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Antibiofilm Activity of Erigeron floribundus (Kunth) against Staphylococcus aureus and Pseudomonas aeruginosa

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ABSTRACT

Antibiotic-resistant biofilm is a complex microbial community associated in the high risk of morbidity and mortality of hospitalized patients. The current study aims to determine the antibiofilm activity of invasive Erigeron floribundus plant extracts often known as "abas-abas" against Staphylococcus aureus and Pseudomonas aeruginosa. Bacterial strains were grown in M1 medium supplemented with fructose and cultivated for 24 and 48 hours at 30°C. Higher % biofilm inhibition was observed in gram-positive bacteria Staphylococcus aureus in most of the solvent use in extraction. Qualitative phytochemical screening was also evaluated and revealed the presence of tannins, flavonoid, saponins, steroid and the absence of alkaloid. The existences of these phytochemicals in the plant could be used to generate synthetic medications as a source of precursors. Moreover, this study also reveals that E. floribundus extract had antibiofilm action against the isolated nosocomial bacteria, implying that it could be used as an alternative to prevent microbial biofilm development.

Keywords: antibiofilm agent, invasive plants, Pseudomonas aeruginosa, Staphylococcus aureus

INTRODUCTION

Universities play a critical role in producing human HED) imOver 30% of cases were found to have bacterial coinfection during the 2009 H1N1 influenza pandemic, despite antibiotic therapy (Rice et al., 2012; Westblade et al., 2021). Viral lung infection weakened the immune system of the host, and altered the makeup and functions of the respiratory microbiota, predisposing hosts to bacterial coinfections (Feldman and Anderson, 2021). Several retrospective studies based on cases from various geographical regions have also shown bacteria coinfecting with SARS-CoV-2. The common co-infecting bacterial species include Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae, Mycoplasma pneumonia, Streptococcus pneumoniae, and Pseudomonas aeruginosa (Fattorini et al., 2020; Hughes et al., 2020; Chen et al., 2020; Bashir et al., 2021; de Buhr & von Köckritz-Blickwede, 2021). Bacterial coinfections worsen respiratory viral infections and are a common cause of death in influenza pandemics, but they're not well understood in individuals with coronavirus disease (COVID-19). One factor for co-infection is its ability to form a microbial biofilm. They are highly resistant to treatments and the immune systems of their hosts, making them a primary virulence factor that causes long-term infections (Muhammad et al., 2020). Biofilm formation composing of exopolysaccharide-protected bacteria up to 1,000 times more resistant to antibiotics than planktonic (free-floating) bacteria, posing substantial therapeutic challenges and complicating treatment alternatives (Sanchez et al., 2013). An estimated 75 percent of bacterial infections are estimated to be caused by biofilms, which are protected by an extracellular matrix. Biofilm inhibition is a popular treatment target for a range of bacterial and fungal infections, and the pharmacological development of these medicines is now being studied extensively (Ramanathan et al., 2021). In several pathogens, this process results in the generation of virulence factors and/or a change in bacterial lifestyle, which is a crucial determinant of the infection's outcome and severity. Recent advancements in our understanding of the genetic and molecular foundation of bacterial community behavior indicate potential treatment targets for biofilm infection.

Pseudomonas aeruginosa is frequently isolated from the airways of patients with cystic fibrosis or with respiratory infections, it most often establishes chronic infections that usually persist for the rest of the lives of the patients (Davies, 2002). This bacterium is a leading cause of death and morbidity, and it has been extensively researched. On the other hand, the Staphylococcus aureus biofilm-associated burden is challenging to the field of medicine to eradicate or avoid. Even though several S. aureus biofilm mechanisms have been established, there is still a need to know more and require the development of new therapeutic strategies. In this viewpoint, we investigated the potential use of invasive Erigeron floribundus, locally known as abas-abas on how they affect the biofilm formation inhibition in nosocomial infections.

