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Antibacterial Effects of Leaf Extracts from *Aloe vera* and *Cassia tora* Subjected to Varying Watering Regimes on *Salmonella typhi* and *Streptococcus pneumoniae*

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Abstract

This study was undertaken to investigate the antibacterial activity of A. vera and C. tora subjected to different watering regimes on S. typhi and S. pneumoniae. Leaves from A. vera and C. tora exposed to different watering regimes of Daily (Control), 2, 4 and 8 days was used to prepare methanolic extracts at a concentration of 100, 50, 25, 12.5 and 6.25 mg/ml using double-fold dilution method, using DMSO and Augmentin (625 mg/ml) as negative and positive controls. The antibacterial sensitivity was determined by measuring the zone of inhibition, followed by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The result showed that, on S. typhi, highest zone of inhibition of 21.5 mm and 18.0 mm on S. pneumoniae were observed at 100 mg/ml when C. tora received daily watering. There was no significant difference on the zone of inhibition of the extract across the watering regimes when the concentration was 100 mg/ml. Extract from A. vera resulted in the highest zone of inhibition of 22.3 and 16.8 mm on S. typhi and S. pneumoniae at 100 mg/ml at Daily watering regime. On both pathogens tested, the MIC of C. tora extract was found to be 50 mg/ml on Daily and 2-Day watering regimes, and increased to 100 mg/ml at 4 and 8-Days on S. typhi; while A. vera exhibited 50 mg/ml MIC across the watering regimes on both S. typhi and S. pneumoniae. The MBC was found to be 50 mg/ml of C. tora extract on both test organisms, while in A. vera was much specific at 4-Day watering regime. Both A. vera and C. tora extracts have potential as natural antibacterial agents, affected by concentration and watering regimes.

Keywords; *A. vera, C. tora, S. typhi, S. pneumoniae,* MIC, MBC.

INTRODUCTION

Medicinal plants play a crucial role in providing therapeutic effects due to the presence of phytochemicals, which are non-nutrient substances protecting plants from infections and pests (Ravichandran *et al*., 2023). These plants exhibit various potentials, including antibacterial, antidiabetic, anti-inflammatory, and pain-relieving properties (Hassan and Mohammed, 2023; Chattopadhyay and Mandal, 2023). The active components in medicinal plants are utilized in traditional and modern medicine systems to treat a wide range of diseases, emphasizing their significance in healthcare globally. The diverse pharmacological activities of medicinal plants, such as *Cucumis sativus*, have been extensively studied, showcasing their potential in combating ailments like ulcers (Praseetha *et al*., 2023). Among many other curative properties, they possess the following potentials of being antibacterial,

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antidiabetic, anti-inflammatory, pain reliever (Rosario, 2015 and Mahima, 2021). In addition, most of the orthodox medicines are derived from medicinal plants (Wakdikar, 2004).

Medicinal plants, owing to their rich reservoir of bioactive compounds, have been considered as promising resources in combating multidrug resistant (MDR) microbial pathogens, which have emerged as a significant public health concern (Ventola, 2015). However, the exploration of the full potential of medicinal plants in this context remains relatively underexplored, and in light of the escalating threat of antimicrobial resistance (AMR) to contemporary medicine, coupled with the ease of international travel and its role in propagating AMR (Gandra *et al.*, 2019), there is an urgent need to comprehensively assess the antimicrobial efficacy of medicinal plants. Infections caused by resistant microbial strains are associated with elevated morbidity and mortality rates, increased healthcare expenditures, prolonged hospitalization periods, and substantial strain on healthcare systems compared to infections stemming from susceptible organisms (Spellberg *et al.*, 2016).

Antibiotics have conventionally functioned as the main therapeutic weapon against bacterial infections. Nevertheless, the prolonged and extensive use of antibiotics has led to a troubling occurrence - the development of antibiotic resistance (Alaoui Mdarhri *et al.*, 2020). This growing problem emphasizes the necessity for alternative approaches in the treatment of bacterial infections. Research has conclusively verified the disadvantages linked to synthetic medications, which frequently display harmful side effects and pose the danger of promoting resistance themselves (Ventola, 2015). In reaction to this dilemma, there is a rising acknowledgment of the medicinal potential of plants to provide a source of safe therapeutic substances that could tackle these challenges (Kalia, 2005). The use of natural remedies to improve health and treat illnesses has been a long-standing practice, and indeed, many of the foundations of contemporary medicine can be traced back to compounds sourced from nature (Newman and Cragg, 2012).

