CHEMICAL ENGINEERING TRANSACTIONS
VOL. 122, 2025
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Bohong Wang, Guo Ren Mong, Petar S. Varbanov

ISBN 979-12-81206-23-6; **ISSN** 2283-9216
DOI: 10.3303/CET25122060

The Processing of Antibiotic-Resistant Bacterial Resources from Sludge in the Cattle Wastewater through *Cratoxylum Pruniflorum* Extract in Vietnam

Thanh Thi Ngoc Lan Mai*, Huy Anh Tran, Dat Tan Tran, Thuy-Nga Huynh Nguyen

Thu Dau Mot University, 6 Tran Van On, Phu Loi District, Ho Chi Minh City.
thanhmtnl@tdmu.edu.vn

Water disinfection is a critical issue for maintaining a healthy life, especially in developing countries. Disinfection of water also demands destruction or removal/inactivation of pathogenic microorganisms from drinking water/raw water/groundwater to prevent recontamination of water in the distribution system and maintain the safest quality of water. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most commonly reported bacteria in disinfection studies. In the next century, the rise of antibiotic-resistant bacteria has raised concerns worldwide. Sludge from the cattle wastewater is a source of antibiotic-resistant bacteria. In this study, the antibiotic-resistant bacteria were isolated from sludge, which is reported. Next, these antibiotic-resistant bacteria were exposed to the plant extract. The results showed that these bacterial strains were killed by the plant extract, and the water quality was then rechecked. The developed approach emphasises the importance of returning to natural methods not only for traditional water disinfection but also for eliminating hazardous antimicrobial-resistant (AMR) bacteria. Additionally, this method may be useful in various other applications aimed at removing antimicrobial bacteria. In this research, plant extract was collected from the *Cratoxylum* sp. species using the maceration method. The effectiveness of the plant extract against antibiotic-resistant bacteria was evaluated through the diffusion method. The water quality was evaluated for the absence of antibiotic-resistant bacteria, which was verified using the spreading method. The study successfully extracted *Cratoxylum pruniflorum* D. with an efficiency of 50.01 %. It also established the optimal conditions for obtaining this extract to test its effectiveness against MRSA and *Staphylococcus* sp. isolated from livestock wastewater. When applied in a laboratory wastewater treatment model, the extract significantly reduced bacterial cell density 5 x 10^6 times. Additionally, the Sulforhodamine B (SRB) test indicated that the *Cratoxylum pruniflorum* D. extract was not toxic to the two tested cell lines, highlighting its potential for use in wastewater treatment.

1. Introduction

Over the past years, antimicrobials have been widely used as growth promoters in breeding practices, as well as in human and veterinary medicine. With the rapid growth of cattle farming to meet society's rising consumption demands, concerns about environmental pollution from this industry are becoming increasingly significant. One major issue is livestock wastewater, which is a serious source of pollution. This wastewater often contains numerous pathogenic microorganisms, particularly opportunistic bacteria like *Staphylococcus aureus* (Lépesová et al., 2018)

S. aureus is a bacterium that exists in the environment and the human body as an opportunistic microorganism. In nature, *S. aureus* carries antibiotic resistance genes and can transfer these genes to other microorganisms, so the presence of antibiotic-resistant bacteria in livestock wastewater poses a risk of spreading to the environment and humans, presenting a serious threat to public health and the ecosystem. Vancomycin is the preferred medication for treating *S. aureus* infections. Vancomycin has a strong affinity for the D-ala-D-ala portion at the C-terminus of the pentapeptide, which is a precursor for cell wall synthesis in MRSA. By binding to this site, vancomycin inhibits cell wall synthesis in MRSA. One important characteristic of vancomycin is that it cannot penetrate the cytoplasmic membrane. As a result, it only interacts with its drug target after the precursor has been embedded in the membrane's surface. In Japan, vancomycin-resistant *Staphylococcus aureus* was

Paper Received: 27 July 2025; Revised: 1 October 2025; Accepted: 12 December 2025
Please cite this article as: Mai T.T.N.L., Tran H.A., Tran D.T., Nguyen T.-N.H., 2025, The Processing of Antibiotic-Resistant Bacterial Resources from Sludge in the Cattle Wastewater Through Cratoxylum Pruniflorum Extract in Vietnam, Chemical Engineering Transactions, 122, 355-360 DOI:10.3303/CET25122060

first reported in 2001 (Hiramatsu, 2001). While there have been no reported cases of vancomycin-resistant *S. aureus* in Vietnam, monitoring for such cases and implementing measures to prevent their emergence is urgently needed.

