2025

Research Article

Antimicrobial Activity of *Uvaria rufa* (Annonaceae) Leaf Extracts

Lawrence R. Delgado, Rane Angelica G. Alarcon, Kyla Shane C. Aldea, Trisha Anne B. Samaniego, Rogie Royce Carandang & Kevin Jace Miranda

Received: October 26, 2023
Revised: April 24, 2024
Accepted: August 22, 2025
Published: September 30, 2025
Similarity index: 0%

Abstract

Antimicrobial resistance is considered a threat to global health. Given the use and benefits of herbal medicine, the healthcare community has been investigating plant-derived compounds for their potential antimicrobial activity. *Susong kalabaw (Uvaria rufa)* was studied for its antibacterial activity using fractionated leaf extracts. *U. rufa* leaves were extracted using 70% ethanol and fractionated using five solvents, including water, methanol/water, sec-butanol, DCM, and hexane. Phytochemical screening was performed on crude extracts. Moreover, the inhibitory activity of crude and fractionated leaf extracts of *U. rufa* was evaluated using the Kirby-Bauer test and MIC assay. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids in crude extracts. The Kirby-Bauer test showed that crude extracts inhibited the growth of *Escherichia coli* (8.3 mm), *Bacillus subtilis* (10.0 mm), and *Staphylococcus aureus* (14.5 mm). The fractions, including water (10.3 mm), methanol/water (11.2 mm), and sec-butanol (17.8 mm), also revealed inhibitory activity against *S. aureus*. Moreover, *Pseudomonas aeruginosa* was susceptible to the sec-butanol fraction, showing an average zone of inhibition of 9.3 mm. For the MIC assay, the methanol/water fraction inhibited the growth of S. aureus at a substantially lower concentration of 67.35 µg/mL. On the other hand, *B. subtilis* and *S. aureus* were susceptible to water and sec-butanol fractions, showing lower concentrations of 68.1 µg/mL and 26.21 µg/mL, respectively. These results emphasized that crude and fractionated leaf extracts of *U. rufa* demonstrated promising antibacterial activity against bacterial samples in the Kirby-Bauer test and MIC assay.

Keywords: antimicrobial resistance, Kirby-Bauer test, MIC assay, plant-derived compounds, *Uvaria rufa*

Author Information:

[1]**Lawrence R. Delgado**
Alumnus
lawrence.delgado@adamson.edu.ph
orcid.org/0009-0009-0958-1925

[1]**Rane Angelica G. Alarcon**
Alumna
rane.angelica.alarcon@adamson.edu.ph
orcid.org/0009-0007-1174-6421

[1]**Kyla Shane C. Aldea**
Alumna
kyla.shane.aldea@adamson.edu.ph
orcid.org/0009-0002-2736-0666

[1]**Trisha Anne B. Samaniego**
Alumna
trisha.anne.samaniego@adamson.edu.ph
orcid.org/0009-0004-1215-3466

[1,2]**Rogie Royce Carandang**
[1]Senior Lecturer, [2]Assistant Professor
carandangr@uthscsa.edu
orcid.org/0000-0001-6668-911X

[3]**Kevin Jace Miranda**
Associate Professor
kamiranda@up.edu.ph
orcid.org/0000-0001-7324-3802

[1]College of Pharmacy
Adamson University
Manila, Philippines

[2]Department of Health, Behavior, and Society
University of Texas School of Public Health San Antonio
San Antonio, TX, USA

[3]College of Arts and Sciences,
University of the Philippines—Manila
Manila, Philippines

https://doi.org/10.53899/spjrd.v30i2.301

Antimicrobials are medicines that prevent and treat infections in humans, animals, and plants. These include antibiotics, antivirals, antifungals, and antiparasitics (World Health Organization [WHO], 2021). The discovery and implementation of antimicrobial drugs in general clinical applications is one of modern medicine's breakthrough points, transforming human health and well-being (Uddin et al., 2021; Wright, Seiple & Myers, 2014). Chemical synthesis enabled the development of the first antimicrobial agents, which were shortly followed and surpassed by more powerful and complex agents, including penicillin, tetracycline, and streptomycin, among others (Wright et al., 2014). However, bacteria, fungi, and other pathogens have constantly evolved to withstand the agents used to combat them. This factor has led to the inevitable emergence and rise of antimicrobial resistance (Aly & Balkhy, 2012; O'Neill, 2014; Uddin et al., 2021). Consequently, efforts must be made to address this issue, such as controlling antimicrobial use, studying the genetic mechanisms of resistance, and developing new therapeutic techniques and approaches to create new antimicrobial agents (Ginovyan, Petrosyan, & Trchounian, 2017; Valle et al., 2015).

Plant-derived substances have been widely utilized in traditional medicine across various regions. With the increasing number of cases involving drug-resistant pathogens, the healthcare community has focused on studies examining the potential antimicrobial activity of plant-derived compounds (Savoia, 2012). The emergence of drug-resistant pathogens poses a significant threat to the successful treatment of microbial infections. The utilization of various plant-derived natural products as antibacterial and antifungal agents is a promising technique for developing drugs that could become effective therapeutic tools in the years to come (Savoia, 2012; Upadhyay et al., 2014).

Uvaria rufa, locally known in the Philippines as *Susong kalabaw*, is a short climbing shrub belonging to the Annonaceae family that usually grows abundantly in the tropics of Africa and Asia, particularly in Indonesia, Thailand, Malaysia, and in low and medium-altitude forests in the Philippines (Hilaria et al., 2016; Macabeo et al., 2012; Padma, Don, & Josthna, 2014; Rosandy et al., 2013). Traditionally, the different parts of the plant are used as herbal medicine in Asia (Hilaria et al., 2016; Rosandy et al., 2013). The leaves are used as an antidiabetic (Hilaria et al., 2016; Pamok, Saenphet, & Buncharoen, 2018) and antitubercular (Buncharoen et al., 2016; Macabeo et al., 2012; Pamok et al., 2018), as an antioxidant (Hilaria et al., 2016), and as a stimulant for uterine contractions in laboring women (Hilaria et al., 2016). Other parts of the plant, such as its roots, stems, and fruits, were used to treat fever, gastrointestinal disorders, and skin allergies, respectively (Buncharoen et al., 2016; Buncharoen, Saenphet, & Saenphet, 2019).

