JOURNAL OF INTERDISCIPLINARY PERSPECTIVES

ISSN Print: 2984-8288, ISSN Online: 2984-8385
Vol. 2, No. 12, pp. 206-215, December 2024

Antimicrobial Activity of Kangkong and Paragis Leaf Extracts against *Klebsiella oxytoca*

Raymund J. Capagas*, Daren Cian D. Capuno, Mary Joy E. Gabao, Arian R. Mamposte, Jullia Franchesca R. Panganiban, Marc Robin S. Pantino
National University, Philippines

*Corresponding Author Email: rjcapagas@nu-moa.edu.ph

Date received: September 3, 2024
Date revised: October 21, 2024
Date accepted: November 7, 2024

Originality: 86%
Grammarly Score: 99%
Similarity: 14%

Recommended citation:
Capagas, R., Capuno, D.C., Gabao, M.J., Mamposte, A., Panganiban, J.F., Pantino, M.R. (2024). Antimicrobial activity of Kangkong and Paragis leaf extracts against *Klebsiella oxytoca*. *Journal of Interdisciplinary Perspectives*, 2(12), 206-215. https://doi.org/10.69569/jip.2024.0469

Abstract. The global spread of multidrug resistance challenges antimicrobial therapy, necessitating the search for alternative approaches. The present study assessed the antimicrobial efficacy of the methanolic leaf extracts of *Ipomoea aquatica* Forssk. (Kangkong), *Eleusine indica* (L.) Gaertn. (Paragis), Moreover, its combination at varying concentrations of 25%, 50%, 75%, and 100% against *Klebsiella oxytoca* using established microbiological techniques. Fifty (50) grams of powdered leaves from the *Ipomoea aquatica* Forssk. (Kangkong) plant was soaked in 375 mL of 95% methanol for 48 hours (2 days) with intermittent stirring—similarly, 50 grams of *Eleusine indica* (L.) Gaertn. (Paragis) were processed using the same method. The macerated powdered leaf samples were filtered using a Whatman filter paper No. 1 and extracted using a rotary evaporator. The obtained extracts were utilized to prepare varying concentrations of 25%, 50%, 75%, and 100% of methanolic leaf extracts for each plant. Positive control (Tigecycline) and negative control (10% DMSO), along with the preparation of extracts, were also prepared. The antimicrobial efficacy of these methanolic leaf extracts at different concentrations and the controls were evaluated against *Klebsiella oxytoca* using the disk diffusion method. The results obtained indicate that the methanolic leaf extracts of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis) did not exhibit any inhibitory effects on *Klebsiella oxytoca*. Both plants' combined methanolic leaf extracts also showed non-inhibitory effects, indicating that *Klebsiella oxytoca* is resistant to both plant extracts and, thus, did not yield a synergistic effect. This study provides a scientific understanding of the antimicrobial efficacy of the plants' properties. Further investigation is needed to assess alternative extraction and methods to determine the antimicrobial efficacy of the plant extracts.

Keywords: Antimicrobial Efficacy; *Eleusine indica*; *Ipomoea aquatica*; *Klebsiella oxytoca*.

1.0 Introduction

Microorganisms' resistance to antibiotics continuously increases over time (Singh et al., 2016). According to Aslam et al. (2018), the appearance and stability of multi-drug-resistant bacteria is an increasing concern for global human health. Among these several types of multi-drug-resistant bacteria are *Klebsiella* species, which is emerging as a significant human pathogen related to the increasing morbidity rates in healthcare settings. This bacterium is isolated from varying clinical specimens, primarily from respiratory secretions and blood; as a result, it is presently invading intensive care units. Thus, hospital infection control committees must monitor the *K. oxytoca* antibiotic susceptibility pattern for better healthcare service (Singh et al., 2016). Shaikh et al. (2015) state that beta-lactamases have made *Klebsiella oxytoca* more resistant to penicillin and ampicillin. The bacterium produces Extended-Spectrum Beta-Lactamase (ESBL), making it resistant to broad-spectrum medicines like cephalosporins and ceftazidim. According to the study of Biswas et al. (2013), Gram-negative bacteria often exhibit an increased resistance to plant-origin antimicrobials due to their distinct cell wall structure, as this serves as a protective barrier that resists the entry of plant extract's component. Singh et al. (2016) found that *Klebsiella oxytoca* was susceptible to tigecycline and colistin.

Due to the progressive resistance of harmful pathogens to synthetic antibiotics, phytomedicine or the science of plant medicine regains interest in current research (Vaou et al., 2021). Naturally occurring plants have secondary metabolites rich in bioactive components, eliciting many beneficial effects in man and animals (Zhao et al., 2015). These active chemicals possess antibacterial properties that hinder the growth of certain bacteria, making them natural antibiotics (Allemailem, 2021). Henceforth, this study looked at the efficacy of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis or Goosegrass) as an antimicrobial agent. *Ipomoea aquatica* Forssk. (Kangkong) belongs to the Convolvulaceae family. It is a tropical crop cultivated for its soft stem and leaves (Sasikala & Sundaraganapathy, 2017). The presence of flavonoids, tannins, glycosides, and saponins was identified through a phytochemical examination of its leaf extracts (Konwar et al., 2021). Additionally, *Ipomoea aquatica* Forssk. (Kangkong) methanolic extract exhibits antimicrobial activity against certain gram-positive and gram-negative bacteria (Sivaraman et al., 2010). Moreover, *Eleusine indica* (L.) Gaertn. (Paragis or Goosegrass) is a perennial grass typically growing in tufts or clumps (Green Institute, 2023). It is historically utilized for illnesses related to the liver and kidneys. Al-Zubairi et al. (2011) stated that Paragis produces many naturally existing secondary metabolites. The methanol, ethanol, and aqueous extracts of *Eleusine indica* (L.) Gaertn. leaves are rich in alkaloids and flavonoids. Among these extracts, the methanol extract stands out as it also contains a small number of tannins. All the extracts share the presence of anthraquinones, while saponins are absent in all of them (Alaekwe et al., 2015).