MRSA resisted cefoxitin, which is a second-generation cephalosporin antibiotic used to treat a variety of infections, including serious ones. It works by inhibiting the synthesis of bacterial cell walls, preventing bacterial growth (Brogden et al., 1979). Microbes are powerful bioconversion agents that play important roles in the degradation of both natural and synthetic compounds, including drugs and antibiotics, a characteristic that contributes to their antibiotic resistance (Mora-Gamboa et al., 2022). Land application and disposal of both human and cattle waste of faecal origin are becoming routine procedures in many regions around the world (Anand et al., 2021). Faecal sludge can contain disease-causing microorganisms even after being undisturbed in the pit for months. Antibacterial and antimicrobial properties have been reported in various plant extracts. Mai et al. have made a significant discovery by identifying MRSA-resistant plants within the phylum *Cratoxylum*, highlighting their potential to combat antibiotic-resistant strains and revolutionise our approach to infection control (Mai, 2024). *Cratoxylum pruniflorum* D. is known in Vietnam as "Nganh nganh long". Flavonoids are a group of compounds that represent a significant portion of the extract from *Cratoxylum pruniflorum* D., such as quercetin, kaempferol, rutin, apigenin, luteolin, and epicatechin. These compounds are well-known for their strong antioxidant properties, which help protect cells from damage caused by free radicals. Additionally, they exhibit antibacterial, anti-inflammatory, and anti-cancer effects through various biological mechanisms, such as inducing apoptosis, inhibiting inflammatory enzymes, and interacting with bacterial cell membranes. The application of naturally derived methods and materials in environmental remediation, particularly for the control of bacterial contamination in livestock wastewater, represents a promising research direction. Such approaches are environmentally sustainable, capitalise on locally available resources, and contribute to ecological improvement while supporting the advancement of green and sustainable agriculture.

2. Material and methods

Sludge specimens were obtained from a manhole at the livestock farm. *Cratoxylum pruniflorum* D., locally referred to in Vietnam as "Nganh nganh long," was collected from the Ma Da forest in Dong Nai Province, Vietnam (11°08'39.2" N, 107°02'56.6" E). The MRSA reference strain ATCC 33591 was procured from Microbiologics (USA). Hepatic cancer (G2) cells and fibroblast cells were sourced from the American Type Culture Collection (ATCC, USA).

2.1 Isolation of bacteria resistant to cefoxitin and vancomycin

1 g of the sludge sample was weighed and added to 99 mL of distilled water. Then, 1 mL of this mixture was taken out and added to 9 mL of distilled water. This process was repeated until the fifth dilution. From each dilution bottle, 0.1 mL was streaked onto an agar plate. A hockey stick was used to spread the dilution evenly on the medium. The plates were sealed with parafilm, labelled, and then placed in an incubator at 30 °C for 24 h (Galler et al., 2018).

2.1.1 Isolation of microorganism

Soil samples were collected from municipal sewage and municipal sludge. The isolation of microorganisms was performed until single colonies were obtained using the serial dilution method and the streaking method. Twenty agar plates were prepared aseptically. Once the agar plates were ready, a plate with fast-growing bacteria was selected. Bacteria of different shapes on the plate were chosen for further analysis. Using aseptic techniques, the selected bacteria on the agar plate were streaked onto the plate, which was then divided into four sections. The streaked agar plate was sealed and incubated at 30 °C for growth. After 24 h, bacterial purification was conducted using another spread plate technique followed by additional incubation. Additionally, Gram staining was performed on samples from the previous plate. The samples were observed under a microscope and characterised based on their appearance (Lozano et al., 2009).