Previous studies have shown that various parts of *U. rufa* possess different phytochemicals. In the extract of the leaves of *U. rufa*, flavonoids, saponins, and tannins were identified (Hilaria et al., 2016). It has been studied that flavonoids present in *U. rufa* aid and act as an antioxidant (Hilaria et al., 2016). It has also been reported that the flavonoids present exhibit antimycobacterial activity by inhibiting enzymes involved in fatty acid and mycolic acid biosynthesis, and have isoniazid-modulating activities (Macabeo et al., 2012). Other phytochemicals from various parts of *U. rufa* include flavonols, alkaloids, flavonolrutin, kaempferol, quercitin, and lignan glycoside (Buncharoen et al., 2016; Buncharoen et al., 2019). While reports on the phytochemistry of *U. rufa* have been published, the pharmacological activities of only a few phytochemicals are known (Buncharoen et al., 2016; Buncharoen et al., 2019; Macabeo et al., 2012). Additionally, there is no detailed information available regarding its antibacterial and antifungal activity. This study investigated the potential antibacterial and antifungal activity of the fractionated leaf extracts of *U. rufa*.

Methodology

Plant Collection, Extraction, and Fractionation

Five (5) kg of *Uvaria rufa* leaves were collected from Barangay Bucana, Ternate, Cavite, and authenticated at the University of the Philippines Diliman—Jose Vera Santos Memorial Herbarium in Quezon City.

The fresh leaves of *U. rufa* were washed with clean water, chopped, air-dried, and pulverized (Buncharoen et al., 2016; Klionsky et al., 2021). Then, the powdered leaves were percolated with 70% ethanol for 48 hours. The collected crude extracts were concentrated using a rotary evaporator, and moisture and excess ethanol were removed using a water bath at 40 °C (Saptarini & Wardati, 2020). The concentrated crude extract of *U. rufa* leaves was placed in three tightly closed scintillation vials and stored in a laboratory refrigerator at 8 °C. The two scintillation vials of concentrated crude extract from *U. rufa* leaves were lyophilized for fractionation.

Moreover, the modified Kupchan method was conducted for the liquid-liquid partitioning of dried crude extract of *U. rufa* leaves (Chua et al., 2019; Cunha et al., 2016; Hamed et al., 2020). The 35 g of dried crude extract of *U. rufa* leaves were suspended in distilled water to obtain 437.5 mL of mother solution and partitioned using organic solvents including 437.5 mL of secondary butanol, 437.5 mL of methanol and water, 437.5 mL of dichloromethane and 437.5 mL of hexane (Chua et al., 2019; Cunha et al., 2016; Hamed et al., 2020). Each fraction was partitioned three times. The 10 mL of 100% methanol in each fraction was added to dissolve the emulsion. Each fraction was concentrated in a rotary evaporator, and the excess solvents were removed by subjecting the samples to a water bath at 40 °C. The water fraction, secondary butanol fraction, methanol/water fraction, DCM fraction, and hexane fraction have total masses of 38.51 g, 45.26 g, 41.37 g, 30.42 g, and 3.36 g, respectively. The collected, fractionated leaf extracts of *U. rufa* were placed in a closed container and stored in a laboratory refrigerator at 8 °C until further analysis.

Phytochemical Screening

The following tests were conducted for the phytochemical screening of the crude extracts of *U. rufa* leaves: Dragendorff's test (alkaloids), Ferric chloride test (tannins), Froth test (saponins), Shinoda test (flavonoids), Liebermann-Burchard's test (steroids), Salkowski test (teroids and terpenoids), and Modified Borntrager's test (glycosides) (Abubakar & Haque, 2020; Auwal et al., 2014; BaoDuy, Pham, & Trang, 2015; Buncharoen et al., 2016; Saptarini, Herawati, & Permatasari, 2016). Each test consisted of two sets of crude extracts of *U. rufa*. The experimental group was the set of samples that used primary reagents. On the other hand, the control group did not apply primary reagents to the samples. The results were analyzed and compared using the Adobe color scheme. Moreover, the result was then interpreted conforming to the established guidelines (Pieroni, 2002).

Antimicrobial Susceptibility Test

The antibacterial activity of the crude extract and fractionated leaf extracts of *U. rufa* was determined using the Kirby-Bauer test, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) with some modifications (Marasini et al., 2015). The turbidity of bacterial suspension of *S. aureus* ATCC® 25923, *B. subtilis* ATCC® 6633, *E. coli* ATCC® 25922, and *P. aeruginosa* ATCC® 27853 was standardized, meeting the 0.5 McFarland standard. Then, the standard bacterial suspensions were cultured in Mueller-Hinton Agar (MHA) plates. Clindamycin (150 mg/mL) and ciprofloxacin (2 mg/mL) were used as positive controls for gram-positive and gram-negative bacteria, respectively, and were diluted in 1% dimethyl sulfoxide (DMSO) (Bhalodia & Shukla, 2011; Kibungu et al., 2021). The pure crude extract and fractionated leaf extracts of *U. rufa* were used as an experimental group, while 1% DMSO was used as a negative control (Bhalodia & Shukla, 2011; Jamkhande et al., 2015; Kibungu et al., 2021).

Sterile Whatman No. 1 filter paper discs, treated with 20 µL of antibiotics, crude extract, and fractionated leaf extracts of *U. rufa*, and 1% DMSO, were placed on MHA plates and incubated for 18 to 24 hours at 35 °C (Bhalodia & Shukla, 2011; Kibungu et al., 2021). The formation of the zone of inhibition on the plates was measured in millimeters (Bhalodia & Shukla, 2011; Kibungu et al., 2021; Jamkhande et al., 2015), and its interpretation was based on established guidelines (Bhalodia & Shukla, 2011). The Kirby-Bauer test was performed in triplicate (Bhalodia & Shukla, 2011; Jamkhande et al., 2015; Kibungu et al., 2021).

On the other hand, the turbidity of fungal suspension of Candida albicans ATCC® 10231 was standardized, meeting the 0.5 McFarland standard. Then, the standard fungal suspension was cultured in Mueller-Hinton Agar (MHA) plates. Fluconazole (2 mg/mL) was diluted in 1% DMSO and used as a positive control. The pure crude extract and fractionated leaf extracts of *U. rufa* were used as an experimental group, while 1% DMSO was used as a negative control. The sterile Whatman No. 1 filter paper discs treated with 20 µL of fluconazole, crude extract, and fractionated leaf extracts of *U. rufa* and 1% DMSO were placed on the MHA plates and incubated for 24 to 48 hours at 35 ^{0}C (Alastruey-Izquierdo et al., 2015; Berkow, Lockhart, & Ostrosky-Zeichner, 2020). After incubation, the formation of the zone of inhibition was measured in millimeters and interpreted according to established guidelines (Bhalodia & Shukla, 2011). The Kirby-Bauer test was performed in triplicate (Bhalodia & Shukla, 2011; Kibungu et al., 2021; Jamkhande et al., 2015).