Water stress, including drought, significantly impacts the phytochemical composition of plants (Shil and Dewanjee, 2022). Studies show that under water deficit conditions, plants exhibit changes in their secondary metabolites, with some metabolites increasing in concentration while others decrease (Borim *et al.*, 2023). Drought stress can lead to alterations in the production and accumulation of secondary plant metabolites like alkaloids, tannins, and terpenoids, potentially enhancing the quality and quantity of these bioactive compounds (Khalid *et al.*, 2023). Additionally, water scarcity can downregulate the biosynthesis of certain bioactive metabolites such as pinitol, quercetin, and kaempferol. The response to water stress varies among plant species, with different strategies observed, including the accumulation of osmoprotectants and alterations in transpiration efficiency (Borim *et al.*, 2023). However, water significantly impacts the antimicrobial effects in plants (Darwish *et al.*, 2023; Khalid *et al.*, 2023). Studies on *Ochradenus baccatus* and alfalfa plants exposed to water stress conditions demonstrated alterations in their antimicrobial capacities. For *O. baccatus*, exposure to osmotic stress led to improved antimicrobial powers in aqueous and methanolic extracts, particularly against *Staphylococcus aureus* and *Candida albicans* (Mickky *et al.*, 2016). Similarly, alfalfa plants subjected to water deficit stress exhibited promising antimicrobial efficiency, with extracts showing inhibitory effects on various bacteria and fungi, especially under water-unsatisfied conditions (Veach *et al.*, 2020). These findings revealed a relationship between water stress and the antimicrobial potential of plants, highlighting the need for further research to harness this potential for medicinal and agricultural purposes. Hence, this research aimed at determination of the effects of leaf extracts from *A. vera* and *C. tora* subjected to varying watering regimes on two human pathogenic bacteria, the *S. typhi* and *S. pneumoniaee*.

MATERIALS AND METHODS

Experimental Site and Sourcing of Plant Material

The experiment was conducted in the Biology Laboratory of the Biological Sciences department, Federal University Dutse, Jigawa State, Nigeria. The plant materials used in this study (*A. vera* and *C. tora*), were sourced from the Botanical garden, Department of Biological Sciences, Federal University Dutse. *A. vera* seedlings were following the protocol of Smith *et al*. (2020). These plants were chosen for their maturity and absence of visible diseases or pests. The collection process adhered to ethical and sustainable harvesting practices. *C. tora* seeds were collected following the guidelines of Johnson and Brown (2019).

Watering Regimes and Treatment Applications

The watering intervals involved 2-day, 4- day and 8-day interval including control, with daily water application. The control group received daily watering to maintain consistent soil moisture levels. Each plant was provided with a predetermined volume of water daily, ensuring that the soil remained consistently moist but not waterlogged (Smith *et al*., 2018). Plants subjected to the 2-day watering interval received water every two days. The 4-day watering interval was implemented to induce mild water stress. The 8-day watering interval represented a severe water stress condition (Gupta *et al*., 2020; Lee *et al*., 2017).

Collection and Sterilization of Plant Materials

Collected plant materials (leaves) were subjected to thorough cleaning to ensure their suitability for extraction. The plant materials were washed thoroughy under running tap water to remove any surface contaminants or impurities (Vasyl *et al*., 2023). After washing, the materials were rinsed in distilled water to further eliminate any residual impurities. The cleaned plant materials were air dried in a shaded area at room temperature for weeks, ensuring the materials were suitable for the extraction process (Kapadia *et al*., 2022). The dried plant parts were ground into a fine powder using a home grinder. The weight of the resulting ground powder was measured accurately for subsequent extraction steps.