2.2 Preparation of ethanol extract of *Cratoxylum pruniflorum* D

After collecting the plant samples, which were washed with water to remove dirt, they were then rinsed with distilled water. The *Cratoxylum pruniflorum* D includes leaves and twigs that were air-dried naturally at approximately 30 ºC ± 2 ºC. After drying, the leaves and twigs of *Cratoxylum pruniflorum* D were ground into powder using a medicinal herb grinder. Samples were weighed over 2 to 3 days to confirm a constant mass. Use 99.5 % ethanol to soak the medicinal powder for plant extract isolation at a ratio of 1 g of powder to 10 mL of ethanol. These extract solutions were soaked in a thermostatic shaker at 50 ºC with the mixture shaken at 100 rpm for 24 h. After extraction, any residues were removed from the mix by Whatman filter paper. The plant extract was collected by a rotary evaporator at 30 ºC. The crude extract of *Cratoxylum pruniflorum* D was then

dried in a cabinet at 50 ºC until a constant mass was achieved and stored at 4 ºC for future use (Mai et al., 2023). The extraction efficiency is determined as the ratio of the mass of the obtained extract to the mass of the dry medicinal material, according to Eq(1).

$$E = \frac{m1(g)}{m0(g)} x100\% \quad (1)$$

E: The extraction efficiency (%); m_1: Weigh of plant extract (g); m_0: Weigh of dried plant extract (g)

2.3 Assessment of antibacterial activity from extracts of *Cratoxylum pruniflorum* D. against MRSA and antibiotic-resistant bacteria in cattle wastewater using the disc diffusion method

The Kirby-Bauer method was employed in the experiment. 100 µL of a standardized inoculum suspension with a concentration of 10^6 CFU/mL was added to each well. Three wells, each with a diameter of 4 mm, were created in the plates. In wells No. 1,2,3 100 µL of crude extract (with concentration equals 16.648 mg/mL) was added. The plates were incubated for 18 h-24 h, at 37 °C. After 18 h-24 h, the diameters of the inhibition zones were measured in millimetres (mm), were validated using CLSI documents (Mai et al., 2020). The experiment was practiced in three times.

2.4 Testing of microbial treatment in water on wastewater model contaminated with antibiotic-resistant bacteria

Evaluation of the growth inhibition efficiency of bacterial strains isolated from cattle wastewater using extract. The evaluation metric is turbidity (OD_{600}) measured after 18 h to 24 h of incubation, compared to the control experiment to determine the percentage of inhibition. 10 mL *Staphylococcus* sp. solution was added at a concentration of 10^9 CFU/mL into a flask containing 126 mL of sterile water and 64 mL of a 50 mg/mL *Cratoxylum pruniflorum* D. extract solution. 136 mL of wastewater was added to the second flask, which already contained 64 mL of a 50 mg/mL *Cratoxylum pruniflorum* D. extract solution. Each experiment was conducted three times using six triangular flasks. Place six 250 mL conical flasks in a microbiological incubator at 37 °C for 18 h to 24 h. Afterward, measure the optical density (OD) to determine the bacterial cell density in the sample following treatment.

2.5 The cell toxicity of *Cratoxylum pruniflorum* D.

Sulforhodamine B (SRB) is used to test the toxicity of drugs on cells. This negatively charged dye binds to the positively charged portions of proteins, allowing researchers to determine the total protein content of cells based on the amount of remaining dye after washing. Cells were meticulously fixed, washed, and stained with SRB, resulting in a strikingly clear binding solution that exhibited an appealing pink hue. This clarity and vibrant color not only highlight the effectiveness of the staining process but also emphasize the precision involved in our methodology. The optical density (OD) value of this solution accurately quantifies the total protein content within the cells. The number of live cells in test samples compared to control samples assesses the drug's toxicity. E-MEM medium is utilized for the culture of hepatic cancer cells (G2) and fibroblast cells, which supplemented with L-glutamine (2 mM), 10 % (v/v) fetal bovine serum (FBS), HEPES (20 mM), penicillin G (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (0.025 µg/mL). The cultures were incubated in an atmosphere containing 5 % CO_2 at 37 °C. Each well of a 96-wells microplate contained 10^4 cells. After 24 h, additional culture medium was added, and the cells were incubated for another 48 h. The total protein is precipitated by the cold trichloroacetic acid method, followed by staining with 0.2 % sulforhodamine B. The plates were measured using a plate reader at 492 nm and 620 nm. The percentage of growth inhibition (Inh %) was determined using Eq(2):

$$\text{Inh } \% = \left(1 - \frac{ODt}{ODc}\right) x\ 100\% \quad (2)$$

In which ODt: the OD of sample; ODc: The OD of control; Camptothecin: a positive control. Prism software was used to identify IC50 using non-linear regression method multivariable and R2>0.9 (Nguyen and Ho-Huynh, 2016). Experiments were repeated three times, presented as mean ± standard deviation. STATGRAPHICS Centurion XV software is used to analysize the data (Statgraphics Centurion, 2006).