The emerging resistance of multi-drug-resistant K. oxytoca is increasingly becoming a prominent bacterial strain (Singh et al., 2016). This result is due to its capability to acquire antibiotic resistance (Yang et al., 2022). *Klebsiella oxytoca* resistance to antibiotics is increasingly alarming, as it can acquire antimicrobial resistance by carrying many carbapenemase genes as well as β-lactamase genes; it can also carry multiple virulence genes, making the bacteria a potentially significant detriment to human health (Yang et al., 2022). Naturally, plants have secondary metabolites, considered biologically active chemicals that can exhibit antimicrobial effects (Zhao et al., 2015). Hence, this study assessed the antimicrobial efficacy of the methanolic leaf extracts of *Ipomoea aquatica* Forssk. and *Eleusine indica* (L.) Gaertn. against *Klebsiella oxytoca.* Specifically, this study determined the antimicrobial efficacy of the two plants by measuring the efficacy of the plants to inhibit the growth of *K. oxytoca.* It determined the minimum inhibitory concentrations that yielded potent results. Lastly, this study determined the synergistic activity of the tested natural plants.

2.0 Methodology

2.1 Research Design

The study used an experimental design with methods related to medical technology. According to Frost (2024), an experimental design is a systematic strategy for gathering and organizing data to determine causal connections between variables. It is a scientific examination performed in a controlled setting to observe how an independent variable impacts a dependent variable (Zubair, 2023). This design is a crucial part of the research process as it aids the researchers in achieving the study's objectives (Kelkar, 2024). Hence, the study emphasized the in vitro analysis of the antimicrobial efficacy of methanolic extracts of *Ipomoea aquatica* Forssk. (Kangkong), *Eleusine indica* (L.) Gaertn. (Paragis), and their combination against *Klebsiella oxytoca*.

2.2 Experimental Procedure

Pre-experimentation Phase

To prevent contamination, all the materials required for the sterilizing procedure were subjected to an autoclave at 121 degrees Celsius for about 15 minutes at 15 psi.

Plant Extraction

The following procedures were adapted from the study of Caccam et al. (2020) with modifications. The plants were washed using tap water. The rinsed samples were shade-dried for 14 days. Shade drying provides better advantages as it can preserve light-sensitive phytochemicals and reduce light-induced reactions like oxidation (Naz et al., 2017). Leaves were powdered using a grinder before extraction. The extraction process of the two plants was adapted to the method of Sanchez et al. (2016). *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis) powdered leaves (50 grams each) were soaked in the solvent of 95% Methanol (375 mL) to increase the polarity and to dissolve the components of the leaves. The extraction took 48 hours with occasional mixing using a stirring rod. The solution was filtered using Whatman filter paper No. 1. Finally, the crude extracts of each plant were obtained using a rotary evaporator for 45 minutes at 80 rpm at 40 degrees Celsius. The extracts were stored in a container wrapped with aluminum foil at refrigerator temperature. It was left at room temperature for an hour before its usage.

Preparation of Concentration

Prior to preparing the varying concentrations, one (1) mL of 10% DMSO was added to dissolve the crude extract due to its viscosity. The researchers used volume per volume (v/v) to calculate the concentrations of methanolic extract (experimental control) utilized for the susceptibility testing against *K. oxytoca.*

The researchers utilized volume per volume (v/v) with the given formula:
Concentration = volume of solute ÷ volume of solution
Concentration (C) = 25%, 50%, 75%, 100%
Total volume = 10% DMSO (μL) + extract (μL) = 500 μL
Volume of Solute (μL) = Crude extract of I. aquatica / E. indica

Table 1 shows the measurements of solute, solvent, and total volume at varying concentrations of the methanolic extracts of I. aquatica and E. indica

Table 1. Concentration of *Ipomoea aquatica* and *Eleusine indica*

Concentration (%)	Solute (μL)	Solvent (μL)	Total volume (μL)
25	125	375	500
50	250	250	500
75	375	125	500
100	500	0	500

To prepare the varying concentrations of combined methanolic extracts of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis), the researchers utilized the following ratios: 1:1:6 (25%), 2:2:4 (50%), 3:3:2 (75%), and 4:4 (100%).

Table 2 shows the amounts of solute, solvent, and the total volume of varying concentrations of the combined methanolic extracts of I. aquatica and E. indica with their ratios, respectively.

Table 2. Concentrations of the combined *Ipomoea aquatica* and *Eleusine indica*

Concentration (%)	Solute (μL) I. aquatica	Solute (μL) E. indica	Solvent (μL)	Total volume (μL)
25	62.5	62.5	375	500
50	125	125	250	500
75	187.5	187.5	125	500
100	250	250	0	500

Agar for Disk Diffusion

Mueller-Hinton Agar was poured into 150 mm plates with a uniform depth of 4 mm (McPherson & Pincus, 2021). The plates with agar were left slightly open to avoid moisture collection. The medium was allowed to harden. One sample plate was incubated at 37 degrees Celsius overnight to check for sterility. The plates were refrigerated and placed at room temperature prior to their usage.

Subculturing of Bacteria

The acquired *Klebsiella oxytoca* was delivered to the laboratory in an agar slant. The bacteria were subcultured every week in a MacConkey agar plate. Before inoculum preparation, it was subcultured in two MacConkey agar plates a day before the testing, as 24-hour young culture is required for a better outcome (Mahon & Lehman, 2022).