Minimum Inhibitory Concentration (MIC) Assay

Twenty microliter (µL) of fractionated *U. rufa* extracts were suspended in 50% DMSO and 80 µL of Mueller-Hinton Broth (MHB), to give a final DMSO concentration of 10%. Then, 10 µL of each sample, 10% DMSO, clindamycin, ciprofloxacin, and fluconazole were dispensed in designated wells using a micropipette. The prepared black 96-well plates were stored in the refrigerator before use (Clinical and Laboratory Standards Institute, 2012; Cockerill & Clinical, 2008).

The *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* were subcultured in MHB for 18 to 20 hours at 35°C, while the *C. albicans* was subcultured in MHB for 24 to 48 hours at 35°C. The turbidity of each test microorganism was standardized to meet the 0.5 McFarland standard. The standard bacterial suspensions were cultured on MHA plates for 18 to 20 hours at 35°C (Clinical and Laboratory Standards Institute, 2012; Breijyeh , Jubeh, & Karaman, 2020). On the other hand, the standard fungal suspension was cultured on MHA plates for 24 to 48 hours at 35°C (Clinical and Laboratory Standards Institute, 2012). The cultivation of test microorganisms on MHA plates was performed in triplicate.

Meanwhile, 100 µL of the prepared bacterial and fungal seeding cultures were dispensed into designated wells. The 100 µL of MHB II were placed in blank wells. The black 96-well plates containing the bacterial samples were incubated for 23 hours at 35°C. On the other hand, the black 96-well plates containing the fungal sample were incubated for 23 to 46 hours at 35°C. Then, 10 µL of a 0.1% resazurin solution was added to all wells and incubated for an additional hour (Breijyeh et al., 2020; Clinical and Laboratory Standards Institute, 2012).

The fluorescence reader was used to measure the fluorescence emission after the incubation period had elapsed. The percent inhibition was computed using the formula:

$$\% \text{ Inhibition} = (1 - (\frac{^{A}\text{sample} - {}^{A}\text{100\% Inhibition}}{^{A}\text{0\% Inhibition} - {}^{A}\text{100\% Inhibition}})) \times 100$$

The > 90% shows significant inhibition of bacterial and fungal growth after the incubation period (Clinical and Laboratory Standards Institute, 2012; Breijyeh et al., 2020).

Results and Discussion

This section presents the highlights of the study conducted on the fractionated extract of susong kalabaw and its potential for therapeutic and pharmaceutical development.

Phytochemical Screening

In this study, the leaves of the *U. rufa* plant were evaluated. Although there have been numerous studies on the plant sample, little is known about the plant's antimicrobial activity (Buncharoen et al., 2016; Buncharoen et al., 2019; Hilaria et al., 2016; Macabeo et al., 2012; Padma et al., 2014; Pamok et al., 2018; Rosandy et al., 2013). Building on this gap, the results showed that *U. rufa* possesses antibacterial properties, highlighting the potential significance of these findings for further exploration of the plant's therapeutic uses.

The crude extracts of the *U. rufa* leaves were subjected to phytochemical analysis, which revealed the presence of alkaloids, tannins, saponins, flavonoids, and terpenoids (Table 1). However, steroids and glycosides were not detected. These findings are summarized in Table 1 below and are considered in context with related research.

Table 1

The Phytochemical Screening Results for the Crude Extract of Uvaria rufa Leaves[1]

Phytochemicals	Test	Results
Alkaloids	Dragendorff's Test	(+++)
Tannins	Ferric chloride Test	(+++)
Saponins	Froth Test	(+++)
Flavonoids	Shinoda Test	(+++)
Steroids	Liebermann-Burchard's Test	(-)
	Salkowski Test	(-)
Terpenoids	Salkowski Test	(+++)
Glycosides	Modified Borntrager's Test	(-)

The abundance of phytochemicals, including alkaloids, tannins, saponins, flavonoids, and terpenoids, in the crude extracts of *U. rufa* could potentially be a significant factor contributing to its antibacterial activity. Notably, the phytochemicals present in *U. rufa* resemble the secondary metabolites found in the root extracts of *Hydnora africana* (Cockerill & Clinical, 2008). The root extracts of *Hydnora africana* are attributed to good antibacterial activity due to their bioactive potency or high concentrations of phytochemicals (Cockerill & Clinical, 2008). Consequently, these extracts are used in traditional medicine as an antidysenteric agent (Cockerill & Clinical, 2008). In addition to *Hydnora africana*, the abundance of phytochemicals and the potential antibacterial activity of the crude extracts of *U. rufa* can be compared with those of other plants in the Annonaceae family. For instance, a previous study on the leaves of *Cassia fistula* found that the crude extracts contain tannins and phenolic compounds (BaoDuy et al., 2015), and these extracts significantly inhibit the bacterial growth of *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa*. Furthermore, the crude extracts of *Cassia fistula* also inhibit the fungal growth of *A. niger*, *A. clavatus*, and *C. albicans* (BaoDuy et

[1]The interpretation of results for the phytochemical screening of *U. rufa* leaf extracts: (1) Traces (+); (2) Moderate (++); (3) Abundant (+++); and (4) Absence of phytochemicals (-)

al., 2015). Similarly, a study found that *Annona reticulata* contains alkaloids, saponins, flavonoids, carbohydrates, glycosides, steroids, amino acids, and tannins, which inhibit the growth of *S. aureus* and *E. coli* (Paul et al., 2018).

These similarities suggest that the plant species possess promising antibacterial activity and may be potentially used in combating bacterial infections. The correlation between their pharmacological activity is seemingly related to the presence of phytochemicals in the crude extracts, as indicated by phytochemical tests that revealed the presence of bioactive compounds, including alkaloids, terpenes, and flavonoids. Furthermore, this result may also serve as guidelines for selecting a potential candidate for plant-based compounds in antibacterial agents through bioassay-guided screening and isolation.