3. Results and discussion

3.1 Isolation of bacteria resistant to cefoxitin and vancomycin

Cattle wastewater was added to TSA medium containing cefoxitin at a concentration of 256 µg/mL and vancomycin at a concentration of 64 µg/mL after 24 h of incubation, resulting in the appearance of bacterial colonies. Individual colonies will be re-cultured on TSB medium with cefoxitin at 256 µg/mL and vancomycin at

64 µg/mL, and overnight for 18 h to 24 h in an incubator, at 37 ºC. The overnight culture was diluted to a concentration of 10^{10} and spread onto TSA medium containing the antibiotics cefoxitin and vancomycin. The plates were then incubated for 18 h to 24 h at 37 ºC. After incubation, the colonies were purified until only pure colonies remained on TSA medium containing cefoxitin at a concentration of 256 µg/mL and vancomycin at 64 µg/mL. The pure colonies were observed fresh, then subjected to Gram staining before being examined under a microscope. Isolated cultures were next evaluated on Mannitol Salt agar and Blood agar to identify the genus *Staphylococcus*. The results are presented in Figure 1.

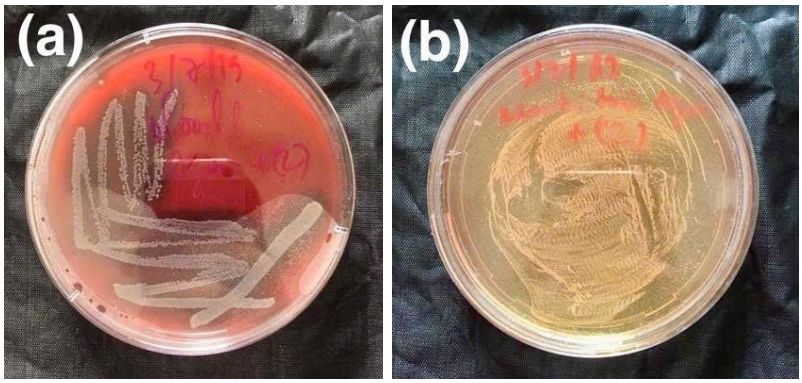

Figure 1: The presence of (a) bacteria on blood agar; (b) bacteria on Manitol Salt Agar

The study identified a strain of *Staphylococcus* sp. that is resistant to cefoxitin and vancomycin, isolated from cattle wastewater.

3.2 Preparation of ethanol extract of *Cratoxylum pruniflorum* D.

After the extraction of plant samples with 96 % ethanol at 50 °C for 24 h, the dry mass obtained after drying was 14.443 g respectively from 28.878 g of initial dry sample, corresponding to an average extraction efficiency of about 50.01 %. The results are presented in Table 1.

Table 1: The extraction efficiency

Plant's name	Weight of plant extract	extraction efficiency
Cratoxylum pruniflorum D.	4.814 ± 0.001	50.01± 0.02

The values are expressed as mean ± SD (n=3)

Ethanol 96 % is a widely used solvent in medicinal research due to its effectiveness in dissolving various biological compounds, including flavonoids, alkaloids, phenols, tannins, and glycosides. However, it is important to note that ethanol is unable to completely dissolve non-polar components, such as lipids, or polymer structures like cellulose and lignin. This limitation accounts for the relatively low extraction efficiency of certain compounds. The extraction process achieved a high efficiency of 50.01 %, successfully collecting bioactive compounds that are soluble in ethanol. The optimal extraction conditions used were a temperature of 50 °C, a duration of 24 h, and a shaking speed of 100 rpm. These parameters not only enhanced solubility but also prevented the thermal decomposition of sensitive active ingredients. When compared to reference documents and similar studies, this recovery efficiency falls within the optimal range, ensuring both the quantity and quality of the extract. Consequently, high-concentration ethanol can be utilised in subsequent research steps, such as the qualitative and quantitative analysis of active substances, biological activity assessments, or the enhancement of product testing protocols.

3.3 Assessment of antibacterial activity from extracts of *Cratoxylum pruniflorum* D. against MRSA and antibiotic-resistant bacteria in cattle wastewater using the disc diffusion method

The antibacterial activity from extracts of *Cratoxylum pruniflorum* D. against MRSA and antibiotic-resistant bacteria in cattle wastewater are presented in Figure 2.