Impregnation of Blank Disk

Impregnation of 115 blank disks (6 mm) followed the method used in the study of Vineetha et al. (2015) with modifications. The disks were made from Whatman filter paper no. 1 using a puncher and were autoclaved before usage. Each disk was impregnated with a fixed volume of 20µL of 10% dimethyl sulfoxide (negative control), tigecycline (positive control), and varying concentrations of methanolic leaf extracts of 25%, 50%, 75%, and 100% inside the biosafety cabinet and was left for two (2) hours until it dried.

Inoculum Preparation

The inoculum was prepared based on the study of Suurbaar et al. (2017) by suspending the subcultured bacteria in a sterile normal saline solution (NSS), as it is imperative to use fresh colonies for accurate results. A cotton swab was used to touch two to four isolated colonies in the MHA agar plate to be tested (McPherson & Pincus, 2021). The cotton swabs with colonies were suspended in 3 ml NSS. A vortex mixer was utilized to achieve the 0.5 McFarland turbidity standard. The inoculum was used within 15 minutes of preparation (Mahon & Lehman, 2022).

Inoculation of MHA Plates

The cotton swab was dipped into the tube using the prepared inoculum, and excess fluid was removed. The MHA agar plates were inoculated with a moist swab streaked four times over the surface. The plates are then rotated roughly 60 degrees to achieve even distribution of the inoculum. Each plate was streaked with a different sterile cotton swab. The swabs were discarded in the designated container. After the inoculation of the plates, they were covered with their lids. The disks with varying concentrations of 25%, 50%, 75%, and 100% were placed using sterile forceps in each plate. On the other hand, in a separate plate, the Tigecycline disks (positive control) and 10% DMSO disks (negative control) were placed using sterile forceps. Incubation at 37 degrees Celsius lasted for 24 hours, using a caliper, and the zone of inhibition in millimeters was measured (McPherson & Pincus, 2021).

Kirby-Bauer Disk Diffusion Method

The researchers focused on the antimicrobial efficacy of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis) methanolic leaf extracts against *K. oxytoca*. Therefore, the researchers conducted antimicrobial susceptibility testing (AST). To further analyze the data, the Kirby-Bauer test was utilized to assess the sensitivity or resistance of *K. oxytoca* to the methanolic extract of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis).

The Kirby-Bauer test determined the resistance or sensitivity of the pathogen to numerous antimicrobial compounds. The researchers interpreted the results as S (susceptible), I (intermediate), and R (resistant). A result categorized as sensitive shows that the bacteria will die when exposed to the methanolic leaf extract of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis). An intermediate result suggests that the maximum recommended dosage is required to inhibit the bacteria. The resistance result shows that the bacteria is resistant to the antimicrobial compounds of the methanolic extract leaf of *Ipomoea aquatica* Forssk. (Kangkong), *Eleusine indica* (L.) Gaertn. (Paragis). Moreover, combined based on the standard zone of inhibition, FDA breakpoints of the Kirby-Bauer method set by the Food and Drug Administration.

Waste Disposal

After the experiment, the researchers placed all laboratory solid waste contaminated with biohazardous materials in the cabinet. Afterward, the research staff autoclaved all the contaminated materials for 60 minutes at 121°C and 15 psi.

2.3 Ethical Considerations

The laboratory implemented various considerations to ensure a safe environment in the microbiology laboratory. The Center for Basic Biomedical Research (CBBR) has collated laboratory safety information into one manual as a reference for personnel using the laboratories of CBBR. The Laboratory Safety Manual of De La Salle Health Sciences Institute Research Division Center for Basic Biomedical Research is the basis for the following ethical and safety considerations.

The student researchers were well-informed about the bacteria handled in the laboratory. Upon acquiring the bacteria, the Philippine National Collection of Microorganisms (PNCM) UP-LB included the authenticity of

Klebsiella oxytoca. The researchers utilized a class II biosafety cabinet as *Klebsiella oxytoca* is a risk group 2 microorganism. Materials and supplies stayed in the laboratory to prevent contamination and the spread of potential infection. Student researchers and the laboratory staff were well-informed about the biosafety practices and techniques used in the laboratory.

3.0 Results and Discussion

3.1 Inhibition Zone of the Methanolic Leaf Extract of *I. aquatica*

The study used the disk diffusion method to evaluate the antimicrobial efficacy of the methanolic leaf extracts of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis) against *Klebsiella oxytoca* with trials one (1), two (2), and three (3), and replicates one (1), two (2), and three (3) using varying concentrations (25%, 50%, 75%, and 100%). The researchers used the measurement of the disk diameter, which measures 6 mm, as an indicator of no diameter of inhibition. Thus, the result of the methanolic leaf extract of *Ipomoea aquatica* Forssk. (Kangkong) against *Klebsiella oxytoca* demonstrated a 6 mm diameter of inhibition, which falls on the resistance breakpoints set by the FDA.

Table 3. Diameter inhibition of the methanolic leaf extract of *I. aquatica* compared with the positive control and negative control against *k.*

Trials	25%			50%			75%			100%			(+)	(-)
	Replicates			Replicates			Replicates			Replicates				
	1	2	3	1	2	3	1	2	3	1	2	3		
1	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R
2	R	R	R	R	R	R	R	R	R	R	R	R	32.65mm (S)	R
3	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R

Note: Food and Drug Administration Disk Diffusion Breakpoints for Tigecycline Susceptible (S) = ≥19mm Intermediate (I) = 15-18mm Resistant (R) = ≤14mm + = Positive Control, - = Negative Cont