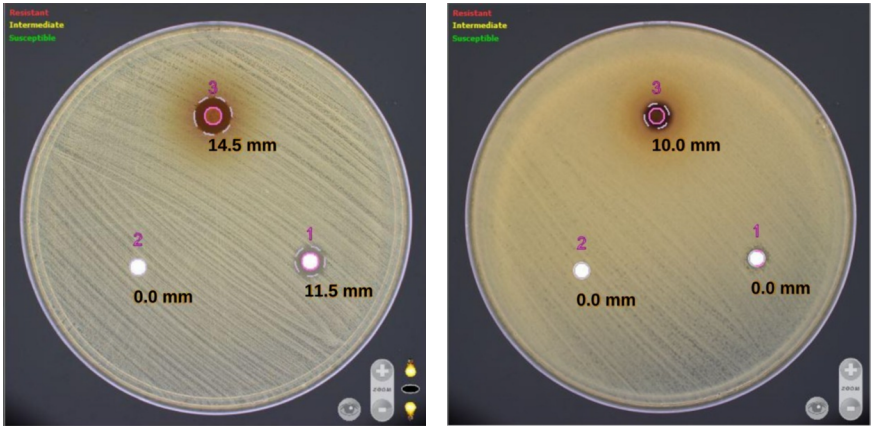
Antibacterial and antifungal activity of the crude extracts of *Uvaria rufa* leaves

For the gram-positive bacterial isolates, the average zone of inhibition of *S. aureus* for the crude extract was 14.5 mm (0.06 ± SD), while the antibiotic control, clindamycin, had an average zone of inhibition of 11.5 mm (0 ± SD). On the other hand, the average zone of inhibition for *B. subtilis* for the crude extract was 10.0 mm (0.10 ± SD), while there was no zone of inhibition for the antibiotic control used (Figure 1).

Figure 1

Antibacterial Activity of Crude Extracts of Uvaria rufa Leaves against Gram-positive Bacteria Tested based on Disc Diffusion Method[2]

S. aureus *B. subtilis*



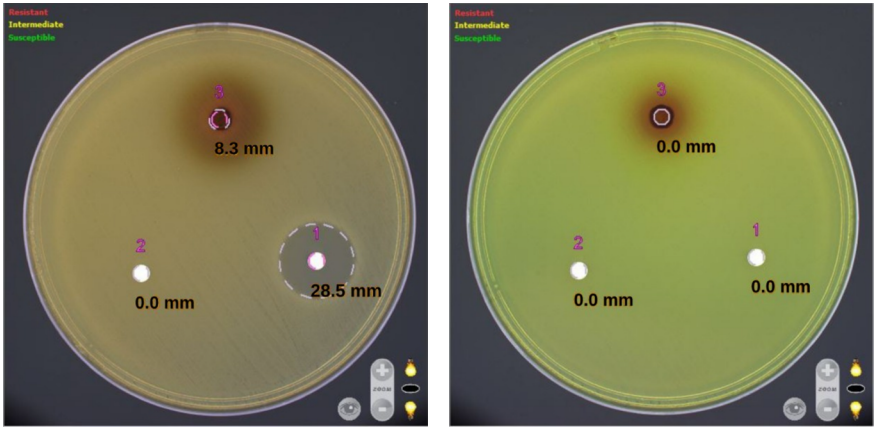
For the gram-negative bacterial isolates, the results of *E. coli* yielded an average zone of inhibition of 8.17 mm (0.12 ± SD), while the antibiotic control, ciprofloxacin, generated the highest average zone of inhibition of 28.5 mm (0 ± SD). The last bacterial strain, *P. aeruginosa*, showed an absence of a zone of inhibition for both the crude extract and the antibiotic control sample (Figure 2).

[2]Samples used for the disc diffusion test of crude extracts of *Uvaria rufa* leaves: (1) Antibiotic and antifungal control; (2) Blank paper disc; and (3 *Uvaria rufa* sample

Figure 2

Antibacterial Activity of Crude Extracts of Uvaria rufa Leaves against Gram-Negative Bacteria Tested based on Disc Diffusion Method

E. Coli *P. aeruginosa*



Lastly, for the fungal isolate, there was no zone of inhibition in the crude extract, indicating resistance. In contrast, the antifungal control, fluconazole, showed a zone of inhibition with an average diameter of 20.6 mm (0 ± SD), indicating susceptibility (Figure 3).

Figure 3

Antifungal Activity of Crude Extracts of Uvaria rufa Leaves against a Fungal Strain Tested based on Disc Diffusion Method

C. albicans

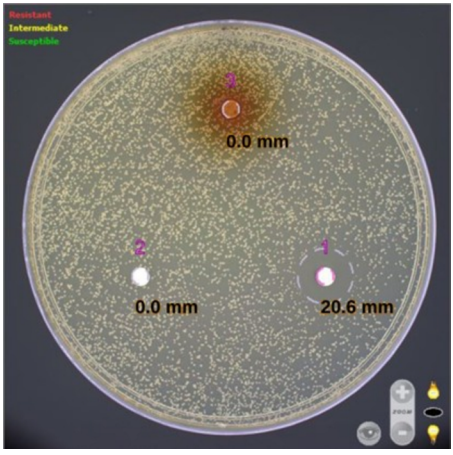


Table 2

Summary of the Results of the Antibacterial and Antifungal Activity of Crude Extracts of Uvaria rufa Leaves[3]

Samples	Average Zone of Inhibition (mm) ± SD				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Uvaria rufa	14.5 (0.06)	10.0 (0.10)	8.17 (0.12)	0	0
Clindamycin	11.5 (0)	0	-	-	-
Ciprofloxacin	-	-	28.5 (0)	0	-
Fluconazole	-	-	-	-	20.3 (0.46)

The crude extracts of *U. rufa* leaves exhibited bioactivity against Gram-positive bacteria, resulting in a noticeable zone of inhibition (Table 2). The crude extracts of *U. rufa* formed an average zone of inhibition of 14.5 mm (0.06 ± SD) against *S. aureus* and 10.0 mm (0.10 ± SD) against *B. subtilis*. Hence, the gram-positive bacteria are susceptible to crude extracts of *U. rufa*. However, the crude extracts of *U. rufa* did not inhibit the growth of gram-negative bacteria, forming a small zone of inhibition to none, indicating that the crude extracts are not viable to use for gram-negative pathogens that can attributed to the outer membrane of gram-negative bacteria, which makes it hard for the antibacterial agents to penetrate and elicit its effect than gram-positive bacteria (Breijyeh et al., 2020; Uddin et al., 2021). Although the crude extract of *U. rufa* contains phytochemicals responsible for its antimicrobial activity, it may not be enough to form a zone of inhibition in the gram-negative samples, as it is intrinsically less permeable than gram-positive bacteria (Breijyeh et al., 2020). Meanwhile, the crude extract of *U. rufa* showed no inhibitory activity against *C. albicans*.

