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Antibacterial activities from aqueous and ethanolic extracts of selected succulent plants

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Abstract

Succulents are admired for their beauty and have been popular among plant enthusiasts during the pandemic. However, succulents can also be tapped as potential sources of antibacterial compounds. Thus, this study aimed to determine the phytochemical compounds and antibacterial activity of selected succulents, *Aloe maculata, Agave potatorum*, and *Graptopetalum mendozae* against two Gram-negative bacteria, *Aeromonas hydrophila* and *Vibrio harveyi* and a Gram-positive bacterium, *Bacillus albus*. Aqueous and ethanolic extracts of these succulents were prepared and screened for the presence of some phytochemical substances. Antibacterial activity was assayed using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MIC was carried out based on turbidity using the microdilution method and the MBC was determined based on the ability of the substance to kill 99.9% of the target pathogen by streaking onto agar plates. The phytochemical test showed that all three succulents contained carbohydrates, phytosterols, flavonoids, phenols and tannins. *A. maculata* and *A. potatorum* showed the presence of glycosides while only *G. mendozae* exhibited the presence of alkaloids. MIC and MBC results for *A. hydrophila* showed that only the ethanolic extract of *A. maculata* can inhibit growth at 0.5g mL^{-1}. Both MIC and MBC results for *V. harveyi* showed that all extracts are effective in inhibiting the bacterium at 0.25 g mL^{-1} except ethanolic extracts of *G. mendozae* at 0.125g mL^{-1} and *A. potatorum* at 0.5g mL^{-1}. All extracts did not show any inhibition against *B. albus*. In conclusion, succulents possessed antibacterial properties against Gram-negative bacteria such as *A. hydrophila* and *V. harveyi* that could lead to the discovery of novel antibacterial medicines.

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Introduction

One of the concerns of global health is the evolution of various strains of microbes and our ability to treat these diseases depends on the discovery of new drugs through some traditional medicine (Frey and Meyers, 2010). According to Walsh (2003) and Roberts and Zembower (2021), there are 14 million deaths worldwide annually caused by these different disease-causing microbes and there is a phenomenon of antimicrobial resistance because antibiotics are overused and misused. These problems can be solved by the discovery of new antimicrobials through plants being screened for their antibacterial properties (Cowan, 1999).

There has been a surge of interest in the search for antimicrobial drugs derived from natural products over the past few decades, owing to the belief that drugs derived from plants are safer and more dependable than synthetic drugs, which may have adverse effects on the host in addition to their high cost (Debalke *et al.*, 2018). Thus, it is an urgent need to explore and discover new antimicrobials.

For thousands of years, nature has provided medical substances, and a remarkable number of modern medications have been identified from natural sources. Plant-based drugs have long dominated pharmaceutical medicine for the treatment of a variety of diseases (Arullappan *et al.*, 2009; Caipang *et al.*, 2019). The chemical and medicinal contents found in their natural form and secondary metabolites are a large reservoir of structural moieties that collaborate to exhibit a wide range of biological activities (Mangaiyarkarasi and Muhammad, 2015).

Succulents are known for their elegant beauty and are admired by most plant enthusiasts as it is poor in maintenance and flexes as an aesthetic. It became a trend as a hobby during the pandemic when lockdowns were implemented, and people tried to shift their minds to alleviate their anxiety about the effects of COVID-19 by taking care of plants. The rising demand for cultivation was a big hit during the season up until now when the disease was no longer rampant as before. In the study of Griffiths and Males (2017), succulent plants are known for their ability to store water and survive drought for long periods, on which 90-95% of the cell contents of the plant are water. Succulents are natively grown or cultivated, and have fleshy, thick leaves or stems; curiosity is about the shape and form of the plant and not about the flowers (Aileen, 2020). One of the succulent's most potent medicinal uses is its antimicrobial properties (Berniyanti and Mahmiyah, 2015). Additionally, it contains various compounds, including saponin, aluin, ligin, antraquinones, vitamins, and minerals. The biological capabilities of crude extracts of plant components and phytochemicals are well recognized, and they can be beneficial in medicinal treatments (Khan *et al.*, 2013).

The present study established the medical significance of succulent plants, namely the *Agave potatorum, Aloe maculata* and *Graptopetalum mendozae* from a collectors' perspective. It aimed to evaluate the phytochemical properties and antibacterial activities of extracts obtained from selected succulent plants against Gram-positive and Gram-negative bacteria. The findings will make it possible to discover some medically important and pharmacologically active constituents from these succulent plants.