According to Figure 2, the results show that the extract of Cratoxylum pruniflorum D. demonstrates resistance to MRSA, with a resistance zone measuring 15.17 ± 1.31 mm. Additionally, it is also resistant to *Staphylococcus* sp. isolated from cattle wastewater, with a resistance zone size of 12.83 ± 0.76 mm. Based on these results

which can conclude that the extract of *Cratoxylum pruniflorum* D. exhibits anti-MRSA and anti-*Staphylococcus* sp. activity, which was isolated from livestock wastewater.

Previous studies have reported the antibacterial activity of the *Cratoxylu*m genus. For example, Thanh et al., 2024 investigated the clinical anti-MRSA activity of *Cratoxylum cochinchinensis* L. in Vietnam. The results of the study determined the *C. cochinchinensis* L. extract against the clinical MRSA with effective concentrations ranging from 1.625 mg/mL to 3.250 mg/mL (Mai, 2024). In this research, the concentration of *Cratoxylum pruniflorum* D. extract against MRSA and *Staphylococcus* sp was isolated from livestock wastewater, equaling 1.6648 mg/mL. These results, compared with the other results about anti-MRSA of the *Cratoxylum* genus, showed that the anti-bacterial activity of *Cratoxylum pruniflorum* D. extract is similar results. Moreover, the anti-MRSA activity of *Cratoxylum pruniflorum* D. extract has not yet been reported.

a) b)

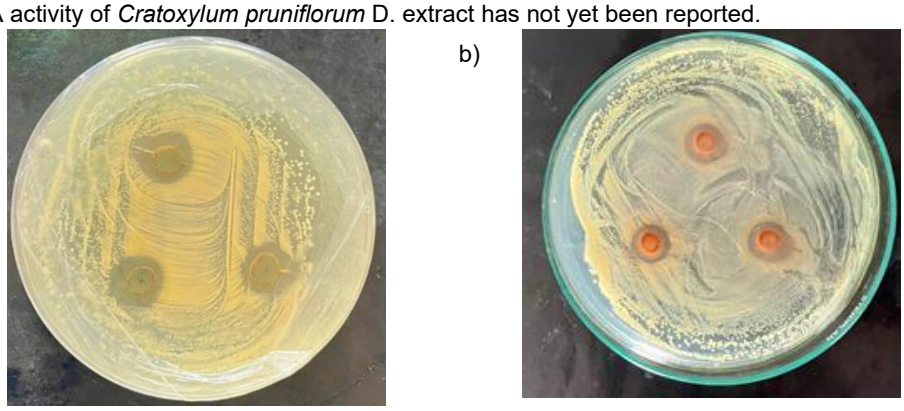

Figure 2: The antibacterial activity from extracts of Cratoxylum pruniflorum D. against (a) MRSA; (b) antibiotic-resistant bacteria in cattle wastewater

3.4 Testing of microbial treatment in water on wastewater model contaminated with antibiotic-resistant bacteria

To assess the effectiveness of the extract in treating wastewater using a pilot model, the project designed the following experiments: Experiment 1, sterilised water supplemented with isolated antibiotic-resistant bacteria; and Experiment 2, wastewater collected directly from the livestock farm's pit treated with *Cratoxylum pruniflorum* D. extract. The optical density of the treatments was measured, and the results are presented in Table 2.

Table 2: Results of OD measurements for water samples treated with Cratoxylum pruniflorum D. *extract*

Sample	OD/Cell density	
	OD value	Cell density (CFU/mL)
Sterilized water supplemented with *Staphylococcus sp* at a concentration of 10^9 CFU/mL	0.1534	5 x 10^7
Wastewater enriched with an extract of *Cratoxylum pruniflorum* D. (16 mg/mL).	0.0388	10^1

According to the results shown in Table 2, the cell density after treatment with *Cratoxylum pruniflorum* D. extract decreased by 5 x 10^6 times. This significant reduction in bacterial content in wastewater demonstrates the antibacterial properties of *Cratoxylum pruniflorum* D. extract against MRSA and *Staphylococcus* sp. isolated from livestock wastewater.

3.5 The cell toxicity of *Cratoxylum pruniflorum* D.

The results of the cell toxicity of *Cratoxylum pruniflorum* D. extract, as shown in Table 3.

Table 3: Percentage of cytotoxicity of the sample on the cell line.