Antibacterial and antifungal activity of the fractionated leaf extracts of *Uvaria rufa*

The fractionated leaf extracts of *U. rufa* showed promising inhibitory activity in the disc diffusion susceptibility test (Table 3). The water, methanol/water, and sec-butanol fractions of *U. rufa* inhibit the growth of *S. aureus*, with average zones of inhibition of 10.3 mm (SD = 0.20), 11.5 mm (SD = 0.26), and 17.8 mm (SD = 0.10), respectively. Additionally, *P. aeruginosa* was susceptible to the sec-butanol fraction, with an average zone of inhibition of 9.0 mm (SD = 0.26). The aforementioned fractions of *U. rufa* showed promising zones of inhibition, indicating that bacterial growth of *S. aureus* was significantly inhibited.

Table 3

Summary of the Results of the Antibacterial and Antifungal Activity of the Fractionated Leaf Extracts of Uvaria rufa[4]

Samples	Average Zone of Inhibition (mm) ± SD				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Water	10.3 (0.20)	0	0	0	0
DCM	0	0	0	0	0

[3]The results of Kirby-Bauer Test of crude extracts of *U. rufa*: (-) Blank

[4]The results of Kirby-Bauer Test of fractionated leaf extracts of *U. rufa*: (-) Blank

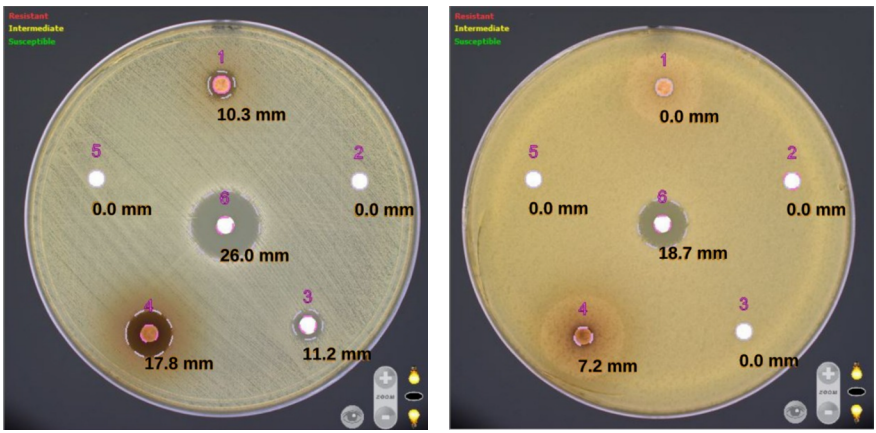
Samples	Average Zone of Inhibition (mm) ± SD				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
MeOH/H2O	11.5 (0.26)	0	0	0	0
Sec-butanol	17.8 (0.10)	7.5 (0.31)	6.9 (0.44)	9.0 (0.26)	0
Hexane	0	0	0	0	0
Clindamycin	26.0 (0)	18.7 (0)	-	-	-
Ciprofloxacin	-	-	17.4 (0)	0	-
Fluconazole	-	-	-	-	33.9 (0)

Clindamycin also prevents the growth of *S. aureus*, forming an average zone of inhibition of 26.0 mm (SD = 0). However, *S. aureus* was resistant to dichloromethane (DCM) and hexane fractions (Figure 4). None of the fractions, including clindamycin and ciprofloxacin, showed inhibitory activity against *B. subtilis* (Figure 4) and *E. coli* (Figure 5). For *P. aeruginosa*, apart from the sec-butanol fraction, the other fractions and ciprofloxacin were resistant (Figure 5). None of the fractions had any inhibitory effects against *C. albicans*; however, fluconazole showed inhibitory activity, with an average zone of inhibition of 33.9 mm (SD = 0) (Figure 6).

Figure 4

Antibacterial Activity of the Fractionated Leaf Extracts of Uvaria rufa against Gram-Positive Bacteria Tested based on Disc Diffusion Method[5]

S. aureus *B. subtilis*



[5]Samples used for the disc diffusion test of fractionated extracts of *Uvaria rufa* leaves: (1) Water; (2) DCM; (3) MeOH/H2O; (4) Sec-butanol; (5) Hexane; and (6) Antibiotic and antifungal control

Figure 5

Antibacterial Activity of the Fractionated Leaf Extracts of Uvaria rufa against Gram-Negative Bacteria Tested based on Disc Diffusion Method

E. coli *P. aeruginosa*

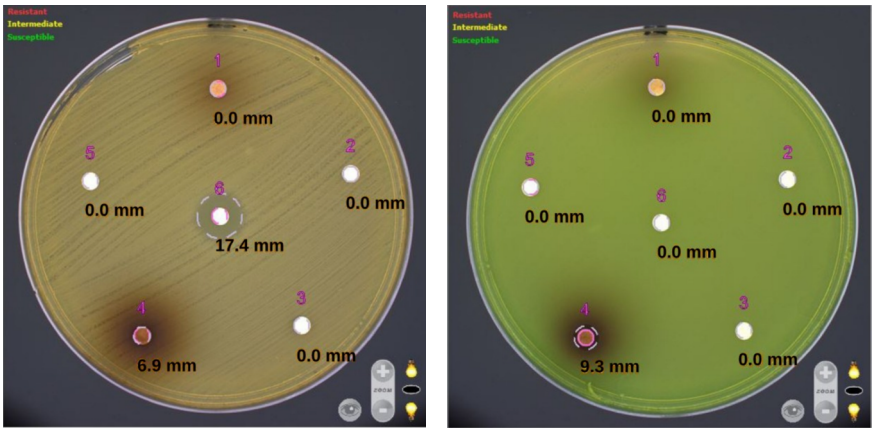
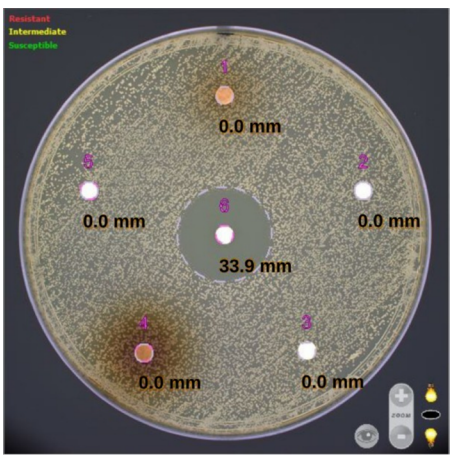


Figure 6

Antifungal Activity of the Fractionated Leaf Extracts of Uvaria rufa against a Fungal Strain Tested based on Disc Diffusion Method