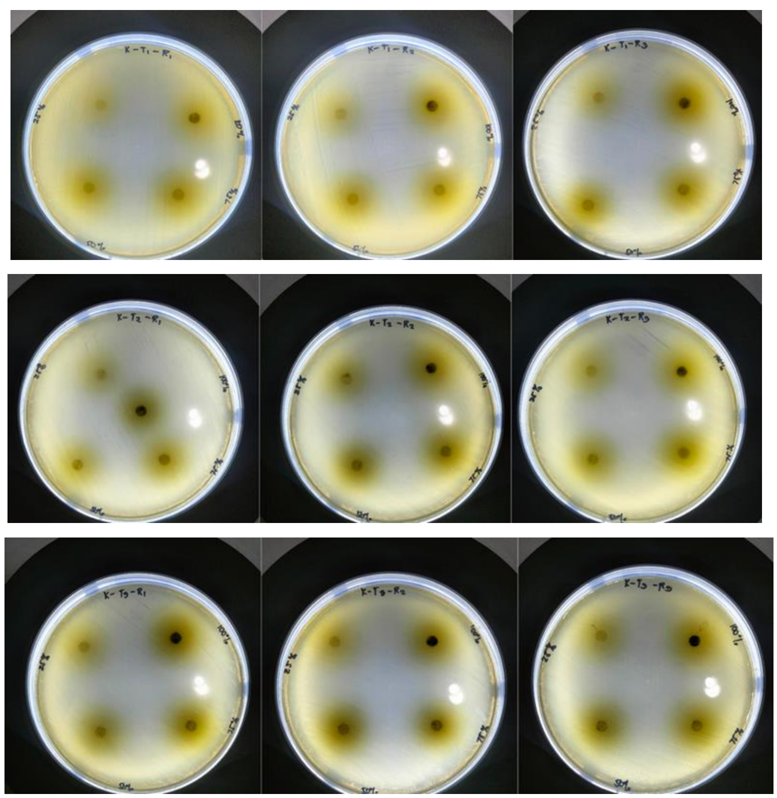

Figure 1. Trial 1,2,3, with Replicates 1-3, of Agar Disk Diffusion of the Varying Concentrations of Methanolic Leaf Extract of *Ipomoea aquatica*

This finding suggests that the extract, at these concentrations, is ineffective against this specific bacterial strain. Thus, this indicates that establishing the minimum inhibitory concentration is not possible. A study by Yoon et al. (2018) demonstrated the methanolic leaf extract of *Ipomoea aquatica* Forssk. (Kangkong) is effective against certain gram-negative bacteria. The study is limited to *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

In addition to the previous research of Yoon et al. (2018), the researcher's study indicates that the methanolic leaf extract of *Ipomoea aquatica* is not a highly effective antimicrobial against *K. oxytoca*.
Tigecycline, the positive control, exhibited a diameter inhibition of 33.21mm (Trial 1), 32.65mm (Trial 2), and 33.21mm (Trial 3) with an average of 33.02 mm (Figure 2) against *Klebsiella oxytoca*, indicating its susceptibility to the antibiotic mentioned above. The results of the two plants, specifically Ipomoea aquatica Forssk. (Kangkong) and Eleusine indica (L.) Gaertn. (Paragis), strongly suggest that establishing the minimum inhibitory concentration is not possible.

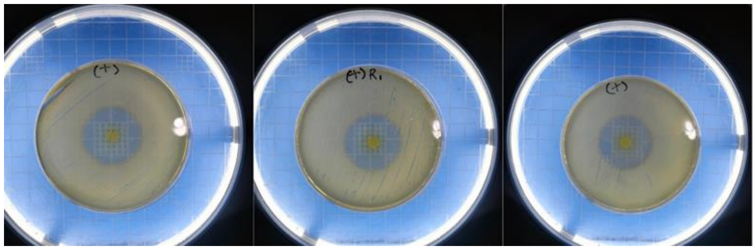

Figure 2. Three (3) trials of Agar Disk Diffusion of the positive control (Tigecycline)

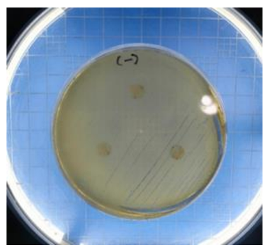

Figure 3. Agar Disk Diffusion of the negative control (10% DMSO).

3.2 Inhibition zone of the Methanolic Leaf Extract of *E. indica*

The researchers observed a 6 mm diameter in the results of the methanolic leaf extract of *Eleusine indica* (L.) Gaertn. (Paragis) against *K. oxytoca*. This result suggests the ineffectiveness of the extract against *Klebsiella oxytoca* at varying concentration levels. A study conducted by Adoho et al. (2021) showed that the methanolic and chloroform extracts of *Eleusine indica* (L.) Gaertn. (Paragis) exhibits antibacterial activity against certain gram-negative bacteria, including *Klebsiella aerogenes, Proteus vulgaris, Enterobacter aerogenes, Pseudomonas aeruginosa, and Escherichia coli*. Conversely, these findings suggest that the methanolic extract is not a potent antimicrobial against *Klebsiella oxytoca*.

Table 4. Diameter Inhibition of the Methanolic Leaf Extract of *E. indica* in Comparison with the Positive Control and Negative Control against *K. oxytoca*

Trials	25%			50%			75%			100%			(+)	(-)
	Replicates			Replicates			Replicates			Replicates				
	1	2	3	1	2	3	1	2	3	1	2	3		
1	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R
2	R	R	R	R	R	R	R	R	R	R	R	R	32.65mm (S)	R
3	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R

Note: Food and Drug Administration Disk Diffusion Breakpoints for Tigecycline Susceptible (S) = ≥19mm Intermediate (I) = 15-18mm Resistant (R) = ≤14mm + = Positive Control - = Negative Control