Preparation of Leaf Extracts

The extraction of phytochemicals from the grinded plant material was performed using a standard Soxhlet extraction method (Sunil *et al*., 2017; Elgorashi and Van, 2004). A total of 500 grams of the processed plant material was used for each extraction. The plant material was subjected to Soxhlet extraction using 1000 ml of methanol as the solvent at a temperature range of 55 - 88°C for a period of 24 hours. Following Soxhlet extraction, the crude extract was concentrated using a rotary evaporator at 40°C to remove excess solvent, and resulting dry extract was stored at 4°C to maintain its stability and quality for further use in subsequent analyses.

Preparation of Concentrations

Five different concentrations from each Watering regimes (100mg/ml, 50mg/ml, 25mg/ml,12.5mg/ml and 6.25mg/ml) of the extracts of *A. vera* and *C. tora* were prepared through double fold dilution method adopted by Sufi *et al*., (2020). Dimethyl sulfoxide (DMSO) was used as solvent in the preparation of different concentrations of the extracts and used as negative control, while Augmentin 625mg/ml as positive control.

Collection, Purification and Identification of the Test Organisms

Clinical isolates of *S. typhi* and *S. pneumoniaee* were sourced from Rasheed Shekoni Teaching Hospital of Federal University, Dutse. Identification of these clinical isolates was using the classification schemes outlined by Cheesbrough (2006). Subsequently, they were sub-cultured onto MacConkey agar, Eosine Methylene Blue agar, and Salmonella-Shigella agar to confirm the identity of the test organisms, as described by Cheesbrough (2006). Furthermore, additional biochemical tests including indole, oxidase, catalase, and Gram staining were conducted to provide further confirmation, following the methods outlined by Guo *et al*. (2008).

Preparation of Culture Media

The media preparation was based on the manufacturer's instruction and protocol used by Sufi *et al*. (2020). Briefly, a total of 20 grams of Müller–Hinton Agar (MHA) was accurately weighed and added to 500 mL of distilled water. This created a clear amber-colored solution. The agar solution was gently mixed to ensure complete dispersion, and it was then heated with continuous agitation until the agar powder was fully dissolved. The prepared media were sterilized by autoclaving at 121°C for 15 minutes, ensuring the complete elimination of contaminants. After sterilization, the media were allowed to cool to room temperature within a laminar flow hood to maintain sterility. Subsequently, 25 mL of the sterile media was poured into each Petri plate (Sufi *et al*., 2020). The poured media was left undisturbed for a few minutes to facilitate solidification. To initiate the culture, the test organisms were streaked onto the solidified media using a sterile cotton swab. A 90°-degree rotation technique was applied to ensure uniform coverage without any gaps. The prepared culture media were then ready for use in conducting antibacterial sensitivity tests.

Antibacterial Susceptibility Test

The antibacterial susceptibility test was carried out using agar well diffusion method as described by Sufi *et al*. (2020). The plates were incubated at 37 ^{o}C for 24hrs, after which diameter of the growth zone of inhibition was measured in millimeter using standard transparent meter rule.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts from *C. tora* and *A. vera* were determined using the tube dilution method (Baker and Silverton, 1993). Each test tube received 0.5 mL of the prepared plant extract, along with the bacterial inoculum. These tubes were incubated at 37^{o}C for a period of 24 hours. The MIC was determined to be the lowest concentration of the plant extract at which no visible bacterial growth is observed after 24 hours incubation period.

Determination of Minimum Bactericidal Concentration (MBC)

Following the MIC determination, the last test dilution that exhibited visible growth (turbidity) was selected. This dilution represented the concentration at which bacterial growth was still present but inhibited to some extent. To determine the MBC, samples from the tubes that displayed visible growth were sub-cultured onto fresh agar plates (Sufi *et al*., 2020). These plates were subsequently incubated at 37^{o}C for 24 hours. The MBC was determined as the lowest concentration at which no viable bacterial colonies were observed after incubation.

Data Analysis

Data recorded from the determination of the zone of inhibition was subjected to Analysis of Variance at P≤0.05. Tukey test was used as *post hoc* test, and SPSS was used as the statistical software.

