the sidelong chain (Sakihama et al., 2002; Rock, 2017; Silva et al., 2014; Auezova et al., 2020;). The number and position of hydroxyl bunches and other substituents contribute to the differing qualities of HCAs. The foremost plenteous HCAs in nature are para-coumaric, caffeic, ferulic, and sinapic acids (Figure 1). In nature, all four acids are once in a while show in a free shape and are as a rule esterified with quinic and tartaric acids or different derivatives of carbohydrates. Chlorogenic acids are one the foremost inexhaustible esters counting the entire set of HCAs esters with quinic corrosive, to be specific caffeoyl-, feruloyl-, dicaffeoyl- and coumaroylquinic acids. The foremost common agent is 5-O-caffeoylquinic acid regularly alluded to as chlorogenic acid. An ester of caffeic corrosive and 3,4-dihydroxyphenyllactic corrosive is called rosmarinic corrosive, which is one of the foremost abundant caffeic corrosive ester within the plant kingdom other than chlorogenic acids.

Conclusion

Based from the data collected, the writer concluded that using the 10 percent of *Hanguana malayana* inhibit the growth of *Staphylococcus aureus* even the researcher unable to measure the size of the inhibition zone due to the effect of the plant extract. The bioactive compounds exhibits synergistic effect that made it have an effective antibacterial property against *Staphylococcus aureus*. In this study, it was only proven to possess antibacterial property against gram-positive bacteria, *Staphylococcus aureus*.

References

- Almeida, A. A. P., Farah, A., Silva, D. A., Nunan, E. A., & Glória, M. B. A. (2006). Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. *Journal of agricultural and food chemistry*, *54*(23), 8738-8743.
- Anter, J., Romero-Jiménez, M., Fernández-Bedmar, Z., Villatoro-Pulido, M., Analla, M., Alonso-Moraga, A., & Muñoz-Serrano, A. (2011). Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid. *Journal of medicinal food*, *14*(3), 276-283.
- Auezova, L., Najjar, A., Kfoury, M., Fourmentin, S., & Greige-Gerges, H. (2020). Antibacterial activity of free or encapsulated selected phenylpropanoids against *Escherichia coli* and *Staphylococcus epidermidis*. *Journal of Applied Microbiology*, *128*(3), 710-720.
- Babich, H., Sedletcaia, A., & Kenigsberg, B. (2002). In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress. *Pharmacology & toxicology*, *91*(5), 245-253.
- Bassoli, B. K., Cassolla, P., Borba-Murad, G. R., Constantin, J., Salgueiro-Pagadigorria, C. L., Bazotte, R. B., ... & de Souza, H. M. (2008). Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*, *26*(3), 320-328.
- Clifford, M. N., Johnston, K. L., Knight, S., & Kuhnert, N. (2003). Hierarchical scheme for LC-MS n identification of chlorogenic acids. *Journal of agricultural and food chemistry*, *51*(10), 2900-2911.

- Dhananjaya, B. L., Nataraju, A., Rajesh, R., Gowda, C. R., Sharath, B. K., Vishwanath, B. S., & D'Souza, C. J. (2006). Anticoagulant effect of *Naja naja* venom 5' nucleotidase: demonstration through the use of novel specific inhibitor, vanillic acid. *Toxicon*, 48(4), 411-421.
- Greenberg, J. A., Boozer, C. N., & Geliebter, A. (2006). Coffee, diabetes, and weight control. *The American journal of clinical nutrition*, 84(4), 682-693.
- Grosvenor, P. W., Gothard, P. K., McWilliam, N. C., Supriono, A., & Gray, D. O. (1995). Medicinal plants from Riau Province, Sumatra, Indonesia. Part 1: Uses. *Journal of Ethnopharmacology*, 45(2), 75-95.
- Guan, S., Ge, D., Liu, T. Q., Ma, X. H., & Cui, Z. F. (2009). Protocatechuic acid promotes cell proliferation and reduces basal apoptosis in cultured neural stem cells. *Toxicology in Vitro*, 23(2), 201-208.
- Huang, H. L., Li, D. L., Li, X. M., Xu, B., & Wang, B. G. (2007). Antioxidative principals of *Jussiaea repens*: an edible medicinal plant. *International journal of food science & technology*, 42(10), 1219-1227.
- Lin, H. H., Chen, J. H., Huang, C. C., & Wang, C. J. (2007). Apoptotic effect of 3, 4-dihydroxybenzoic acid on human gastric carcinoma cells involving JNK/p38 MAPK signaling activation. *International Journal of Cancer*, 120(11), 2306-2316.
- Muskiet, F. A., & Groen, A. (1979). Urinary excretion of conjugated homovanillic acid, 3, 4-dihydroxyphenylacetic acid, p-hydroxyphenylacetic acid, and vanillic acid by persons on their usual diet and patients with neuroblastoma. *Clinical chemistry*, 25(7), 1281-1284.
- Nakamura, Y., Torikai, K., Ohto, Y., Murakami, A., Tanaka, T., & Ohigashi, H. (2000). A simple phenolic antioxidant protocatechuic acid enhances tumor promotion and oxidative stress in female ICR mouse skin: dose-and timing-dependent enhancement and involvement of bioactivation by tyrosinase. *Carcinogenesis*, 21(10), 1899-1907.
- Oboh, G., Agunloye, O. M., Akinyemi, A. J., Ademiluyi, A. O., & Adefegha, S. A. (2013). Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain-in vitro. *Neurochemical Research*, 38(2), 413-419.
- Ooh, K. F., Ong, H. C., Wong, F. C., Sit, N. W., & Chai, T. T. (2014). High performance liquid chromatography profiling of health-promoting phytochemicals and evaluation of antioxidant, anti-lipoxygenase, iron chelating and anti-glucosidase activities of wetland macrophytes. *Pharmacognosy Magazine*, 10(39), 443.
- Panda, A., & Misra, M. K. (2011). Ethnomedicinal survey of some wetland plants of South Orissa and their conservation. *Indian Journal of Traditional Knowledge*, 10(2), 296-303.
- Perron, N. R., & Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell biochemistry and biophysics*, 53(2), 75-100.
- Rock, C. D. (2017). Phenylpropanoid Metabolism. *eLS*, 1-18.

- Sakihama, Y., Cohen, M. F., Grace, S. C., & Yamasaki, H. (2002). Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, 177(1), 67-80.
- Santosh, K., & Satya, N. (2010). Herbal remedies of wetlands macrophytes in India. *International Journal of Pharma and Bio Sciences*, 1(2).
- Sarkar, T. (2018). Role of hesperdin, luteolin and coumaric acid in arthritis management: A Review. *International Journal of Physiology, Nutrition and Physical Education* 3(2), 1183-1186.
- Silva, T., Oliveira, C., & Borges, F. (2014). Caffeic acid derivatives, analogs and applications: A patent review (2009–2013). *Expert opinion on therapeutic patents*, 24(11), 1257-1270.
- Teixeira, J., Gaspar, A., Garrido, E. M., Garrido, J., & Borges, F. (2013). Hydroxycinnamic acid antioxidants: an electrochemical overview. *BioMed research international*, 2013.
- Watanabe, T., Arai, Y., Mitsui, Y., Kusaura, T., Okawa, W., Kajihara, Y., & Saito, I. (2006). The blood pressure-lowering effect and safety of chlorogenic acid from green coffee bean extract in essential hypertension. *Clinical and experimental hypertension*, 28(5), 439-449.
- Watson, R. R., Preedy, V. R., & Zibadi, S. (Eds.). (2013). *Polyphenols in human health and disease*. Academic press.