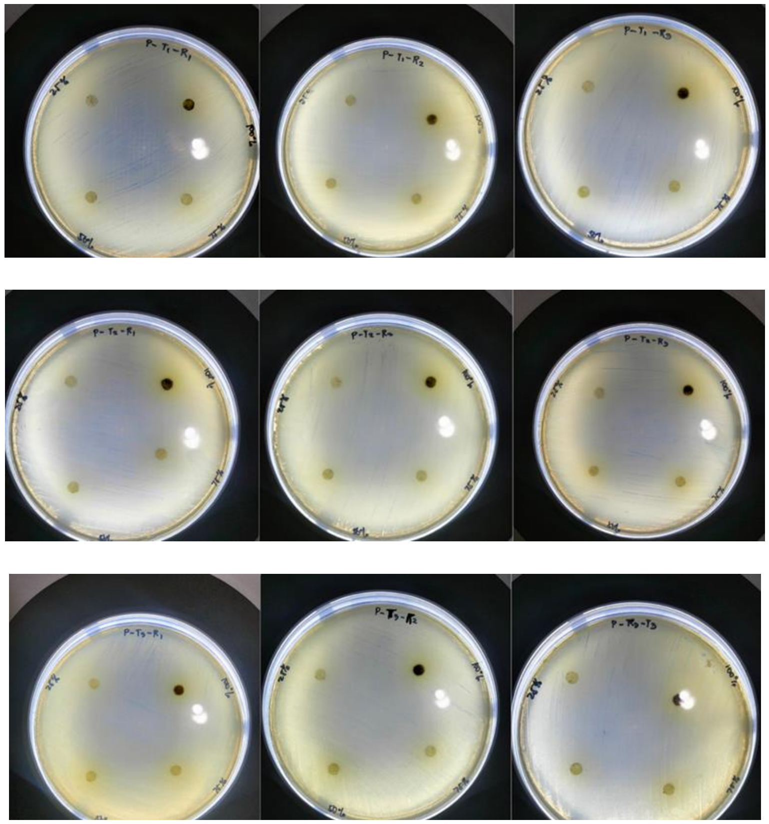

Figure 4. Trial 1,2,3, with Replicates 1-3, of Agar Disk Diffusion of the Varying Concentrations of Methanolic Leaf Extract of *Eleusine indica*.

3.3 Inhibition zone of the Combined Methanolic Leaf Extracts of the I. aquatica and E. indica

The researchers combined the methanolic leaf extracts of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis). The disk diffusion method results exhibit a 6 mm diameter of inhibition on all three trials and three replicates of varying concentrations. Thus, it was concluded that *Klebsiella oxytoca* is resistant to the combined methanolic leaf extracts. Therefore, the findings show no synergistic effect between the methanolic leaf extracts of the two plants, specifically *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis). It was observed that the combination of the two plants does not enhance their antimicrobial action.

Table 5. Diameter Inhibition of the Combined Methanolic Leaf Extracts of the I. aquatica and E. indica in Comparison with the Positive Control and Negative Control Against K. oxytoca

Trials	**25%**			**50%**			**75%**			**100%**			**(+)**	**(-)**
	Replicates			**Replicates**			**Replicates**			**Replicates**				
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**		
1	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R
2	R	R	R	R	R	R	R	R	R	R	R	R	32.65mm (S)	R
3	R	R	R	R	R	R	R	R	R	R	R	R	33.21mm (S)	R

Note: Food and Drug Administration Disk Diffusion Breakpoints for Tigecycline Susceptible (S) = ≥19mm Intermediate (I) = 15-18mm Resistant (R) = ≤14mm + = Positive Control - = Negative Control

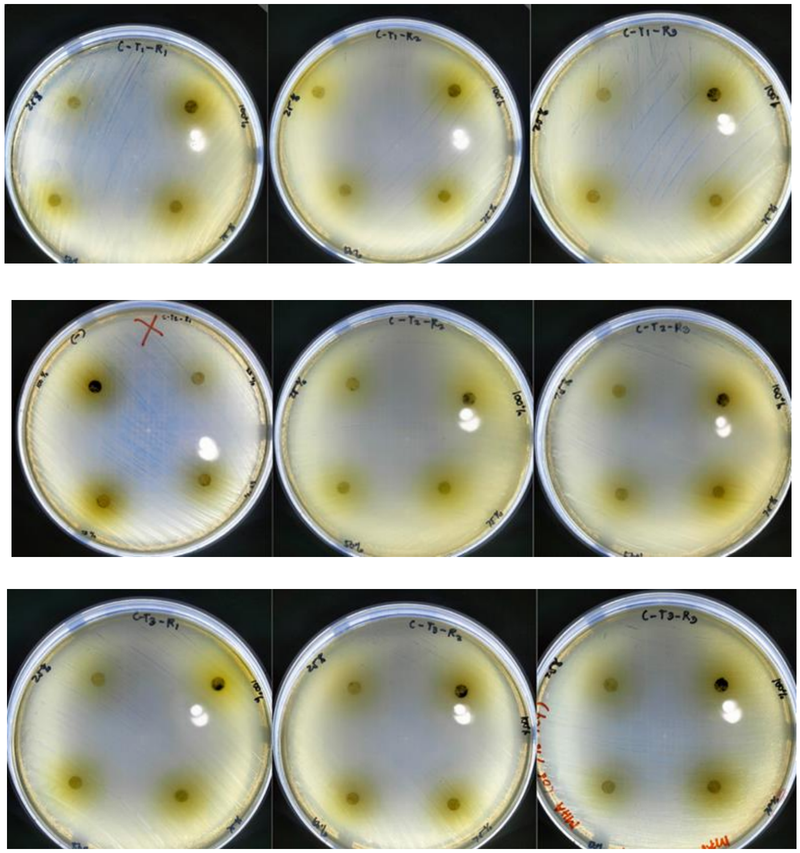

Figure 5. Trial 1,2,3, with Replicates 1-3, of Agar Disk Diffusion of the Varying Concentrations of Combined Methanolic Leaf Extracts of *I. aquatica* and *E. indica*