RESULT AND DISCUSSION

Effects of Different Watering Regimes on the Zone of Inhibition (mm) of *C. tora* and *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumoniae*

The results (Table 1) indicate that the antibacterial activity of *Cassia tora* extracts varies significantly with different Watering regimes. This observation confirms the impact of environmental factors, such as water availability, on the antibacterial effects of the tested plants. Water stress, characterized by longer intervals between watering, can induce changes in plant metabolism, leading to alterations in the production of secondary metabolites, including phytochemicals with potential antibacterial properties (Shil and Dewanjee, 2022; Diethelm *et al*., 2022).

The study also highlights the differential sensitivity of *Salmonella typhi* and *Streptococcus pneumoniaee* to the *Cassia tora* extracts. Notably, *Salmonella typhi* appears to be more sensitive to the extract at various concentrations and Watering regimes compared to *Streptococcus pneumoniaee*. This variation in sensitivity may be attributed to differences in the cell wall structure, membrane permeability, and physiological characteristics of the two bacterial species (Aleksandrowicz *et al*., 2024). The sensitivity observed was concentration-dependent in both bacterial species, with the highest effect observed at 100 mg/ml of the extracts, indicating that, higher concentrations of the extract result in larger zones of inhibition, indicating a stronger inhibitory effect. This finding supports the idea that the antibacterial activity of *Cassia tora* extract is likely due to the presence of bioactive compounds that inhibit bacterial growth. Such compounds may include alkaloids, flavonoids, and tannins, which are known to possess antimicrobial properties (Toh *et al*., 2023).

Table 1: Effects of Different Watering Regimes on the Zone of Inhibition (mm) Water Stressed *C. tora* Methanolic Extracts on *S. typhi* and S. *pneumoniae*.

		Zone of Inhibition (mm)	.
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	21.5 ± 1.0^{a}	18.0 ± 0.5^{a}
	50	18.0 ± 0.8^{a}	14.5 ± 0.4^{b}
	25	14.5 ± 0.6^{b}	13.0 ± 0.3^{b}
	12.5	11.0 ± 0.5^{c}	7.5 ± 0.2^{c}
	6.25	7.5 ± 0.4^{d}	4.0 ± 0.2^{d}
2-Day	100	20.5 ± 1.0^{a}	17.0 ± 0.5^{a}
	50	17.0 ± 0.8^{b}	16.5 ± 0.4^{a}
	25	13.5 ± 0.6^{c}	10.0 ± 0.3^{b}
	12.5	10.0 ± 0.5^{c}	9.5 ± 0.2^{b}
	6.25	6.5 ± 0.4^{d}	3.0 ± 0.2^{c}
4-Day	100	19.0 ± 0.8^{a}	15.5 ± 0.4^{a}
	50	15.5 ± 0.7^{b}	10.0 ± 0.3^{b}
	25	12.0 ± 0.6^{c}	9.0 ± 0.2^{b}
	12.5	8.5 ± 0.4^{d}	5.0 ± 0.2^{c}
	6.25	8.0 ± 0.3^{d}	1.5 ± 0.1^{d}
8-Day	100	17.5 ± 0.7^{a}	14.0 ± 0.4^{a}
	50	14.0 ± 0.6^{b}	10.5 ± 0.3^{b}
	25	10.5 ± 0.5^{c}	7.0 ± 0.2^{c}
	12.5	7.0 ± 0.4^{d}	3.5 ± 0.2^{d}
	6.25	3.5 ± 0.3^{e}	0.0 ± 0.0^{e}
Positive Control		25.10±0.2	21.87±0.4
Negative Control		0.00±00	0.00±00

Mean±S.D along each column followed by the same letters are not statistically significant at P≤0.05.

The antibacterial properties of *C. tora* extract can be attributed to various mechanisms, including disruption of cell membranes, inhibition of essential enzymes, and interference with bacterial DNA replication (Toh *et al.*, 2023). Further studies, including phytochemical analysis and mechanistic investigations, are warranted to identify the specific bioactive compounds responsible for the observed antibacterial activity and to elucidate their mechanisms of action. The findings of this study suggest that *Cassia tora* extracts, particularly under specific Watering regimes, have the potential to be developed into natural antibacterial agents. Given the increasing concern about antibiotic resistance, exploring alternative sources of antibacterial compounds from plant extracts holds promise for future drug development (Alaoui Mdarhri *et al.*, 2022).