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One promising alternative is the search for naturally occurring plant-derived chemicals that can block biofilm formation (Rojita et al., 2020). Historically, plant extracts and their physiologically active compounds have long been a rich source of natural goods that have aided in illness prevention, treatment, and health maintenance. Furthermore, they are widely accepted due to the perception that they are safe and have a long history of use in folk medicine to cure diseases and illnesses since ancient times. This study evaluated the antibiofilm activity of an aqueous extract of Erigeron floribundus (Kunth) or (syn": Conyza sumatrensis (Retz) E.K. Walker) a terrestrial herbaceous plant of the family Asteraceae. Folkloric use of the plant shows multiple traditional benefits to treat several diseases, including rheumatism, gout, cystitis, nephritis, dysmenorrhoea, and dental pain. Since the plant is used traditionally in the treatment of painful illnesses, it is valuable to evaluate its antimicrobial biofilm activity. In vitro, the biological activities of E. floribundus essential oil displayed antioxidant, antimicrobial, and antiproliferative activities (Petrelli et al., 2016). Furthermore, it showed inhibitory effects on nicotinate mononucleotide adenylyltransferase (NadD), a promising new target for developing novel antibiotics. It also possesses interesting bioactivity in anti-inflammatory, immunomodulatory, antiplasmodial, antioxidant, antiproliferative, and antimicrobial activities (Tra Bi et al., 2008; Menan et al., 2006; Kuiate et al., 2005). Given the plant's widespread usage in traditional medicine as well as its in vitro biological activities, it's worth investigating the causes for its bioactivity by evaluating its biofilm inhibitory activity against nosocomial pathogens in various extraction solvents.

METHODOLOGY

Bacterial Strains: Pseudomonas aeruginosa BIOTECH 1335 and Staphylococcus aureus BIOTECH 1582 strains were obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Banos, Philippines.

Plant Materials: Fresh and healthy plants growing around the Bukangliwayway community, located at Kibawe, Bukidnon (7.4815 N, 125.0384 E), were collected between February to May 2020. Samples of vouchers were deposited at the herbarium of Central Mindanao University Herbarium (CMUH) while duplicates were sent to Philippine National Herbarium (PNH). Collected plants were rinsed severally with clean tap water to make them dust and debris free. Then, the leaves were spread evenly and dried in the shady condition for 3 to 4 days until they became crispy while still retaining the greenish coloration at room temperature (25±2°C). Finally, dried materials were ground in an electric chopper to get fine powder form for further analysis.

Medium: Nutrient-rich (NR) medium was used in seeding, maintenance, and storage of the bacteria and mineral salts (M1) medium of the same composition as that reported by Gutierrez et al., (2013 for biofilm formation analysis). The solvents used were purchased from were purchased from RCI Labscan.

Preparation of plant extracts: The dried and powdered samples (each 50g were extracted successively with double distilled water, ethanol, and methanol (each 400ml) for 10 12 hrs. Then, the collected solutions were filtered through Whatman No-1 filter paper. The extracts were evaporated to dryness under reduced pressure at 50°C using a rotating vacuum evaporator, and then stored in a freeze condition at 180°C until used for further analysis.

Instrumentations: The samples were evaporated through the rotary vacuum evaporator from 60 - 100°C according to the B.P. of supplied solvents. Absorbance spectrophotometry was carried out using a UV-vis spectrophotometer (EI, model-1371). Wavelength scans and absorbance measurements were in 1ml quartz cells of 1cm path length.

Biofilm formation assay: Biofilm formation was determined using a protocol modified by Gutierrez et al., (2013), cells were grown in 4 ml of M1 medium with 70 mM fructose in the presence or absence of crude extract at 30°C in glass tubes without agitation for 24 and 48 h. Static biofilm formation was measured by visual inspection of the air-liquid interface of the cultures. Coverage of the air-liquid interface of the culture by a layer of cells and matrix material was considered a biofilm. The tubes were washed with distilled water and stained with 0.1% crystal violet solution for 20 min. After the addition of 4.5 ml of 95% ethanol to each tube, the adsorbed dye was quantified from the OD readings at 600 nm.

$$(\%)\ \text{inhibition} = \frac{OD\ control - OD\ sample\ x100}{OD\ control}$$

Qualitative Phytochemical Screening. The E. floribundus ethanolic extracts were subjected to standard phytochemical tests to evaluate their chemical composition for different active constituents; for these extracts (3–5 mg/mL), they were separately suspended in 1 mL of absolute ethanol or distilled water using clean test tubes.