According to Izah (2018), several factors influence the antimicrobial susceptibility pattern of plant extracts, including adaptability, physiology, and microorganism metabolism. Gram-negative bacteria often exhibit increased resistance to plant-origin antimicrobials due to their distinct cell wall structure, which serves as a protective barrier that resists the entry of plant extract components (Biswas et al., 2013). *Klebsiella oxytoca* is a gram-negative, encapsulated bacteria (Al-Khikani et al., 2020). The bacteria's resistance to plant extracts is due to the capsule, which obstructs the penetration of the methanolic leaf extracts of *Eleusine indica* and *Ipomoea aquatica*. According to Yang et al. (2022), current research discovered that *Klebsiella oxytoca* is not a single species, but a complex one composed of other *Klebsiella* species, which are *K. oxytoca, K. grimontii, K. michiganensis, K. pasteurii, K. spallanzanii, K. huaxiensis* and other three unnamed novel species based on the microorganism's genomic taxonomy. The current study highlighted the genetic flexibility of *Klebsiella oxytoca*, which is highly diverse, and the data regarding the species is limited; however, the study suggests that *Klebsiella oxytoca* can acquire antimicrobial resistance independently (Moradigaravand et al., 2017). The outcome of the current research shows that the tested bacteria (*Klebsiella oxytoca*) is resistant to the methanolic leaf extract of *Ipomoea aquatica* Forssk. (Kangkong), *Eleusine indica* (L.) Gaertn. (Paragis) and their combination.

4.0 Conclusion

The methanolic leaf extract of *Ipomoea aquatica* Forssk. (Kangkong), in varying concentrations of 25%, 50%, 75%, and 100%, showed a diameter inhibition of 6mm, compared to the positive control (tigecycline) with a diameter inhibition of 33.21mm, 32.65mm, and 33.21mm with an average of 33.02 mm. Thus, this result indicates resistance of *Klebsiella oxytoca* to the extract. Furthermore, the methanolic leaf extract of *Eleusine indica* (L.) Gaertn. (Paragis), also exhibited a diameter inhibition of 6mm at concentrations of 25%, 50%, 75%, and 100%, in contrast to the positive control (Tigecycline), with a diameter inhibition of 33.21mm, 32.65mm, and 33.21mm with an average of 33.02mm. Thus, this outcome implies that *Klebsiella oxytoca* is resistant to the extract. In addition, the combined methanolic leaf extracts of *Ipomoea aquatica* Forssk. (Kangkong) and *Eleusine indica* (L.) Gaertn. (Paragis) produced a diameter inhibition of 6mm at varying concentrations of 25%, 50%, 75%, and 100%, in contrast to the positive control (tigecycline), which showed a diameter inhibition of 33.21mm, 32.65mm, and 33.21mm with an average of 33.02mm. As a result, this implies that the *Klebsiella oxytoca* is resistant to the extract. The conclusion was reached that there is no synergistic effect between the methanolic leaf extracts of the two plants.

This study concluded that there was low antimicrobial efficacy against *K. oxytoca* and that a minimum inhibitory concentration could not be established on both plants and their combination. Further investigation is needed to assess the bacteria's minimum inhibitory concentration, such as utilizing alternative extraction techniques and methods used in antimicrobial susceptibility testing. Furthermore, this study would recommend that policymakers or pharmaceutical companies explore other plants that will help combat the ongoing resistance of the bacteria to various antibiotics. In addition, future researchers may find the gaps in this study. They could improve or explore other procedures that could exhaust the possible applications of *I. aquatica* and *E. indica.*

5.0 Contributions of Authors

The following are the author's contributions; RJC – conceptualizing, writing, editing, supervising, data analysis, finalizing. DCDC – conceptualizing, writing, editing, data analysis, funding, finalizing. MJEG - conceptualizing, writing, editing, data analysis, funding, finalizing. ARM - conceptualizing, writing, editing, data analysis, funding, finalizing. JFRP - conceptualizing, writing, editing, data analysis, funding, finalizing. MRSP - conceptualizing, writing, editing, data analysis, funding, finalizing.

6.0 Funding

This work has not received funding from any funding agency.

7.0 Conflict of Interest

The authors declare no conflict of interest.

8.0 Acknowledgment

The authors would like to thank all who have provided assistance and technical assistance in conducting this study.
.

9.0 References

Adoho, A. C. C., Zinsou, F. T. A., Olounladé, P. A., Azando, E. V. B., Hounzangbé-Adoté, M. S., & Gbangboche, A. B. (2021). Review of the literature of Eleusine indica: phytochemical, toxicity, pharmacological and zootechnical studies. Journal of Pharmacognosy and Phytochemistry, 10(3), 29–33. https://doi.org/10.22271/phyto.2021.v10.i3a.14060

Alaekwe, Ajiwe, Ajiwe, Aningo, A., I. O, Ajiwe, V. I. E, Ajiwe, A. C. &. Aningo, G. N. (2015). Phytochemical and anti–microbial screening of the aerial parts of Eleusine indica. Indian Journal of Pure & Applied Biosciences, 3(1), 257-264. https://tinyurl.com/4dw368s9

Allemailem, K. S. (2021). Antimicrobial potential of naturally occurring bioactive secondary metabolites. Journal of Pharmacy and Bioallied Sciences, 13(2), 155. https://doi.org/10.4103/jpbs.jpbs_753_20

Al-Khikani, F. H. O., Abadi, R. M., & Ayit, A. S. (2020). Emerging carbapenemase Klebsiella oxytoca with multidrug resistance implicated in urinary tract infection. Biomedical and Biotechnology Research Journal, 4(2), 148. https://doi.org/10.4103/bbrj.bbrj_165_19