Materials and methods

Study site

This study was conducted at the Biological Laboratory of the University of San Agustin, located at Gen. Luna St., Iloilo City, Philippines.

Collection of samples

The following succulent plants, namely, *Aloe maculata*, *Agave potatorum*, and *Graptopetalum mendozae* were utilized for this study. The identity of the succulents was confirmed by the staff at the Regional Office, Department of Agriculture, Iloilo City, Philippines. Leaf samples of succulent plants were collected, then thoroughly washed with tap water and distilled water to remove all the impurities on the surface. The leaves were sundried for 48 h to reduce the moisture content of the leaves by <10%.

Phytochemical screening

Standard techniques in the phytochemical analysis were utilized to test the leaf extracts for the presence of bioactive compounds: alkaloids, carbohydrates, glycosides, phenols and tannins, phytosterols, and flavonoids as described in Khalid *et al.* (2018), and Shaikh and Patil (2020).

Preparation of extracts

Aqueous extract

Sun-dried leaves were weighed, cut into small pieces and added to distilled water at a ratio of 1:5 (w/v). The samples were then placed inside a blender to thoroughly grind the leaves. The ground mixture was transferred into a sterile amber bottle and placed at 4°C for 24 hrs. After 24 hours, the extract was filtered using a sterile filter paper. The filtrate was transferred into a beaker, placed into a water bath and evaporated to dryness. The residue of the aqueous extracts of each plant was weighed, added with normal saline solution to obtain a stock solution of 2 g mL^{-1}, transferred to a 10 ml centrifuge tube and placed at -20°C for subsequent analyses. A working solution containing 1 g mL^{-1} of each extract was prepared using a normal saline solution as a diluent and two-fold serial dilutions were done for the antibacterial assays.

Ethanol extract

The ethanolic extracts from the leaves were obtained following the procedures used in the preparation of the aqueous extracts. However, instead of water, 80% ethanol was used for this purpose at a ratio of 1:4 (w/v).

Antimicrobial assays

Bacteria and culture media

The antimicrobial properties of plant extracts were tested against Gram-negative bacteria, *Aeromonas hydrophila* (Pakingking *et al.*, 2020) and *Vibrio harveyi* (Pakingking *et al.*, 2018) and Gram-positive bacterium, *Bacillus albus*, which was provided by the Biological Laboratory of the University of San Agustin. All the bacteria were grown in nutrient broth with an additional 1% NaCl for *V. harveyi* and incubated for 24 h. After incubation, the plate count (CFU mL^{-1}) was determined. Ten-fold serial dilutions were prepared using normal saline solution to obtain a final concentration of 1 x 10^4 CFU mL^{-1}. This bacterial concentration was used in all antibacterial assays.

Minimum inhibitory concentration (MIC)

The standard broth dilution method was used to determine the antibacterial activity from the aqueous and ethanolic extracts of the selected succulent plants by evaluating the visible growth of microorganisms in the microplate. Serial two-fold dilutions of the aqueous and ethanolic extracts in concentrations ranging from 0.125 g mL^{-1} to 1 g mL^{-1} were used to determine the MIC. Each concentration was done in triplicate and added with a similar volume of the bacteria at a concentration of 1 x 10^4 CFU mL^{-1}. The control contained only inoculated nutrient broth with bacteria and incubated for 24 h at 32°C for *A. hydrophila,* 28°C for *V. harveyi* and 35°C for *B. albus*. The visual turbidity of the tubes was observed before and after incubation to determine the MIC values.

Minimum bactericidal concentration (MBC)

Minimum Bactericidal Concentration (MBC) is the measure to check the growth of bacteria for 24 h of incubation on the lowest concentration of an antibacterial agent (Parvekar *et al.*, 2020). MBC values for the different extracts of succulents were determined using growth inhibition. Using the streak plate method, a sterile loop was dipped in the various concentrations (1g mL^{-1}, 0.5g mL^{-1}, 0.25g mL^{-1}, and 0.125g mL^{-1}) from the microplate and streaked onto the agar plates. Nutrient agar was used for testing bacterial inhibition of *A. hydrophila* and *B. albus*, while the Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar was used for *V. harveyi.*

The plates were incubated at 32°C for 18–24 h. The MBC was determined as the lowest concentration which inhibited the growth of the respective microorganisms, as indicated by the absence of bacterial growth on the agar plates. All assays were performed in triplicate. Nutrient broth inoculated with the bacteria served as a negative control for all the assays.