Cell line	Percentage of Cytotoxicity (%)	
Fibroblast	16.94	0.83
HepG2	-9.89	3.43

The values are expressed as mean ± SD (n=3)

The cytotoxic effects of *Cratoxylum pruniflorum* D. extract were evaluated on two fibroblast cell lines and HepG2 liver cancer cells. The results indicated that the extract of *Cratoxylum pruniflorum* D. was not toxic to the two cell lines tested, suggesting its potential application in treating contaminated wastewater. *Cratoxylum pruniflorum* D., traditionally consumed in Vietnam as a sedative tea for alleviating insomnia and stress, represents a valuable medicinal resource. Reassessing the cytotoxicity of its extract is therefore of scientific importance and may provide the basis for expanded applications in water treatment, pharmaceuticals, and functional foods. These findings highlight the scientific value of *Cratoxylum pruniflorum* D. extract

4. Conclusions

The study successfully extracted *Cratoxylum pruniflorum* D. with an efficiency of 50.01 %. It also established the optimal conditions for obtaining this extract to test its effectiveness against MRSA and *Staphylococcus sp.* isolated from livestock wastewater. When applied in a laboratory wastewater treatment model, the extract significantly reduced bacterial cell density 5 x 10^6 times. Additionally, the SRB test indicated that the *Cratoxylum pruniflorum* D. extract was not toxic to the two tested cell lines, highlighting its potential for use in wastewater treatment. MRSA is a community-acquired antibiotic-resistant bacterium. Treating MRSA with plant-derived extracts contributes to environmental protection and reduces the risk of drug resistance. This method also has the potential to become a sustainable technology for wastewater treatment.

References

Anand U., Reddy B., Singh V.K., Singh A.K., Kesari K.K., Tripathi P., Simal-Gandara J., 2021, Potential environmental and human health risks caused by antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs) and emerging contaminants (ECs) from municipal solid waste (MSW) landfill, Antibiotics, 10(4), 374.

Brogden R., Heel R., Speight T., Avery G., 1979, Cefoxitin: a review of its antibacterial activity, pharmacological properties and therapeutic use, Drugs, 17(1), 1-37.

Galler H., Feierl G., Petternel C., Reinthaler F.F., Haas D., Habib J., Zarfel G., 2018, Multiresistant bacteria isolated from activated sludge in Austria, International journal of environmental research and public health, 15(3), 479.

Hiramatsu K., 2001, Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance, The Lancet infectious diseases, 1(3), 147-155.

Lépesová K., Kraková L., Pangallo D., Medveďová A., Olejníková P., Mackuľak T., Birošová L., 2018, Prevalence of antibiotic-resistant coliform bacteria, *Enterococcu*s spp. and *Staphylococcu*s spp. in wastewater sewerage biofilm, Journal of global antimicrobial resistance, 14, 145-151.

Lozano C.J.S., Mendoza M.V., de Arango M.C., Monroy E.F.C., 2009, Microbiological characterization and specific methanogenic activity of anaerobe sludges used in urban solid waste treatment, Waste management, 29(2), 704-711.

Mai T.T.N.L, 2024, The Potency of The Cratoxylum cochinchinensis L. Fractional Extract against Some Clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Viet Nam, Chemical Engineering Transactions, 113, 313-318.

Mai T.T.N.L., Hoang H.A., Truong T.V, 2020, Antibacterial activity of tram tron *Syzygium glomerulatum* extract against methicillin-resistant *Staphylococcus aureus*, Chemical Engineering, 78.

Mai T.T.N.L., Le M., Hoang H.A., Truong T.V., 2023, Anti-Methicillin resistant *Staphylococcus aureus* Activity of *Carallia brachiata* L. Extract, Chemical Engineering Transactions, 106, 25-30.

Mora-Gamboa M.P., Rincón-Gamboa S.M., Ardila-Leal L.D., Poutou-Piñales R.A., Pedroza-Rodríguez A.M., Quevedo-Hidalgo B.E., 2022, Impact of antibiotics as waste, physical, chemical, and enzymatical degradation: Use of laccases, Molecules, 27(14), 4436.

Nguyen M.N.T., Ho-Huynh T.D., 2016, Selective cytotoxicity of a Vietnamese traditional formula, Nam Dia long, against MCF-7 cells by synergistic effects, BMC complementary and alternative medicine, 16(1), 220.