C. albicans



The significant antibacterial activity of the water, methanol/water, and sec-butanol fractions of *U. rufa* against *S. aureus* and *P. aeruginosa* may be attributed to the abundance of bioactive compounds,

particularly the presence of alkaloids, terpenes, and flavonoids in the extract, as evaluated in phytochemical studies. This observation was also observed in a study of one of the members of the Annonaceae family. As observed in the ethyl acetate and hexane fractions of *A. muricata*, wherein they inhibit the growth of *S. aureus* and *E. coli*, forming a zone of inhibition of 42 mm and an activity index of 1.31, and a ZOI of 42 mm and an activity index of 1.40, respectively (Ugwu Okechukwu et al., 2013). Although formation of a zone of inhibition against *B. subtilis* and *E. coli* is observed, the fractionated leaf extracts of *U. rufa* are resistant based on the established guidelines (Bhalodia & Shukla, 2011); it can be used as a baseline for the isolation, purification, structure elucidation, and chemical modification through synthesis to improve the bioactivity against these pathogens. Meanwhile, the five fractions of *U. rufa* did not work against *C. albicans*, which can be attributed to the formation of biofilms, which is one of the mechanisms of antibiotic resistance in *C. albicans*, making it resistant to antifungal agents, similar to some gram-negative pathogens like *P. aeruginosa* (Tsui, Kong & Jabra-Rizk, 2016).

MIC Assay

The methanol/water fraction inhibited *S. aureus* at a concentration of 67.35 µg/mL (SD=0.07) but had no effect on other microorganisms (Table 4). The water and sec-butanol fractions inhibited *S. aureus* and *B. subtilis* at concentrations of 68.1 µg/mL (SD = 0.07) and 26.21 µg/mL (SD = 0.02), respectively; however, they were ineffective against *E. coli*, *P. aeruginosa*, and *C. albicans*. The DCM and hexane fractions had no inhibitory activity against bacterial and fungal samples at the given concentrations. Clindamycin, ciprofloxacin, and fluconazole had inhibitory effects against bacterial and fungal samples at a concentration of 25 µg/mL.

Table 4

Summary of the MIC Assay Results of the Fractionated Leaf Extracts of Uvaria rufa[6]

Samples	MIC Values (µg/mL) ± SD				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Water	68.10 (0.09)	68.10 (0.07)	0	0	0
DCM	0	0	0	0	0
MeOH/H2O	67.35 (0.07)	0	0	0	0
Sec-butanol	26.21 (0.02)	26.21 (0.12)	0	0	0
Hexane	0	0	0	0	0
Clindamycin	25 (0.17)	25 (0.85)	-	-	-
Ciprofloxacin	-	-	25 (0.24)	25 (0.14)	-
Fluconazole	-	-	-	-	25 (0.15)

Among the five fractions of *U. rufa*, the water and the sec-butanol fractions had antibacterial activity against *S. aureus* and *B. subtilis* at lower concentrations. The abundance of phytochemicals, such as flavonoids, tannins, and alkaloids, in the water and the sec-butanol fractions of *U. rufa* had significant inhibitory effects on the growth of gram-positive bacteria (Jamkhande et al., 2015; Shami, 2017). This is primarily attributed to the ability of these phytochemicals to inhibit cell wall synthesis, chelate iron, disrupt quorum sensing, and interfere with the metabolic pathways of these pathogens. (Czerkas et al., 2024; Farha et al., 2020; Kaczmarek, 2020).

[6]The results of MIC of fractionated leaf extracts of *U. rufa*: (-) Blank

Meanwhile, the methanol/water fraction of *U. rufa* showed inhibitory activity against *S. aureus*. These data emphasize that the water, sec-butanol, and methanol/water fractions of *U. rufa* had comparable inhibitory activity with clindamycin against gram-positive bacteria. These results provide new insights into the antibacterial activity of fractionated leaf extracts of *U. rufa*, which can be utilized for the development of isolation and even modification of the bioactive compound for clinical, industrial, and biological applications (Barbieri et al., 2017; Khameneh et al., 2021).

This study has shown that *U. rufa* leaf extracts possess antibacterial properties, which is in line with previous studies on other plant samples from the Annonaceae family (Jamkhande et al., 2015; Shami, 2017). The fractionated leaf extracts of Annona reticulata contain phenolic compounds similar to the fractionated leaf extracts of *U. rufa*. The fractionated leaf extracts of Annona reticulata significantly inhibit bacterial and fungal growth in *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, *S. cerevisiae*, and *C. blanki*, respectively (Jamkhande et al., 2015). The well-studied mechanism of phytochemicals in inhibiting bacterial growth and their bactericidal activity is due to the ability of these compounds to inhibit cell wall, nucleic acid, and protein synthesis, disrupt the cell membrane, intercalate DNA, modify virulence factors, and disrupt quorum sensing (Masyita et al., 2022; Sulaiman et al., 2022; Thawabteh et al., 2024; Yan et al., 2021). In contrast, the fractionated leaf extracts of *U. rufa* showed no antibacterial activity against gram-negative bacteria, and antifungal activity against *C. albicans*, primarily due to their ability to resist such phytochemicals by means of the porin channels, biofilm formation, and the alteration of the target site of antibacterial and antifungal agents (Breijyeh et al., 2020; Gauba & Rahman, 2023; Reygaert, 2018).

Conclusion

The crude extract of *U. rufa* leaves possessed alkaloids, tannins, saponins, flavonoids, and terpenoids, which were associated with the good antibacterial activity of the plant extract. The Kirby-Bauer test showed the inhibitory activity of both crude extract and fractionated leaf extracts of *U. rufa* against bacterial samples. Moreover, for the MIC assay, the water, sec-butanol, and methanol/water fractions of *U. rufa* have potent concentrations that completely inhibit the growth of gram-positive bacteria. Hence, water, sec-butanol, and methanol/water fractions of *U. rufa* exhibited promising antibacterial activity.

The researchers are encouraging future researchers to perform purification, isolation, and structure elucidation of the bioactive compounds responsible for the antibacterial activity of *U. rufa* extracts. Furthermore, the researchers recommend evaluating the antifungal activity using different strains of pathogenic fungi and conducting a study on the other potential pharmacological activities of *U. rufa* based on the identified phytochemicals.

Acknowledgment

The researchers would also like to thank the Chemistry Laboratory, Chemical Engineering Department, and Pharmacy Department of Adamson University for providing the necessary resources, equipment, and research facilities for the study. The researchers are also grateful to the following: the Institute of Biology, College of Science, University of the Philippines-Diliman, for the taxonomical authentication of the plant; the College of Public Health, University of the Philippines-Manila, for the acquisition of pathogenic microorganisms; Mr. Kelvin Malonda of United Laboratories, for conducting the disc diffusion analysis and minimum inhibitory concentration tests. Lastly, the researchers would also like to thank the Adamson University - Center for Research and Development for providing funding for the study.