Statistical Analysis: All experimental results were expressed as mean ± standard deviation (SD) for analysis performed in duplicate at least three times. Using SPSS software version 17.0, statistical analysis of the data was performed using Analysis of Variance (ANOVA) and mean comparison using Student's -test.

RESULTS AND DISCUSSION

E. floribundus is widespread in Brgy. Bukangliwayway, Kibawe, Bukidnon, even along the barangay highway, and in all sampling sites (Figure 1). It prefers undisturbed sites and is a problem in low-tillage systems plantations and crops. Despite these concerns, E. floribundus can sometimes be viewed as having some economic values, e.g., improving soil fertility and controlling soil erosion. The antimicrobial activities of various parts of E. floribundus have not previously been thoroughly studied, and there is a lack of the essential information needed to commercialize its contribution.

Preliminary antimicrobial tests of E. floribundus against S. aureus and P. aeruginosa through monitoring

Figure 1. Erigeron floribundus (Kunth) or (syn": Conyza sumatrensis (Retz) E.K. Walker)

its growth inhibition was quite variable between different solvents. From the different extraction solvents, chloroform extract showed significant inhibitory activity against Gram-negative bacteria P. aeruginosa than in gram positive bacteria S. aureus (Figure 2). This suggests that the type of solvent used in the extraction technique has a significant impact on the success of determining physiologically active chemicals from plant material. The results of our cell density antimicrobial activity assay revealed no significant difference in the test organisms to the five different E. floribundus leaf solvents extracts (acetone, ethanol, chloroform, methanol, and water).

Interestingly, biofilm formation of E. floribundus extracts against S. aureus showed significantly higher % biofilm inhibition using different solvent extraction as shown in Figure 3. Aqueous extractions significantly inhibited biofilm formation for almost 80% inhibition in S. aureus (Figure 3a). In contrast, E. floribundus showed lower % inhibition in Gram-negative bacteria P. aeruginosa (Figure 3b). These results demonstrated that E. floribundus can be a potential antibiofilm agent for Gram-positive bacteria that are responsible for the majority of hospital-acquired infections, such as nosocomial infection. The active compounds of E. floribundus must be investigated to understand the underlying principles of its inhibition. The cell wall of Gram-positive bacteria has a thick peptidoglycan layer that comprises linear polysaccharide chains connected by short peptides, providing a rigid structure that makes extract penetration difficult while Gram-negative bacteria have a thinner peptidoglycan layer. However, in this study, we have successfully found an interesting candidate against gram-positive bacteria.

Moreover, the photochemical analysis of E. floribundus also revealed the presence of tannins, flavonoids, saponins, steroids, and absences of alkaloids in ethanol extracts (Table 1). The existence of these phytochemicals suggests that the plant could be used to generate synthetic medications as a source of precursors. The absence of alkaloids indicates that the plant is not harmful or toxic. The plant provides a readily source for tea, with the caffeoylquinic derivatives being the most abundant constituents (Berto et al., 2014).

Thus, further identification and characterization of E. floribundus active compound responsible for its antibiofilm agent must be quantified to develop formulated products for herbal and medicinal tract.