Results and discussion

Phytochemical analysis

In the present study, results of the phytochemical analysis show that *Agave potatorum, Aloe maculata* and *Graptopetalum mendozae* contained carbohydrates, phytosterols, flavonoids, phenols, and tannins. *A. maculata* and *A. potatorum* showed the presence of glycosides, while only *G. mendozae* exhibited the presence of alkaloids, as shown in Table 1. The results of this study confirm the findings of earlier works on the phytochemical composition of these succulents. For example, *A. potatorum* contained significant amounts of coumarins, alkaloids, flavonoids, saponins, tannins, cardiac glycosides and triterpenoid compounds in a qualitative assays conducted by Soto-Castro *et al.* (2021) and Almazán-Morales *et al.* (2022). *A. maculata*, which belongs in the Aloe family, contains tannins, saponins, flavonoids, steroids, terpenoids, and cardiac glycosides anthroquinones (Noor *et al.*,2008; Cock, 2015). Genova-Kalou *et al.* (2022) observed high levels of phenolic compounds and anthocyanins in *Graptopetalum paraguayense*, and further characterization using high performance liquid chromatography (HPLC) revealed the presence of gallic acid, flavone, genistin, daidzin, and quercetin (Chao *et al.*, 2019). Both *G. paraguayense* and G. *mendozae* belong in the same family, and it would not be surprising of the latter will contain these phytochemicals. The ability of the succulents to biosynthesize phytochemicals could be tapped as good sources of natural antioxidants (Karabourniotis and Fasseas, 1996).

Table 1. Qualitative analyses of phytochemical substances in different extracts of selected succulent plants.

	Alkaloids	Carbohydrates	Glycosides	Phytosterols	Flavonoids	Phenols and Tannins
G. mendozae	++	++	-	++	++	+
A. maculata	-	++	++	+	+	++
A. potatorum	-	++	++	++	+	++

++: intensely present, +: Present, - : Absent.

Antibacterial assays

After a 24-h incubation under aerobic conditions, the aqueous extracts of the three succulents had MIC (Table 2) and MBC (Table 3) of 0.125 g mL^{-1} against *A. hydrophila*. In comparison, the MIC and MBC of the ethanolic extracts of the three succulents against the bacterium were 0.125 g mL^{-1}, 0.5 g mL^{-1}, and 0.125 g mL^{-1} for *A. potatorum*, *A. maculata*, and *G. mendozae*, respectively. After a 24-h incubation under aerobic conditions, the aqueous extracts of the three succulents had MIC (Table 4) and MBC (Table 5) of 0.25 g mL^{-1} against *V. harveyi*. In comparison, the MIC and MBC of the ethanolic extracts of the three succulents against the bacterium were 0.5 g mL^{-1}, 0.25 g mL^{-1}, and 0.125 g mL^{-1} for *A. potatorum*, *A. maculata*, and *G. mendozae*, respectively.

Table 2. Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts against *Aeromonas hydrophila*.

Scientific name	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
Aloe maculata	Aqueous	-	-	-	-
	Ethanol	+	+	-	-
Graptopetalum mendozae	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
Control	Control	+	+	+	+

+ turbid; - not turbid.

After a 24-h incubation, all three plant extracts did not inhibit *B. albus* as shown by the presence of turbidity (Table 6) and bacterial growth (Table 7) in all the concentrations tested. Succulents are plants that are known for their ability to store water and survive drought for extended periods (Griffiths and Males, 2017). These plants were selected for use in this study because these could be potential sources of pharmacological properties due to the presence of various phytochemical compounds that could inhibit microorganisms (Choi *et al.,* 2015).

Table 3. Minimum Bactericidal Concentration (MBC) of aqueous and ethanolic extracts against *Aeromonas hydrophila*.

Scientific name	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
Aloe maculata	Aqueous	-	-	-	-
	Ethanol	+++	+++	-	-
Graptopetalum mendozae	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
Control	Control	+++	+++	+++	+++

*(+/++/+++) indicates bacterial growth on replicate plates; (-) no bacterial growth.

Agave potatorum is distinguished by its compact rosettes, layered with flat sheets, small teeth, and a spike on each leaf (García-Mendoza, 2010). In the study of Soto-Castro *et al.* (2021), fresh leaves of *A. potatorum* were used to test its effect on the mycelial growth of a fungus, *Pleurotus spp,* as it contains secondary metabolites that are capable of accelerating mycelial growth, while in the study of Monterrosas-Brisson *et al.* (2013), leaves of *Agave tequilana*, *Agave angustifolia*, and *Agave americana* were tested for the identification of anti-inflammatory compounds due to these species being used as traditional medicines in Mexico in treating various diseases.