Conflict of Interest Statement

The authors declare no conflict of interest.

AI Disclosure

The authors declare that the manuscript did not use any artificial intelligence (AI) software, nor has the manuscript undergone AI-assisted writing and proofreading.

References

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & BioAllied Sciences*, *12*(1), 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

Alastruey-Izquierdo, A., De Souza Carvalho Melhem, M., Bonfietti, L. X., & Rodríguez-Tudela, J. L. (2015). Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. *Revista Do Instituto De Medicina Tropical De Sao Paulo*, *57*(suppl 19), 57–64. https://doi.org/10.1590/s0036-46652015000700011

Aly, M., & Balkhy, H. H. (2012). The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. *Antimicrobial Resistance and Infection Control*, *1*(1), 1-5. https://doi.org/10.1186/2047-2994-1-26

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*, *5*(2), 95–100. https://tinyurl.com/27wkfre8

BaoDuy, N. L., Pham, N. K. T., & Trang, M. (2015). Preliminary phytochemical analysis of leaf extracts of *Thuja orientalis* (L.) Endl. *International Journal of Research Science & Management*, *2*(1), 21–25. https://tinyurl.com/bd83xw2h

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research*, *196*, 44–68. https://doi.org/10.1016/j.micres.2016.12.003

Berkow, E. L., Lockhart, S. R., & Ostrosky-Zeichner, L. (2020). Antifungal susceptibility testing: current approaches. *Clinical Microbiology Reviews*, *33*(3). https://doi.org/10.1128/cmr.00069-19

Bhalodia, N. R., & Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula l.*: An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(2), 104-109. https://doi.org/10.4103/2231-4040.82956

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-Negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, *25*(6), 1340. https://doi.org/10.3390/molecules25061340

Buncharoen, W., Saenphet, K., Saenphet, S., & Thitaram, C. (2016). Uvaria rufa Blume attenuates benign prostatic hyperplasia via inhibiting 5α-reductase and enhancing antioxidant status. J*ournal of Ethnopharmacology*, *194*, 483-494. https://doi.org/10.1016/j.jep.2016.10.036

Buncharoen, W., Saenphet, S., & Saenphet, K. (2019). Relaxant activities of extracts from *Uvaria rufa* Blume and *Caesalpinia sappan L.* on excised rat's prostate strips. *Journal of Pharmaceutical Research International*, *29*(1), 1–12. https://doi.org/10.9734/jpri/2019/v29i130228

Chua, L. S., Lau, C. H., Chew, C. Y., & Dawood, A. S. (2019). Solvent fractionation and acetone precipitation for crude saponins from *Eurycoma longifolia* extract. *Molecules*, *24*(7), 1416. https://doi.org/10.3390/molecules24071416

Clinical and Laboratory Standards Institute. (2012). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard (9th edition).* CLSI document M07-A9.

Cockerill, F. R., & Clinical. (2008). *Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement.* http://ci.nii.ac.jp/ncid/BB08787049

Cunha, L., de Morais, S., Aquinoa, F., Changa, R., de Oliveira, A., Martins, M., Martins, C., Sousa, L., Barros, T., da Silva, C., & do Nascimento, E. (2016). Bioassay-guided fractionation and antimicrobial and cytotoxic activities of Cassia bakeriana extracts. *Brazilian Journal of Pharmacognosy*, *27*(1), 91-98. https://doi.org/10.1016/j.bjp.2016.08.002

Czerkas, K., Olchowik-Grabarek, E., Łomanowska, M., Abdulladjanova, N., & Sękowski, S. (2024). Antibacterial activity of plant polyphenols belonging to the tannins against *Streptococcus mutans*—Potential against dental caries. *Molecules*, *29*(4), 879s. https://doi.org/10.3390/molecules29040879

Farha, A. K., Yang, Q. Q., Kim, G., Li, H. Bin, Zhu, F., Liu, H. Y., Gan, R. Y., & Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, *38*, 100751. https://doi.org/10.1016/j.fbio.2020.100751

Gauba, A., & Rahman, K. M. (2023). Evaluation of antibiotic resistance mechanisms in Gram-Negative bacteria. *Antibiotics*, *12*, 1590. https://doi.org/10.3390/antibiotics12111590

Ginovyan, M., Petrosyan, M., & Trchounian, A. (2017). Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Complementary and Alternative Medicine*, *17*(1), 50. https://doi.org/10.1186/s12906-017-1573-y

Hamed, A. A., Soldatou, S., Qader, M. M., Arjunan, S., Miranda, K. J., Casolari, F., Pavesi, C., Diyaolu, O. A., Thissera, B., Eshelli, M., Belbahri, L., Luptakova, L., Ibrahim, N. A., Abdel-Aziz, M. S., Eid, B. M., Ghareeb, M. A., Rateb, M. E., & Ebel, R. (2020). Screening fungal endophytes derived from under-explored Egyptian marine habitats for antimicrobial and antioxidant properties in factionalised textiles. *Microorganisms*, *8*(10), 1617. https://doi.org/10.3390/microorganisms8101617

Hilaria, M., Elisma, Dapa, M. Y., & Teti, M. (2016). Antioxidant activities of ethyl acetate extract and hexane extract of Lelak (*Uvaria rufa* Blume) leaves. *Asian Journal of Applied Sciences*, *4*(4), 930–934. https://tinyurl.com/44n479mv

Jamkhande, P. G., Wattamwar, A. S., Kankudta, A. D., Tidke, P. S., & Kalaskar, M. G. (2015). Assessment of *Annona reticulata* Linn. leaves fractions for in vitro antioxidative effect and antimicrobial potential against standard human pathogenic strains. *Alexandria Journal of Medicine*, *52*(1), 19-25. http://dx.doi.org/10.1016/j.ajme.2014.12.007

Kaczmarek, B. (2020). Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-A minireview. *Materials*, *13*(14), 3224. https://doi.org/10.3390/ma13143224

Khameneh, B., Eskin, N. A. M., Iranshahy, M., & Fazly Bazzaz, B. S. (2021). Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics*, *10*(9), 1–33. https://doi.org/10.3390/antibiotics10091044

Kibungu, W. C., Fri, J., Clarke, A. M., Otigbu, A., & Njom, H. A. (2021). Seasonal variation in antimicrobial activity of crude extracts of *Psammaplysilla sp.* 1 from Phillips Reef, South Africa. *International Journal of Microbiology*. https://doi.org/10.1155/2021/7568493