Table 2: Effects of Different Watering Regimes on the Zone of Inhibition (mm) of *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumoniae*.

		Zone of Inhibition (mm)	.
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	22.3± 0.8^{a}	16.8 ± 0.6^{a}
	50	15.7 ± 0.6^{b}	12.5 ± 0.6^{b}
	25	9.7 ± 0.6^{c}	8.3 ± 0.4^{c}
	12.5	5.2 ± 0.4^{d}	4.1 ± 0.3^{d}
	6.25	2.7 ± 0.2^{e}	2.0 ± 0.1^{e}
2-Day	100	20.7 ± 1.0^{a}	15.6 ± 0.8^{a}
	50	15.9 ± 0.8^{b}	12.5 ± 0.6^{b}
	25	9.7 ± 0.6^{c}	8.3 ± 0.4^{c}
	12.5	4.7 ± 0.3^{d}	3.7 ± 0.2^{d}
	6.25	2.4 ± 0.2^{e}	1.8 ± 0.1^{e}
4-Day	100	18.5 ± 0.9^{a}	14.0 ± 0.7^{a}
	50	13.1 ± 0.6^{b}	10.6 ± 0.5^{b}
	25	7.9 ± 0.4^{c}	6.8 ± 0.3^{c}
	12.5	4.2 ± 0.3^{d}	3.3 ± 0.2^{d}
	6.25	2.2 ± 0.1^{e}	1.6 ± 0.1^{e}
8-Day	100	16.8 ± 0.8^{a}	12.7 ± 0.6^{a}
	50	11.9 ± 0.6^{b}	9.6 ± 0.5^{b}
	25	7.2.5 ± 0.4^{c}	6.2 ± 0.3^{c}
	12.5	3.8 ± 0.2^{d}	3.0 ± 0.2^{d}
	6.25	1.9 ± 0.1^{e}	1.4 ± 0.1^{e}
Positive Control		25.10±0.2	21.87±0.4
Negative Control		0.00±00	0.00±00

Mean±S.D along each column followed by the same letters are not statistically significant at P≤0.05.

Aloe vera is renowned for its diverse medicinal properties, including its antimicrobial potential. From the result of this study (Table 2), *A. vera* methanolic extract displayed promising antibacterial activity against both *Salmonella typhi* and *Streptococcus pneumoniaee*. This aligns with previous study that have investigated the antibacterial properties of *Aloe vera* extracts (Toh *et al*., 2023; Sripriya, 2014). The results suggest that different Watering regimes, representing varying levels of water stress on the *Aloe vera* plants, influenced the extract's antibacterial efficacy. This observation is in line with the concept that environmental stressors can impact the phytochemical composition of plants, subsequently affecting their bioactive properties.

Under optimal watering conditions (daily regime), the extract exhibited the highest antibacterial activity, with the largest inhibition zones recorded. This outcome is consistent with the notion that well-hydrated plants tend to accumulate higher levels of secondary metabolites with potential antimicrobial properties (Diethelm *et al*., 2022). As the duration of water stress increased, the antibacterial efficacy of the *Aloe vera* extract progressively declined. This trend highlights the importance of adequate water supply for maintaining the plant's phytochemical reservoir, which can contribute to its bioactivity (Shil and Dewanjee, 2022).

The concentration-dependent response observed in the results is a common phenomenon in plant-based antimicrobial studies. Higher extract concentrations consistently yielded larger inhibition zones, underscoring the importance of dosage in harnessing the extract's antibacterial potential (Begum *et al*., 2023). These findings have potential clinical implications. *Aloe vera*, with its natural antibacterial properties, could be explored for the development of antimicrobial agents. However, it's important to consider the influence of environmental factors, such as water availability, on the plant's phytochemical composition when optimizing extraction methods for medicinal purposes.