CONCLUSION

E. floribundus endowed antibiofilm agent, and this invasive weedy plant species can be a potential candidate

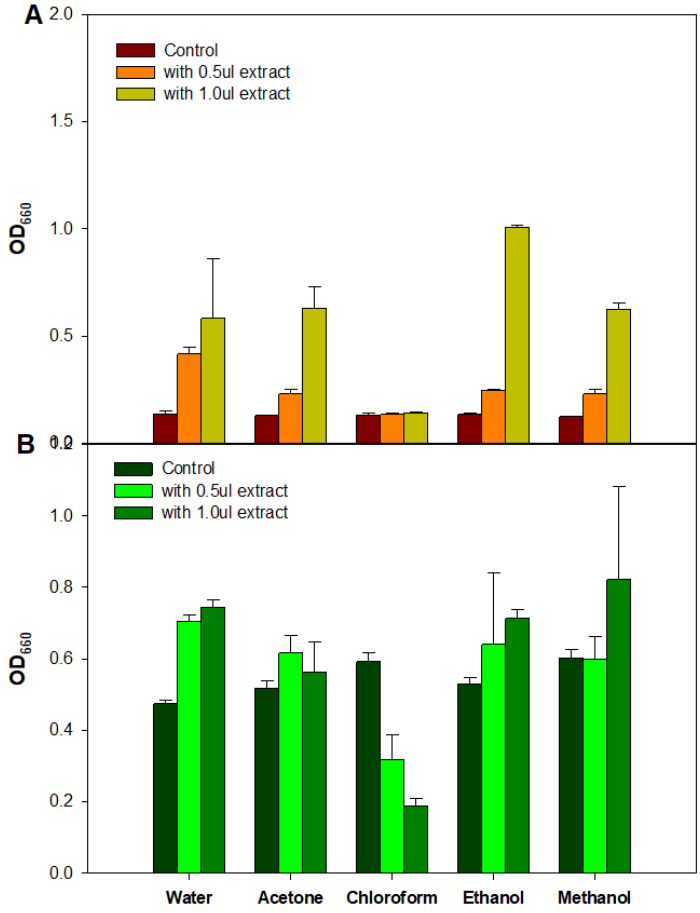

Figure 2: Growth of S. aureus and P. aeruginosa with E. floribundus plant extracts

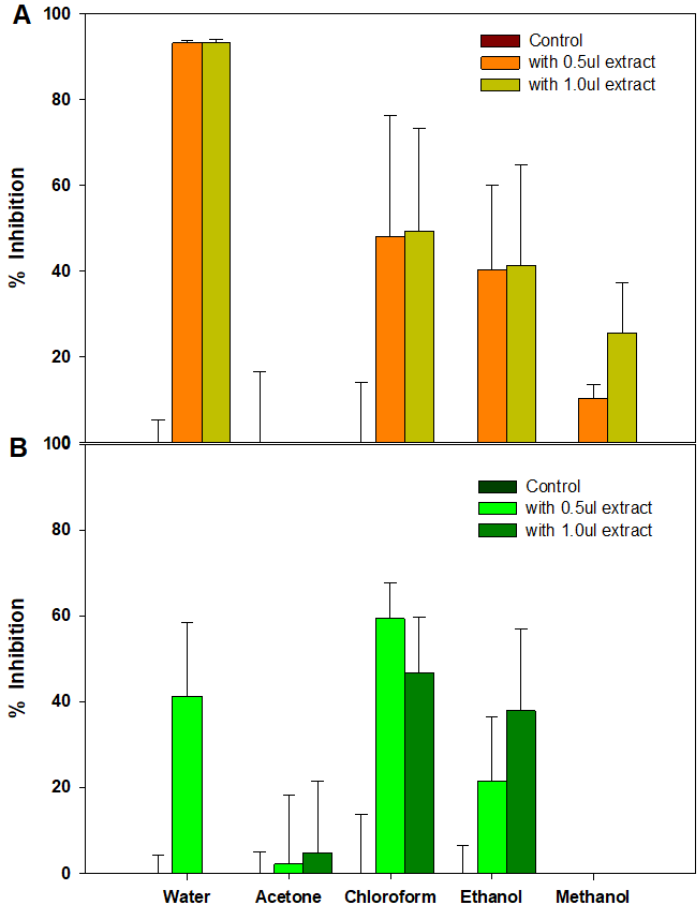

Figure 3: % biofilm inhibition of E. floribundus against (a) S. aureus and (b) P. aeruginosa

Table 1: Qualitative phytochemical analysis of *E. floribundus*

Active principle	Test	*Ethanolic extract*
Tannins	FeCl3	+
Saponins	Frothing test	+
Flavanoids	NaOH	+
Steroids	Salkwoski	+
Alkaloids	Wagner's test	-

as readily available sources of antimicrobial agent, some of which may be useful as microbial biofilm inhibitor and antibiotic drugs. The search for naturally occurring compounds of more invasive plants and developing several green nonlethal strategies capable of blocking biofilm formation is further recommended.

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