Klionsky, D. J., Abdel-Aziz, A. K., Abdelfatah, S., Abdellatif, M., Abdoli, A., Abel, S., Abeliovich, H., Abildgaard, M. H., Abudu, Y. P., Acevedo-Arozena, A., Adamopoulos, I. E., Adeli, K., Adolph, T. E., Adornetto, A., Aflaki, E., Agam, G., Agarwal, A., Aggarwal, B. B., Agnello, M., . . . Tong, C. (2021). Guidelines for the use and interpretation of assays for monitoring autophagy (4th ed.). *Autophagy*, *17*(1), 1–382. https://doi.org/10.1080/15548627.2020.1797280

Macabeo, A. P. G., Tudla, F. A., Krohn, K., & Franzblau, S. G. (2012). Antitubercular activity of the semi–polar extractives of Uvaria rufa. *Asian Pacific Journal of Tropical Medicine*, *5*(10), 777-780. https://doi.org/10.1016/s1995-7645(12)60142-4

Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., & Shrestha, K. (2015). Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *BioMed Research International*, *2015*, 1–6. https://doi.org/10.1155/2015/265425

Masyita, A., Mustika Sari, R., Dwi Astuti, A., Yasir, B., Rahma Rumata, N., Emran, T. Bin, Nainu, F., & Simal-Gandara, J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry: X*, *13*, 100217. https://doi.org/10.1016/j.fochx.2022.100217

O'Neill, J. (2014) Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance.

Padma, K. R., Don, K. R., & Josthna, P. (2014). Herbal plant Uvaria species and its therapeutic potentiality. *World Journal of Environmental Biosciences*, *9*(1), 33–38. https://tinyurl.com/7adr33j5

Pamok, S., Saenphet, K., & Buncharoen, W. (2018). Effects of *Uvaria rufa* Blume on the histology of male reproductive organs of testosterone induced alopecia rats. *Microscopy and Microanalysis Research*, *31*(1), 9-17. https://doi.org/10.14456/microsc-microanal-res.2018.2

Paul, S., Dash, B., Bora, A.J., & Gupta, B. (2018). Preliminary phytochemical screening and in vitro antimicrobial activity of ethanolic extracts of fruits of *Annona reticulata* against standard pathogenic strains. *International Journal of Current Pharmaceutical Research*, *10*(4), 59-63. http://dx.doi.org/10.22159/ijcpr.2018v10i4.28466

Pieroni, A. (2002). Trease and Evans pharmacognosy. *Fitoterapia*, *73*(7-8). https://doi.org/10.1016/s0367-326x(02)00228-9

Reygaert, W. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, *4*(3), 482–501. https://doi.org/10.3934/microbiol.2018.3.482

Rosandy, A. R., Din, L. B., Yaacob, W. Z. W., Yusoff, N. M. I. N., Sahidin, I., Latip, J., Nataqain, S., & Noor, N. M. (2013). Isolation and characterization of compounds from the stem bark of *Uvaria rufa* (Annonaceae). T*he Malaysian Journal of Analytical Sciences*, *17*(1), 50–58. https://tinyurl.com/2n3jjdf8

Saptarini, N. M., & Wardati, Y. (2020). Effect of extraction methods on antioxidant activity of papery skin extracts and fractions of Maja Cipanas onion (*Allium cepa* L. var. *ascalonicum*). *The Scientific World Journal*, *2020*. https://doi.org/10.1155/2020/3280534

Saptarini, N. M., Herawati, I. E., & Permatasari, U. Y. (2016). Total flavonoids content in acidified extract of flowers and leaves of Gardenia (*Gardenia jasminoides* Ellis). *Asian Journal of Pharmaceutical and Clinical Research*, *9*(7), 213–215. https://tinyurl.com/2mnvekvw

Savoia, D. (2012). Plant-derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiology*, *7*(8), 979-990. https://doi.org/10.2217/fmb.12.68

Shami, A. M. (2017). The effect of alkaloidal fraction from *Annona squamosa L.* against pathogenic bacteria with antioxidant activities. *Pharmaceutical Sciences*, *23*(4), 301–307. https://doi.org/10.15171/ps.2017.44

Sulaiman, M., Jannat, K., Nissapatorn, V., Rahmatullah, M., Paul, A. K., de Lourdes Pereira, M., Rajagopal, M., Suleiman, M., Butler, M. S., Break, M. K. Bin, Weber, J. F., Wilairatana, P., & Wiart, C. (2022). Antibacterial and antifungal alkaloids from Asian angiosperms: Distribution, mechanisms of action, structure-activity, and clinical potentials. *Antibiotics*, *11*(9), 1146. https://doi.org/10.3390/antibiotics11091146

Thawabteh, A. M., Ghanem, A. W., AbuMadi, S., Thaher, D., Jaghama, W., Karaman, R., Scrano, L., & Bufo, S. A. (2024). Antibacterial activity and antifungal activity of monomeric alkaloids. *Toxins*, *16*(11). https://doi.org/10.3390/toxins16110489

Tsui, C., Kong, E. F., & Jabra-Rizk, M. A. (2016). Pathogenesis of Candida albicans biofilm. Pathogens and Disease, 74(4), ftw018. https://doi.org/10.1093/femspd/ftw018

Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, K. H., Gajdacs, M., Sahibzada, M. U. K., Hossain, M. J., & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health*, *14*(12), 1750-1766. https://doi.org/10.1016/j.jiph.2021.10.020

Ugwu Okechukwu, P. C., Nwodo Okwesili, F. C., Parker, E. J., Bawa Abubakar, Ossai, E. C., & Odo, C. E. (2013). Phytochemical and acute toxicity studies of *Moringa oleifera* ethanol leaf extract. *International Journal of Life Sciences, Biotechnology, and Pharma Research*, *2*(2), 66–71.

Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A., & Venkitanarayanan, K. (2014). Combating pathogenic microorganisms using plant-derived antimicrobials: A minireview of the mechanistic basis. *BioMed Research International*, *2014*, 1–18. https://doi.org/10.1155/2014/761741

Valle, D. L., Andrade, J. I., Puzon, J. J. M., Cabrera, E. C., & Rivera, W. L. (2015). Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*, *5*(7), 532-540. https://doi.org/10.1016/j.apjtb.2015.04.005

World Health Organization: WHO. (2021) Antimicrobial resistance. *www.who.int*. https://tinyurl.com/27cnzt2w

Wright, P. M., Seiple, I. B., & Myers, A. G. (2014). The evolving role of chemical synthesis in antibacterial drug discovery. *Angewandte Chemie International Edition*, *53*(34), 8840–8869.

https://doi.org/10.1002/anie.201310843

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics*, *10*(3), 318. https://doi.org/10.3390/antibiotics10030318