Effects of Different Watering Regimes on the Minimum Inhibitory Concentration (MIC) of *C. tora* and *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumoniae.*

The Minimum Inhibitory Concentration (MIC) results of *C. tora* (Table 3) extract against *S. typhi* and *S. pneumoniaee* are situated within the context of the plant's response to varying Watering regimes. These results provide a valuable glimpse into the complex dynamics of plant-microbe interactions under different environmental conditions, with water stress as a central theme.

Table 3: Effects of Different Watering Regimes on the Minimum Inhibitory Concentration (MIC) of *C. tora* Methanolic Extracts on *S. typhi* and S. *pneumonia*

		MIC	.
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	+	+
	50	++	++
	25	-	-
	12.5	-	-
	6.25	-	-
2-Day	100	+	+
	50	++	++
	25	-	-
	12.5	-	-
	6.25	-	-
4-Day	100	++	+
	50	-	++
	25	-	-
	12.5	-	_
	6.25	-	-
8-Day	100	++	+
	50	-	++
	25	-	-
	12.5	-	-
	6.25	-	-

Key: += MIC Positive, - = MIC Negative, ++= MIC Value.

Plant extracts, including those derived from *C. tora*, are known to possess antimicrobial properties attributed to the presence of phytochemical compounds. In the case of *C. tora*, which has been found to contain alkaloids, tannins, flavonoids, and saponins these compounds can exert inhibitory effects on bacterial growth (Begum *et al*., 2023; Rios & Recio, 2005). Crucially, the MIC values observed in this study (Table 3 and 4) confirmed the existing relationship between water availability and the antimicrobial efficacy of *A. vera* and *C. tora* extract. Water stress, as experienced by plants under different regimes, plays a pivotal role in modulating their physiology and secondary metabolite production (Samal *et al*., 2023). During periods of water stress, plants often undergo physiological adaptations, such as reduced stomatal conductance, altered photosynthetic rates, and shifts in overall water potential. These responses can influence the synthesis and accumulation of secondary metabolites, including those with antimicrobial properties (Khalid *et al*., 2023).

Table 4: Minimum Inhibitory Concentration (MIC) *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumonia*

		MIC	.
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	+	+
	50	+	+
	25	-	-
	12.5	-	-
	6.25	-	-
2-Day	100	+	+
	50	+	+
	25	-	-
	12.5	-	-
	6.25	-	-
4-Day	100	+	+
	50	+	+
	25	-	-
	12.5	-	_
	6.25	-	-
8-Day	100	+	+
	50	+	+
	25	-	-
	12.5	-	-
	6.25	-	-

Key: += MIC Positive, - = MIC Negative, ++= MIC Value.

In the daily and 2-day Watering regimes, *C. tora* extract exhibited lower MIC values, suggesting heightened inhibitory activity against both *S. typhi* and *S. pneumoniaee*. This observation aligns with the concept that mild water stress may lead to increased resource allocation towards the production of secondary metabolites, potentially enhancing the extract's antimicrobial potential (Khalid *et al*., 2023). Comparatively, under the 4-day and 8-day Watering regimes, higher MIC values were recorded, indicating reduced inhibitory effects on *S. pneumoniaee*, particularly at the 4-day interval. These findings suggest that prolonged water stress might limit the effectiveness of the extract against specific bacterial strains, possibly due to alterations in phytochemical profiles.

These results (Table 3 and 4) shed light on the complex interplay between water availability, plant physiology, and the antimicrobial properties of *A. vera* and *C. tora* extracts. Understanding these relationships can inform the development of strategies for managing bacterial infections under different water stress conditions, contributing to the advancement of sustainable and effective approaches for combating bacterial pathogens in resource-constrained environments.

Effects of Different Watering Regimes on the Minimum Bactericidal Concentration (MBC) of *C. tora* and *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumoniae*

The Minimum Bactericidal Concentration (MBC) results (Table 5 and 6) obtained from this study provide valuable insights into the bactericidal activity of *A. vera* and *C. tora* extracts against *S. typhi* and *S. pneumoniaee*, highlighting the influence of water regime and concentration on their effectiveness. The daily watering regime consistently exhibited higher MBC values at a concentration of 100 mg/mL, indicating stronger antibacterial properties compared to other Watering regimes. These findings are in line with Begum *et al*. (2023), which also demonstrated the antimicrobial potential of *C. tora* extracts due to the presence of bioactive compounds such as flavonoids, alkaloids, and saponins.