Al-Zubairi, A. S., Abdul, A. B., Abdelwahab, S. I., Peng, C. Y., Mohan, S., & Elhassan, M. M. (2011). Eleucine indica possesses antioxidant, antibacterial and cytotoxic properties. Evidence-Based Complementary and Alternative Medicine, 2011(1), 965370. https://doi.org/10.1093/ecam/nep091

Aslam, B., Wang, W., Arshad, M., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance, 11, 1645–1658. https://doi.org/10.2147/idr.s173867

Bhaigybati, T., Sanasam, S., Gurumayum, J., Singh, L. R., & Devi, P. G. (2020). Phytochemical profiling, antioxidant activity, antimicrobial activity and GC-MS analysis of Ipomoea aquatica Forsk collected from EMA market, Manipur. J Pharmacogn Phytochem, 9(1), 2335-2342. https://tinyurl.com/mwnnjscx

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., & Yadav, A. (2013). Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajavaL.) on Two Gram-Negative and Gram-Positive Bacteria. International Journal of Microbiology, 0(0), 1–7. https://doi.org/10.1155/2013/746165

Boakye, Y. D. (2016). Anti-infective properties and time-kill kinetics of phyllanthus muellerianus and its major constituent, geraniin. Medicinal Chemistry, 6(2). https://doi.org/10.4172/2161-0444.1000332

Caccam, G. G., Mendoza, J. M., Rico, N. a. G., Robles, J. a. A., & Taculing, L. D. (2020). Comparative study of Paragis (Eleusine indica) and Guava (Psidium guajava) Methanolic leaf extract against Staphylococcus aureus and Escherichia coli. Health Research Bulletin, 7, 45-64. https://tinyurl.com/3xcaarja

Cadman, B. (2023). Klebsiella oxytoca infection: What you should know. Retrieved from: https://www.medicalnewstoday.com/articles/321072

Cancaya, İ. İ. T., & Somuncuoglu, E. I. (2021). Potential and Prophylactic Use of Plants Containing Saponin-Type Compounds as Antibiofilm Agents against Respiratory Tract Infections. Evidence-based Complementary and Alternative Medicine, 0(0), 1–14. https://doi.org/10.1155/2021/6814215

Dago, T. R., Zewudie, A., Mamo, Y., Feyissa, D., & Geleta, S. (2020). Multi-Drug Resistant Post Corneal Repair Klebsiella oxytoca's Keratitis. International medical case reports journal, 13, 537–541. https://doi.org/10.2147/IMCRJ.S278625

De Paula Barbosa, A. (2014). An overview on the biological and pharmacological activities of saponins. Int J Pharm Pharm Sci, 6(8), 47-50. https://tinyurl.com/2p93yja3

Dias, M. C., Pinto, D., & Silva, A. M. S. (2021). Plant flavonoids: chemical characteristics and biological activity. Molecules, 26(17), 5377. https://doi.org/10.3390/molecules26175377

Ettebong, Ette & Obot, Daniel. (2020). A Systematic review on Eleusine indica (L.) Gaertn.): From ethnomedicinal uses to pharmacological activities. Journal of Medicinal Plants Studies, 8(4), 262-274. https://tinyurl.com/4afystrz

Farha, A. K., Yang, Q.-Q., Kim, G., Li, H.-B., 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

Frost, J. (2024). Experimental Design: Definition and types. Retrieved from https://statisticsbyjim.com/basics/experimental-design/

Gauro, R., & Amatya, P. (2022). Phytochemical screening and biological activities of Ipomoea aquatica forssk. World journal of pharmacy and pharmaceutical Sciences, 11(9). https://doi.org/10.20959/wjpps20229-23165

Holland, K. (2017). What is Klebsiella oxytoca? Retrieved from https://www.healthline.com/health/klebsiella-oxytoca

Izah, S. C. (2018). Some determinant factors of antimicrobial susceptibility pattern of plant extracts. Research and Review Insights, 2(3), 1-4. https://doi.org/10.15761/rri.1000139

Md. Kamruzzaman. (2015). In vitro antimicrobial activity of different extracts of gotu kola and water spinach against pathogenic bacterial strains. Current Research in Microbiology and Biotechnology, 3(4), 663-669. https://doi.org/10.13140/RG.2.1.2180.4642

Kelkar, G. (2024). What is Experimental Design? A Comprehensive Guide for 2023. Retrieved from https://tinyurl.com/mpnfvj9u

Khan, R., Hoque, S. M., Hossain, K.F., Siddique, A.B., Uddin, K., & Rahman, M. (2020) Green synthesis of silver nanoparticles using Ipomoea aquatica leaf extract and its cytotoxicity and antibacterial activity assay. Green Chemistry Letters and Reviews, 13(4), 303-315. https://doi.org/10.1080/17518253.2020.1839573

Konwar, M., Sarma, M. P., Loying, S., Nayak, R., & Bhagawati, P. (2021). Phytochemical analysis and antimicrobial activity of leaves of Ipomoea aquatica forssk. International Journal of Botany Studies, 6(5), 1310-1314. https://tinyurl.com/4e7fhrza

Larghi, E. L., Bracca, A. B., Arroyo Aguilar, A. A., Heredia, D. A., Pergomet, J. L., Simonetti, S. O., & Kaufman, T. S. (2015). Neocryptolepine: A Promising Indoloisoquinoline Alkaloid with Interesting Biological Activity. Evaluation of the Drug and its Most Relevant Analogs. Current topics in medicinal chemistry, 15(17), 1683–1707. https://doi.org/10.2174/1568026615666150427113937

Li, N., Tan, Sn., Cui, J. et al. (2014). PA-1, a novel synthesized pyrrolizidine alkaloid, inhibits the growth of Escherichia coli and Staphylococcus aureus by damaging the cell membrane. J Antibiot 67, 689–696. https://doi.org/10.1038/ja.2014.49

Liu, X., & Lu, M. (2023). Normal saline: Past, present, and future. Science Progress, 106(2), 003685042311688. https://doi.org/10.1177/00368504231168821

Mahon, C. R., & Lehman, D. C. (2019). Textbook of Diagnostic Microbiology (6th ed.) Elsevier.