Table 4. Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts against *Vibrio harveyi.*

Scientific name	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	+	-	-	-
	Ethanol	+	+	-	-
Aloe maculata	Aqueous	+	-	-	-
	Ethanol	+	-	-	-
Graptopetalum mendozae	Aqueous	+	-	-	-
	Ethanol	-	-	-	-
Control	Control	+	+	+	+

+ turbid; - not turbid.

Aloe species are native to South Africa, where the warm and arid climate is favorable; it exhibits a spiky tongue shape, the epidermis of the leaves has a thick cuticle, and the main bulk is mostly Aloe gel wrapped by the mesophyll tissue (Choi *et al.*, 2015). *G. mendozae* was first considered rare in the wild. However, due to various local cultivations, the species are now commonly grown (Cházaro-Basñez and Acevedo-Rosas, 2009). The same study also elucidated that aqueous extract of *G. paraguayense* shows anti-inflammatory and antioxidant activity (Chung *et al.*, 2005) and down-regulates the expression level of some oncoproteins of hepatocellular carcinoma (Hsu *et al.*, 2015).

Table 5. Minimum Bactericidal Concentration (MBC) of aqueous and ethanolic extracts against *Vibrio harveyi.*

Scientificname	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	++	-	-	-
	Ethanol	+	+	-	-
Aloe maculata	Aqueous	++	-	-	-
	Ethanol	+	-	-	-
Graptopetalum mendozae	Aqueous	+++	-	-	-
	Ethanol	-	-	-	-
Control	Control	+++	+++	+++	+++

*(+/++/+++) indicates number of bacterial growth on replicate plates; (-) no bacterial growth.

Table 6. Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts against *Bacillus albus*.

Scientific name	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
Aloe maculata	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
Graptopetalum mendozae	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
Control	Control	+	+	+	+

+ turbid; -not turbid.

In this study, the MIC and MBC of selected succulent plants (*A. potatorum, A. maculata*, and *G. mendozae*) against Gram-negative (*A. hydrophila* and *V. harveyi*) and Gram-positive (*B. albus*) bacteria were determined by microdilution method. It was noted that different bacteria differ in growth preferences wherein in *A. hydrophila*, only 0.125 g mL^{-1} and 0.25 g mL^{-1} tubes containing ethanol extracts of *A. maculata* have bacterial growth, and for *V. harveyi*, all 0.125 g mL^{-1} tubes containing ethanol and aqueous extract have bacterial growth except for the ethanolic extract of *G. mendozae* which has no growth. Also, only ethanolic extract of *A. potatorum* has bacterial growth at a concentration of 0.25g mL^{-1}. It implies that the bacteria will grow at low concentrations of the extract regardless of the extracting solvent that is used. On the other hand, all the concentrations tested were ineffective against Gram-positive bacteria as shown by the bacterial growth on the agar plates.

Table 7. Minimum Bactericidal Concentration (MBC) of aqueous and ethanolic extracts against *Bacillus albus.*

Scientific name	Type of extract	*0.125g/mL*	*0.25g/mL*	*0.5g/mL*	*1g/mL*
Agave potatorum	Aqueous	+++	+++	+++	+++
	Ethanol	+++	+++	+++	+++
Aloe maculata	Aqueous	+++	+++	+++	+++
	Ethanol	+++	+++	+++	+++
Graptopetalum mendozae	Aqueous	+++	+++	+++	+++
	Ethanol	+++	+++	+++	+++
Control	Control	+++	+++	+++	+++

*(+/++/+++) indicates number of bacterial growth on replicate plates; (-) no bacterial growth.

In summary, all three succulent plants contained carbohydrates, phytosterols, flavonoids, phenols, and tannins. However, *A. maculata* and *A. potatorum* showed the presence of glycosides, while the *G. mendozae* exhibited the presence of alkaloids. Both the aqueous and ethanolic extracts of *A. potatorum, A. maculata* and *G. mendozae* inhibited the growth of Gram-negative bacteria such as *A. hydrophila* and *V.*

harveyi. In contrast, the Gram-positive bacterium, *B. albus* was resistant to the extracts. Hence, this study confirmed that succulent plants possessed antibacterial properties against Gram-negative bacteria. The data could be used as baseline information in discovering antimicrobial compounds for future therapeutic applications.

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