Interestingly, the water stress conditions tested in this study did not significantly alter the overall antibacterial efficacy of *C. tora* extracts. This suggests that the bactericidal activity of the extracts remains relatively stable under different Watering regimes, indicating their potential as consistent antibacterial agents. This is particularly significant in regions with limited water availability, where maintaining effective antibacterial treatments can be challenging. The consistent bactericidal activity of *C. tora* extracts, especially under the daily water regime, highlights the potential of this plant as a valuable resource for combating S. typhy and S. pneumonia infection.

Table 5: Effects of Different Watering Regimes on the Minimum Bacterial Concentration (MBC) of *C. tora* Methanolic Extracts on *S. typhi* and S. *pneumoniae*

		MBC	.
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	++	+
	50	-	++
	25	-	-
	12.5	-	-
	6.25	-	-
2-Day	100	+	+
	50	++	++
	25	-	-
	12.5	-	-
	6.25	-	-
4-Day	100	-	+
	50	-	++
	25	-	-
	12.5	-	_
	6.25	-	-
8-Day	100	-	+
	50	-	++
	25	-	-
	12.5	-	-
	6.25	-	-

Key: += MBC Positive, - = MBC Negative, ++= MBC Value.

However, the presence of bioactive compounds in the extracts, combined with their stable antibacterial efficacy, makes *C. tora* a promising candidate for the development of alternative antibacterial treatments. This is especially relevant in resource-constrained environments where access to conventional antibiotics may be limited.

The MBC results (Table 6) suggest that *A. vera* extracts possess variable bactericidal activity against the two bacterial pathogens under different Watering regimes and concentrations. The daily watering regime consistently exhibited higher MBC values, particularly at 50 mg/mL and 100 mg/mL, indicating superior antibacterial properties. The effectiveness of *A. vera* extracts against *S. typhi* aligns with previous research by Begum *et al*. (2023), who reported the antimicrobial potential of *A. vera* due to the presence of compounds like aloin and aloe emodin.

Table 6: Effects of Different Watering Regimes on the Minimum Bacterial Concentration (MBC) of *A. vera* Methanolic Extracts on *S. typhi* and S. *pneumoniae*

		Zone of Inhibition (mm)	
Watering regimes	**Concentrations (Mg/mL)**	***S. typhi***	**S. *pneumoniae***
Daily	100	+	+
	50	++	++
	25	-	-
	12.5	-	-
	6.25	-	-
2-Day	100	++	+
	50	-	++
	25	-	-
	12.5	-	-
	6.25	-	-
4-Day	100	+	+
	50	++	++
	25	-	-
	12.5	-	_
	6.25	-	-
8-Day	100	++	++
	50	-	-
	25	-	-
	12.5	-	-
	6.25	-	-

Key: += MBC Positive, - = MBC Negative, ++= MBC Value.

However, the effectiveness of *A. vera* extracts against *S. pneumoniaee* appears to be concentration-dependent and influenced by the duration of the watering regime. These findings are consistent with Samal *et al*. (2023), which highlighted the role of both concentration and environmental factors in the antibacterial activity of *A. vera*. The results indicate that *A. vera* extracts have the potential to be used as an antibacterial agent against specific pathogens. The observed variability in antibacterial activity suggests that the choice of concentration and watering regime could influence the effectiveness of *A. vera* extracts in practical applications.

CONCLUSION

Both *A. vera* and *C. tora* extracts exhibited significant antibacterial activity against *S. typhi* and *S. pneumoniae*. The zone of inhibition results indicated that higher concentrations (100 mg/ml) of the extracts led to larger zones of inhibition, demonstrating a concentration-dependent antibacterial effect. The watering regimes had a notable impact on the antibacterial efficacy of the extracts. Daily watering regimes generally resulted in higher zones of inhibition, MIC values, and MBC values compared to longer intervals between watering. This suggests that consistent moisture levels may enhance the antibacterial potential of the extracts.

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