McPherson, R. A., & Pincus, M. R. (2021). Henry's Clinical Diagnosis and Management by Laboratory Methods E-Book. Elsevier Health Sciences.

Moradigaravand, D., Martin, V., Peacock, S. J., & Parkhill, J. (2017). Population Structure of Multidrug-Resistant Klebsiella oxytoca within Hospitals across the United Kingdom and Ireland Identifies Sharing of Virulence and Resistance Genes with K. pneumoniae. Genome Biology and Evolution, 9(3), 574–584. https://doi.org/10.1093/gbe/evx019

Nakul, N., Phukan, U., Puzari, M., Sharma, M., & Chetia, P. (2021). Klebsiella oxytoca and Emerging Nosocomial Infections. Current Microbiology, 78(4), 1115–1123. https://doi.org/10.1007/s00284-021-02402-2

Naz, R., Ayub, H., Nawaz, S., Islam, Z. U., Yasmin, T., Bano, A., Wakeel, A., Zia, S., & Roberts, T. H. (2017). Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC Complementary and Alternative Medicine, 17(1), 302. https://doi.org/10.1186/s12906-017-1815-z

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., & Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflammation and Allergy - Drug Targets, 8(3), 229–235. https://doi.org/10.2174/187152809788681029

Safe, S., Jayaraman, A., Chapkin, R. S., Howard, M., Mohankumar, K., & Shrestha, R. (2021). Flavonoids: structure–function and mechanisms of action and opportunities for drug development. Toxicological Research, 37(2), 147–162. https://doi.org/10.1007/s43188-020-00080-

Saikia, K., Dey, S., Hazarika, S. N., Handique, G. K., Thakur, D., & Handique, A. K. (2023). Chemical and biochemical characterization of Ipomoea aquatica: Genoprotective potential and inhibitory mechanism of its phytochemicals against α-amylase and α-glucosidase. Frontiers in Nutrition, 10, 1304903. https://doi.org/10.3389/fnut.2023.1304903

Sánchez, E., Rivas Morales, C., Castillo, S., Leos-Rivas, C., García-Becerra, L., & Ortiz Martínez, D. M. (2016). Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. Evidence-Based Complementary and Alternative Medicine, 2016(1), 1572697. https://doi.org/10.1155/2016/1572697

Sasikala, M., & Sundaraganapathy, R. (2017). Optimization of extraction method for Ipomoea aquatica forssk. [indian river spinach] from its whole plant. International Journal of Recent Scientific Research, 8(5), 16967–16971. https://doi.org/10.24327/ijrsr.2017.0805.0254

Shaaban, M. T., Ghaly, M. F., & Fahmi, S. M. (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. Journal of Basic Microbiology, 61(6), 557–568. https://doi.org/10.1002/jobm.202100061

Shah, P. (2021). Phytochemical analysis and antioxidant activity of Ipomoea aquatica from Ghodaghodi wet land area, Nepal. International Journal of Herbal Medicine, 9(2), 23-27. https://tinyurl.com/4sh6dcjv

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. Mohd. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences, 22(1), 90–101. https://doi.org/10.1016/j.sjbs.2014.08.002

Singh, L., Cariappa, M., & Kaur, M. (2016). Klebsiella oxytoca: An emerging pathogen? Medical Journal Armed Forces India, 72, 59–61. https://doi.org/10.1016/j.mjafi.2016.05.002

Sivaraman, D., Muralidaran, P., & Kumar, S. S. (2010). Evaluation of Anti-microbial and anti-inflammatory activity of methanol leaf extract of Ipomoea aquatica Forsk. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1(2), 258–26. https://tinyurl.com/yexvebux

Sukor, N. S. M., Zakri, Z. H. M., Rasol, N. E., & Salim, F. (2023b). Annotation and Identification of Phytochemicals from Eleusine indica Using High-Performance Liquid Chromatography Tandem Mass Spectrometry: Databases-Driven Approach. Molecules, 28(7), 3111. https://doi.org/10.3390/molecules28073111

Suurbaar, J., Mosobil, R., & Donkor, A. (2017). Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of Ricinus communis against selected pathogens. BMC Research Notes, 10(1). https://doi.org/10.1186/s13104-017-3001-2

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms, 9(10), 2041. https://doi.org/10.3390/microorganisms9102041

Vineetha, N., Vignesh, R., & Sridhar, D. (2015). Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples. International Journal of Applied Research, 1(11), 624–631. https://tinyurl.com/j7rmm8my

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

Yang, J., Long, H., Hu, Y., Feng, Y., McNally, A., & Zong, Z. (2022). Klebsiella oxytoca Complex: Update on Taxonomy, Antimicrobial Resistance, and Virulence. Clinical Microbiology Reviews, 35(1). https://doi.org/10.1128/cmr.00006-21

Yoon, B. K., Jackman, J. A., Valle-González, E. R., & Cho, N. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. International Journal of Molecular Sciences, 19(4), 1114. https://doi.org/10.3390/ijms19041114

Zakri Z.H.M., Suleiman M., Shean Yeaw N., Ngaini Z., Maili S., Salim F. Eleusine indica for Food and Medicine. J. Agrobiotechnol, 12, 68–87. https://doi.org/10.37231/jab.2021.12.2.260

Zhao, Y., Wu, Y., & Wang, M. (2015). Bioactive substances of plant origin. In Springer eBooks.